July 27, 2015

Mr. David Madore  
Board of County Councilors  
1300 Franklin  
Vancouver, WA 98660

Re: Requested data

Dear Mr. Madore:

As per your request at the Council meeting on Tuesday, July 21, 2015, please find attached the data that you requested. It includes the following information:

1. Regarding trees and stream temperatures:
   a. Effects of Clear-Cutting on Stream Temperatures.
   b. Modeling the Effects of Land use Change on Water Temperature in Unregulated Urban Streams.

2. Regarding sustainable issues for groundwater and aquifers:
   a. Sustainable Yield of Groundwater.
   b. Ground-Water Depletion Across the Nation.
   d. Assessment of Trends in Ground Water Levels Across the United States.
   e. Critical Aquifer Recharge Areas
   f. Tapping Unsustainable Ground Water Stores for Agricultural Production in the High Plains Aquifer of Kansas, projections to 2110

3. Issues Regarding Water Quality and Fish:
   a. Meeting Hydrologic and Water Quality Goals through Targeted Bioretention Design.
   b. Landscape Ecotoxicology of Coho Salmon Spawner Mortality in Urban Streams.
   d. Sublethal Exposure to Crude Oil During Embryonic Development Alters
Cardiac Morphology and Reduces Aerobic Capacity in Adult Fish.
e. Natural Sunlight and Residual Fuel Oils Are An Acutely Lethal Combination for Fish Embryos
f. Attenuation of Polycyclic Aromatic Hydrocarbons from Urban Stormwater Runoff by Wood Filters.
g. Defects in Cardiac Function Precede Morphological Abnormalities in Fish Embryos Exposed to Polycyclic Aromatic Hydrocarbons.
h. Fish Embryos Are Damaged by Dissolved PAHs, Not Oil Particles.
i. Coastal Storms, Toxic Runoff, and the Sustainable Conservation of Fish and Fisheries.
j. Chemosensory Deprivation in Juvenile Coho Exposed to Dissolved Copper under Varying Water Chemistry Conditions.
k. Effects of Water Hardness, Alkalinity, and Dissolved Organic Carbon on the Toxicity of Copper to the Lateral Line of Developing Fish.

If you have further questions after reviewing this information, please do not hesitate to contact me.

Linda Conaway

cc: Ms. Jeannne Stewart, Clark County Councilor
Mr. Tom Mielke, Clark County Councilor
Clark County Planning Department
Effects of Clear-Cutting on Stream Temperature

GEORGE W. BROWN AND JAMES T. KRYGIER

Oregon State University, Corvallis, Oregon 97331

Abstract. The principal source of energy for warming streams is the sun. The amount of sunlight reaching the stream may be increased after clear-cut logging. Average monthly maximum temperatures increased by 14°F and annual maximum temperatures increased from 57°F to 65°F one year after clear-cut logging on a small watershed in Oregon's coast range. In a nearby watershed where strips of brush and trees separated logging units from the stream, no changes in temperature were observed that could be attributed to clear-cutting.

INTRODUCTION

Timber, water, and sport and commercial fish are the principal resources in the Oregon coast range. The need for delineating the areas of conflict between logging and utilisation of the other resources led to the establishment of the Alsea Logging-Aquatic Resources Study in 1966. The purpose of this broadly interdisciplinary study was to determine the effect of logging on the physical, chemical, and biological characteristics of small coastal streams.

The purpose of this paper is to describe the long-term effects of two clear-cuttings on the temperature regime of two small streams in Oregon's coast range. One watershed contained three small clear-cuts; the edges of the clearcuts were at least 100 feet from the stream. The second watershed was completely clear-cut. An earlier report [Brown and Krygier, 1967] described the first-year effect of clear-cutting only during the logging operation on the completely clear-cut watershed. This report reviews results from a network of 18 thermograph stations distributed through the watersheds. The observation period extends from two years before logging through the fourth summer after logging.

Temperature is a significant water quality parameter. It strongly influences levels of oxygen and solids dissolved in streams. Temperature changes can induce algal blooms with subsequent changes in taste, odor, and color of a stream. Warm water is conducive to the growth and development of many species of aquatic bacteria, such as the parasitic columnaris disease. Increased populations of these bacteria may cause fish mortality [Brett, 1966]. The growth of fish may be directly affected by water temperature as demonstrated on juvenile coho salmon [Brett, 1966]. In short, water temperature is a major determinant of the suitability of water for many uses.

Research has been limited on temperature changes in small streams from land use, although fishery biologists have long been concerned with the effects of deforestation on water temperature. Meekan et al. [1966] studied the effects of clear-cutting on the salmon habitat of two southeastern Alaska streams. They noted a statistically significant increase in mean monthly temperatures after logging. The maximum increase in average monthly temperature was about 4°F. The increase in maximum temperature was about 9°F during July and August.

During a study of logging and southeastern trout streams, Greene [1950] reported that maximum weekly temperatures recorded during May on a nonforested stream were 13°F higher than those recorded on a nearby forested stream. He noticed also that the maximum temperature dropped from 80°F to 63°F after the nonforested stream meandered through 400 feet of forest and brush cover.

Levno and Rothacher [1967] reported large temperature increases in two experimental watersheds in Oregon after logging. The shade provided by riparian vegetation in a patch-cut watershed was eliminated by scouring after large floods in 1964. Subsequently, mean monthly temperatures increased 7°F to 12°F from April to August. Average monthly maximums increased by 4°F after complete clear-cutting in a second
watershed. The smaller increase in the completely clear-cut watershed was the result of shade from the logging debris that accumulated in the channel.

Postic [1969] compared the effect of two clear-cutting patterns on water quality. Temperatures were unaffected by clear-cutting the upper half of one watershed. Clear-cutting the lower half of the second watershed increased temperatures up to 7°F.

**THE STUDY**

Three experimental watersheds are included in the Alsea Logging-Aquatic Resources Study (Figure 1). These watersheds, which vary in size from 175 to 750 acres, are located in Oregon's coast range about 10 miles from the Pacific Ocean. Each stream is an important rearing area for coho salmon (*Oncorhynchus kisutch* Walbaum) and coastal cutthroat trout (*Salmo clarki clarki* Richardson). In its natural
condition, the study area was densely forested with Douglas fir (Pseudotsuga menziesii [Mirb.] Franco) and red alder (Alnus rubra Bong). The study streams were overgrown with salmonberry (Rubus spectabilis Pursh.), vine maple (Acer circinatum Pursh.), and other species. Although annual precipitation is about 100 inches, the summer months are generally hot and dry. Summer streamflow regularly drops below 0.20 cubic feet per second (cfs) or 0.17 cubic feet per second per square mile (csm) on Deer Creek, the largest watershed, and to 0.01 cfs or 0.04 csm on Needle Branch, the smallest watershed.

The low summer flows described above may seem insufficient to support salmon. The adults, however, spawn in these streams during the high flows of the winter months. Salmon fingerlings live in these streams during the summer. The fingerlings inhabit pools, many of which become nearly isolated during the late summer. In the fall, the yearling fish migrate to the sea when rains again increase streamflow. Before logging, the number of yearling fish passing through the fish trap to the sea ranged from 1809 to 3175 in Deer Creek and from 166 to 630 in Needle Branch [Hall and Lents, 1969].

The study was designed to permit comparison of two different logging patterns. One watershed, Needle Branch (175 acres), was fully clear-cut. A second watershed, Deer Creek (750 acres), was patch-cut; 20% of this area had several clear-cut units. The remainder of the watershed was unlogged. In Deer Creek, strips of vegetation were left along the perennial
streams. A third watershed, Flyan Creek (502 acres), was left unlogged as a control. Two small subwatersheds in Deer Creek also served as unlogged controls.

Eighteen 7-day thermographs, accurate to 0.5°F, were installed in the three watersheds to evaluate the effect of the cutting (Figure 1). Thermographs were placed below each proposed logging unit and at the junction of each major tributary in Deer Creek so that effects within the watershed could be determined. In Needle Branch, thermographs were distributed within the clear-cutting to evaluate the spatial temperature changes occurring in a fully exposed stream. Thermographs were installed in March 1964. Probes were placed in flowing water deep enough to insure complete coverage throughout the year. The years 1964 and 1965 served as control periods, and the years 1966–1969 as treatment periods.

Road building was completed in 1965, but, because the roads were built on ridges, little change occurred that could be interpreted as having any influence on water temperature. Logging began in March 1966 in both watersheds and was completed in August on Needle Branch and in November on Deer Creek. In October 1966, the stream in the clear-cut watershed was cleared of logging debris. After clearing, a well-distributed burn removed most logging debris and streamside vegetation. Reported data extend through September 1969.

RESULTS

The data from this study have been analyzed in two ways. The large changes that occur after clear-cutting are presented graphically. The small changes that occur after patch-cutting required a statistical analysis to ascertain the significance of these changes. The standard statistical technique of regression could not be used because of the nonrandom effects of climate, the lack of independence between successive daily maximums, and the potential alteration of the variance of seasonal temperature distributions by logging. A stationary time series was developed to circumvent these difficulties [Beck, 1965]. Time series techniques are commonly used for analysis of weather data where similar difficulties abound. Jenkins and Watts [1968] describe the application of this technique to several such problems. Our time series compared daily maximum temperatures recorded June 1–October 1 of the pretreatment years with the daily maximums for the same period during each treatment year. The analysis was applied to data from the control as well as from the patch-cut watershed to ascertain the effects of climate.

Diurnal Temperature Regimes

The temperature patterns recorded on the days of the annual maximum on the clear-cut watershed from 1965 to 1969 are illustrated in Figure 2. The values for 1966 and 1968 occurred at the watershed outlet. The values for 1967, 1968, and 1969 occurred within the cutover unit. A thermograph was installed at 1000 feet above the outlet in the spring of 1967, after intensive sampling showed that the maximum occurred at this location. This inconsistency in the temperature pattern was the result of incomplete removal of shade from the lower portion of the stream channel after logging and burning. The variation in temperatures recorded at the outlet of the unlogged watershed for the same days is also shown. Minimums recorded on the clear-cut watershed are about the same as the maximums recorded on the unlogged control. This phenomenon occurs because travel time through the clear-cut watershed is greater than 24 hours during the low flow period. Convection and nocturnal back radiation are insufficient to cool the water to the same minimums measured on the control. The maximum diurnal fluctuation recorded on the clear-cut watershed was 28°F during 1967. The maximum temperature, 88°F during 1967, represents an increase of 28°F over the prelogging maximum of 57°F for 1966. The decline of maximum temperatures after 1967 represents the rapid return of streamside vegetation in this watershed.

The maximum temperatures recorded on the patch-cut watershed were 60° and 81.5°F for 1966 and 1967, respectively. The maximum diurnal fluctuation during both years was 10°F.

A cumulative frequency distribution of the diurnal fluctuations in temperature at the outlet of all three streams from June 1 to October 1 for the years 1965–1969 is shown in Figure 3. The temperature stability of natural, forested streams is illustrated again in this figure. The maximum fluctuations in temperature before logging in 1965 were 6°, 8°, and 10°F on Flyan

015415
Creek, Needle Branch, and Deer Creek. These maximum fluctuations were not exceeded on Flynn Creek or at any station within Deer Creek during or after logging. On Needle Branch, the maximum fluctuation of 8°F was exceeded 28% of the time in 1968 and 82% of the time in 1967, the year immediately following burning and stream clearance. This percentage dropped to 46% in 1968 and 36% in 1969, again reflecting the regrowth of streamside vegetation.

The outlet stations are representative of the changes that occurred within each watershed. Temperature fluctuations generally decreased with distance upstream in the patch-cut watershed. The most remote station (station 15) in the clear-cut watershed performed similarly to the outlet of Deer Creek.

**Monthly Maximum Temperatures**

**Clear-cut.** Maximum daily stream temperatures averaged by month for one year before and four years after logging are shown for the outlets of the clear-cut and unlogged watersheds in Figure 4. Except for the most remote station (station 15), the changes recorded at the outlet station are representative of those occurring at the other stations within the clear-cut watershed. Again the highest temperatures occurred during 1967, the year after stream clearance and slash burning. The mean monthly maximum for July increased from 57°F in 1965 to 71°F in 1967. The trend toward the prelogging condition is shown again in this figure.

**Patch-cut.** The frequency diagram illustrates the nearly constant pattern of daily temperature fluctuation recorded on the patch-cut watershed throughout the study (Figure 3). A time series was required to determine whether the small increases observed initially were the result of logging or climatic differences between years. The results of the time series are presented in Table 1. Significant changes in the summer maximum temperatures were observed at the outlets on the control and patch-cut watershed one year after logging. The larger changes observed on the control indicate that climatic factors, and not the patch-cutting, were responsible for this increase. During 1967 and 1968, summer maximums in the stream of the patch-cut were nearly the same as those observed before logging. The 10 internal stations exhibited smaller changes in temperature than the outlet station. These data show that patch-cuting, which leaves streamside strips of brush and trees, did not alter the temperature patterns of the adjacent stream.

Other inferences about the temperature patterns may be drawn from these data. Clearly the patterns in summer temperatures of forested streams are relatively constant from year to year. The small differences between years listed in Table 1 and the statistical significance of a

---

**TABLE 1. A Time Series Analysis of the Differences between Daily Maximum Temperatures of Streams before and after Patch-Cutting for the Period of June 1 to October 1**

<table>
<thead>
<tr>
<th>Watershed</th>
<th>1966</th>
<th>1967</th>
<th>1968</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flynn Creek</td>
<td>0.3</td>
<td>2.3*</td>
<td>0.3</td>
</tr>
<tr>
<td>(unlogged)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deer Creek</td>
<td>0.4</td>
<td>1.9*</td>
<td>-0.4</td>
</tr>
<tr>
<td>(patch-cut)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at the 5% level of probability.
2° change illustrate little variability in average maximum temperature for a summer.

**DISCUSSION**

A detailed description of the hydrologic and atmospheric factors affecting water temperature was given earlier [Brown, 1969]. The most important environmental factor governing temperature change is solar radiation received at the stream surface.

Temperature differences between watersheds and all of the temperature anomalies within the clear-cut watershed can be explained in terms of shade differences. The patch-cuts on Deer Creek did not produce any significant changes in temperature in the main stream. Strips of timber 100 feet long were left beside each perennial stream; the amount of shade on the stream surface was essentially unchanged. On Needle Branch, little shade remained after the clear-cutting and burning were completed. As a result, large changes in annual and daily patterns of temperature were observed.

The principal cause of high water temperature following logging is stream surface exposure to direct insolation and not increased soil temperature on the clear-cut slopes as suggested by Bechler and Larnopere [1968]. Satisfactory predictions of temperature have been made on Deer Creek and Needle Branch with net radiation as the primary parameter. On these same streams we found that the maximum rate of net thermal radiation added to the unshaded stream in the clear-cut watershed was more than 10 times that added to the shaded stream in the patch-cut watershed. These differences are reflected in the postlogging temperature patterns of each stream and help explain the temperature stability of most forested streams.

For a given level of solar radiation or heat, stream temperature is inversely proportional to volume. As a result the temperature patterns of small, shallow streams typical of headwater regions may be increased significantly by any changes in the solar radiation. Stream discharge in the clear-cut watershed regularly drops to 0.01 cfs during the hot summer months. Thus the large changes in temperature recorded after the shade was removed from this stream were to be expected. The flow regime of this small stream is typical of many western Oregon streams that must support salmon and trout during the period of low flow.

Can the results of this study be classified as typical of western Oregon conditions or were they merely caused by unusually hot summers? The maximum temperature of 85°F recorded for the clear-cut and burned watershed is undoubtedly close to the maximum temperature that could occur for this size of clear-cut watershed and stream. Because of its small size, the stream responded to each clear day with high temperatures, regardless of the previous day's weather. Even if only one clear hot day had occurred, this temperature would have been observed. The records of mean monthly temperatures and the frequencies of diurnal change present data for longer periods. The number of days of sunshine, overcast, fog, and rain influence such results. Long-term records of sunshine are not available for the study area. Records at nearby stations, however, indicate that the period of study was not abnormal.

Duration of temperature effects following clear-cutting is the subject of continued observation in these experimental streams. Temperature amelioration is related to shade development. As stream bank vegetation becomes reestablished, temperatures drop accordingly. In many of the watersheds of the Pacific Northwest, regrowth occurs very rapidly on moist sites. On the basis of our data, it seems that summer maximums may approach prelogging levels within six years after logging has completely exposed the stream if vigorous invasion of such moist-site species as alder, salmonberry, elderberry, and vine maple occurs. The decline of high temperatures noted in Figures 3, 4, and 4 after the 1967 maximum on Needle Branch illustrate this recovery. The stream's temperature patterns are well on the way to returning to the prelogging condition.

Our research reported here and a companion study [Brown, 1969] have illustrated the effect of two patterns of logging on the temperature of small coastal streams and the amelioration of the effect with time. These results, however, have raised several questions about temperature control in the management of fishery, forest, and water resources. Foremost among them is the effect of high temperatures, such as those recorded during 1967 in the clear-cut watershed, on fish. Earlier work [Brett, 1932] indicated
that at 27.5°C (81.5°F) the time required to induce 50% mortality in a population of coho salmon fry is only 70 minutes. But no unusual mortality in coho was observed during this study even though stream temperatures were often above this limit for 4 to 6 hours.

The study illustrated the benefit of strips of vegetation alongside small streams for temperature control. The width, density, species composition, and costs incurred when planting streamside strips are only a few of the questions posed by forest managers.

Finally, this study should encourage water resource agencies to engage in further studies of the aquatic habitat in small streams and its relation to land use. Although the temperature changes recorded are defined as thermal pollution in Oregon's current water quality standards [Oregon Sanitary Authority, 1967], the effect on the coho fishery would suggest that a more precise definition is required. Such a definition, of course, will require a better understanding of the response of the aquatic system to temperatures in the lethal and sublethal ranges.

Acknowledgments. The research was sponsored by the Federal Water Pollution Control Administration under grant WP-438 and by Oregon State University. Other cooperators in the Aina Logging--Aquatic Resources study include: Department of Fisheries and Wildlife, Oregon State University; Oregon Game Commission; U. S. Geological Survey; Federal Water Pollution Control Administration; Water Resources Research Institute, Oregon State University; Georgia Pacific Corporation; U. S. Forest Service; Stokes Lumber Company; and F. W. Williamson. The authors are particularly indebted to L. C. Beck and Dr. F. L. Ramsey, Department of Statistics, Oregon State University, for their assistance in the time series analysis of a portion of our data.

REFERENCES

Beck, L. C., Basic concepts and theory of stationary time series and their application to the problem of determining effects of logging on water temperature, M.S. report in statistics, Oregon State University, Corvallis, 1969.


Oregon Sanitary Authority, Standards of quality for public waters of Oregon and disposal therein of sewage and industrial wastes, Admin. Order SA #8, June 1, 1967.


(Manuscript received January 5, 1970; revised April 14, 1970.)
Modeling the Effects of Land Use Change on the Water Temperature in Unregulated Urban Streams

Robert T. LeBlanc*, Robert D. Brown† and John E. FitzGibbon†

*Faculty of Environmental Design, The University of Canberra, PO Box 1, Belconnen, Australia, ACT 2616 and †School of Landscape Architecture, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Final version received 4 November 1996; accepted 11 November 1996

Streams, in their natural state, are typically diverse and biologically productive environments. Streams subject to urbanization often experience degradation brought about by the cumulative effects of flow alteration, unsanitary discharge and channelization. One of the water quality parameters affected by urbanization is stream temperature. This study offers a model for predicting the impact of land use change on the temperature of non-regulated streams during extreme events.

A stream temperature model was created by considering the gains and losses of thermal energy resulting from radiation, convection, conduction, evaporation and advection. A sensitivity analysis showed that out of 14 variables, shade/transmissivity of riparian vegetation, groundwater discharge, and stream width had the greatest influence on stream temperature. These same three variables are highly influenced by land use. Individual component models were developed to predict how urbanization changes stream width and baseflow discharge. Using 3-D computer modeling, a model was also developed to illustrate the effects of altering the extent and composition of riparian vegetation on streams with different orientations.

By modeling these three variables as a function of urbanization, the results became inputs into the stream temperature model. The critical urban stream temperature model (CrUSTe), an aggregation of these four models, allows the prediction of stream temperature change as a result of amount, type and location of urbanization within a watershed. It has the potential to become a valuable tool for environmental managers.

Keywords: stream temperature, watershed urbanization, riparian vegetation, baseflow, channel morphology.

1. Introduction

The way in which land is developed can seriously compromise the integrity of rivers and streams. Countless studies have focused on the relationship between land use...

*Corresponding author. E-mail: rob.leblanc@ns.sympatico.ca

© 1997 Academic Press Limited
change and the quality and quantity of water in urbanizing basins. These studies suggest that hydrologic changes in urban watersheds can most often be attributed to the modification of stream characteristics which govern discharge, stream chemistry, stream/floodplain interactions and channel morphology. As a result, urbanization is thought to cause reduced baseflows, increased frequency and magnitude of peak discharges, increased sediment loads, reduction in channel and floodplain complexity and impaired water quality (Dunne and Leopold, 1978; Klein, 1979; Halyk et al., 1991).

One of the more significant water quality parameters affected by urbanization is water temperature. Temperature is a major regulator of living systems. Water temperature has been considered one of the most important factors determining the geographic distribution, growth rate and survival of fish and other aquatic organisms (Barthalow, 1989; Holmes and Regier, 1990; Armour, 1991). Temperature regimes are thought to influence migration patterns, egg maturation, incubation success, inter- and intraspecific competitive ability and resistance to parasites, diseases, and pollutants (Armour, 1991). Water temperature also influences the rates of in-stream chemical reactions, the self purification capacity of streams and their aesthetic and sanitary qualities (Feller, 1981). In essence, drastic alterations of the natural temperature regime can cause a cascade whereby the aquatic system can digress from a stable state (with a high assimilative capacity) to an unstable state (with a low assimilative capacity).

1.1. URBANIZATION AND STREAM TEMPERATURE CHANGE

In a study of urbanization and its effect on the temperature of streams on Long Island, New York, Pluhowski (1970) found that modification of the hydrologic environment due to urbanization increased the average stream temperature in summer by as much as 5–8°C. Klein (1979) documented similar changes to the natural stream temperature regime as a result of urbanization.

This paper presents a Critical Urban Stream Temperature (CrUSTe) model which includes the mechanisms by which the critical water temperatures of unregulated streams are affected by land uses within the basin. There were two main reasons for choosing unregulated systems. First, temperature is a function of discharge; so regulated streams (streams with a controlled discharge), by their very nature, have a regulated temperature regime. Daley and Seaders (1966) provide insight into predicting temperatures of regulated systems. Second, and more importantly, the indirect processes that define how stream temperatures are affected by urbanization are not well understood.

“Critical” stream temperatures refer to summer extremes usually associated with low flow periods. It is during these times that the biotic potential of the stream is most at risk. In light of recent global warming predictions (Hengeveld, 1990) and the continuing expansion of urban areas world wide, this study offers valuable insight into stream temperature changes within the context of the developing basin.

Stream temperature is a function of many variables, some of which are directly or indirectly affected by urbanization. This study has identified three main urban factors responsible for altering the thermal environment of streams. These are: (1) changes to the composition of vegetation in the watershed (more specifically, the clearing of riparian vegetation along stream banks), (2) changes to the low flow regime, and (3) changes to the stream’s hydraulic geometry.

Riparian vegetation controls the stream’s thermal character by intercepting short-wave radiation during the day and insulating the stream from long-wave radiation loss at night. Stream discharge also regulates water temperatures. The more water, the more
energy it takes to heat it. The hydraulic geometry, a function of the cross-sectional shape of the stream, influences the surface area of the stream. Since most energy exchanges occur at the air–water interface, the surface area of the stream is directly related to water temperature changes.

To predict how urbanization affects temperature, it is first necessary to model how urbanization influences each of these three variables. Once this is known, the outputs from the individual component models become the inputs into the stream temperature model. This paper is organized in such a way as to present the stream temperature model and each of the three component models (namely, the riparian vegetation removal model, the stream morphology model and the baseflow model). The combination of the component models and the stream temperature model form the basis for the CrUSTe model.

The aforementioned urban variables are not the only variables that can influence water temperature in the urban environment. Other factors not considered by this study include the removal or introduction of wetlands, ponds or lakes, thermal discharge from industrial (or point source) operations, and non point-source thermal discharges (such as storm sewers, urban runoff, agricultural drainage tiles, etc.). The rationale for excluding the first two factors stems from the fact that they are specific to certain watersheds. This study is limited to more generic urban watersheds. The effects on stream temperature resulting from the creation or removal of wetlands is a possible area for further research. Non point-source thermal discharges, having variable heat budget contributions during and after storm events, have been excluded because there is little or no impact on stream temperature during critical periods (which occur during dry periods).

2. Stream temperature model

A physical process model, which estimates the energy inputs and outputs and translates these into temperature changes, was adopted for this study. Although more complex than a regression type model, the physical process model provided a more robust picture of stream temperatures in an urban setting.

The model defined the energy balance in a stream by estimating the radiation, evaporation, conduction, convection and advection in terms of their respective flux densities. The energy balance equation takes the form shown in Equation (1):

$$\Delta H = H_{sr} + H_E + H_C + H_R + H_A$$

(1)

where $\Delta H$ is the net change in energy stored (J/cm$^2$/hr), $H_{sr}$ is the net thermal radiative flux, $H_E$ is the evaporative flux, $H_C$ is the conductive flux, $H_R$ is the convective flux, and $H_A$ is the advective flux. For energy gained by the stream, the sign convention is positive. A net increase or decrease in heat results in a corresponding change in water temperature.

The net change in energy stored is translated into a corresponding water temperature change using the specific heat capacity of water (4184 J/\degree C). The equation takes the form:

$$\Delta T = (\Delta H/A/Q) \cdot 0.000664$$

(2)

where $\Delta T$ is the change in water temperature (\degree C), $A$ is the stream surface area (m$^2$)
obtained by taking the product of the reach length and the average reach width, \( Q \) is the discharge (l/sec) and 0.000664 is a constant having the units (l*°C/J).

The following sections (2.1–2.5) describe the equations which can be used to obtain values for the five respective energy fluxes.

2.1. \( H_{NR} \), NET RADIATIVE FLUX

The net radiative flux is composed of net short-wave and net long-wave radiation, such that \( H_{NR} = R_{SW} + R_{LW} \) in W/m².

The net short-wave radiation \( (R_{SW}) \) is a product of the short-wave radiation at the stream surface \( (R_s) \) and the absorptivity of the water \( (1 - \alpha) \), such that

\[
R_{SW} = R_s(1 - \alpha); \text{ where } \alpha = \text{ the albedo of water}
\]

(3)

The reflectivity of the water's surface, or albedo, can be estimated (Anderson, 1954) by:

\[
\alpha = 1.18 \times 10^{-7} \text{ for } \beta \geq 1.24\degree; \\
\alpha = 1.0 \text{ for } \beta < 1.24\degree; \text{ where } \beta = \text{ the solar elevation angle in degrees}
\]

(4)

The short-wave radiation received at the stream surface can be calculated from:

\[
R_s = (SVF(0.1 - K)) + (S_f Trans(0.9 - K)) + ((1 - S_f)(0.9 - K))
\]

(5)

where \( S_f \) is the average percent of shade striking the surface of the stream per hour throughout the entire reach. \( SVF \) is the sky view factor (as a decimal). The sky view factor is a ratio of the amount of sky visible from a certain point on the surface of the water relative to the maximum possible. If the point is completely enclosed the \( SVF \) equals 0, whereas if the sky is completely visible from a point the \( SVF \) approaches unity. \( K \) is the measured short-wave radiation in the open (W/m²). The \( K \) value can be directly measured using a pyranometer, or it can be estimated from Equation (6) (Brown and Gillespie, 1990). The variable, \( Trans \), is the transmissivity of the riparian vegetation (as a fraction) which represents the percent of short-wave radiation which can penetrate the canopy. The transmissivity values for several hydrophic species of trees have been reported by McPherson (1984).

\[
K = (0.77 - (1360) \times \text{Sin(\beta)})
\]

(6)

All objects emit long-wave radiation. The atmosphere and riparian trees are major contributors. The net long-wave radiation \( (R_{LW}) \) can be estimated using the net long-wave radiation received at the stream surface \( (R_L) \) in W/m² and the temperature of the stream \( (T_s) \) in Kelvin \((°C + 273.15)\) shown in Equation (7). Since it is the purpose of the model to predict water temperature, a non-steady state (dynamic) approach is needed to solve \( R_{LW} \).

\[
R_{LW} = R_L - 0.98 \times (5.67 \times 10^{-8} T_s^4)
\]

(7)

\( R_L \) is the sum of the long-wave radiation from the sky \( (L_s) \) and the trees \( (L_T) \) so that
\[ R_t = L_2 + L_3 \]

\[ L_2 = (1 - C_C)(1.2 \times 10^{-8} T^2 - 171) + (C_C(5.67 \times 10^{-8} T^4 - 9) \cdot SVF) \]  \hspace{1cm} (8)

\[ L_3 = (0.98(5.67 \times 10^{-8} T^4))(1 - SVF) \]  \hspace{1cm} (9)

Where \( T_a \) is the temperature of the air in Kelvin and \( C_C \) is the cloud cover ratio that ranges from 0 (no clouds) to 1 (completely clouded sky). The units of the net long-wave and short-wave radiation are in W/m². To convert W/m² to J/cm²-hr for use in Equation (2), it is necessary to multiply W/m² by 0.36.

2.2. \( H_o \), EVAPORATIVE FLUX

Evaporative heat transfer results from the change in state of water from a liquid to a vapour. Heat can be dissipated from the stream as energy is supplied to the process of evaporation. Evaporative heat transfer can be approximated using Equation (10) (Krajewski et al., 1982).

\[ H_o = -23.42 \cdot W[(1.0646)^T - (1.0646)^W \cdot h] \]  \hspace{1cm} (9)

Where \( W \) is the wind velocity (m/s) at the surface of the water and \( h \) is the relative humidity (%) which can be obtained at a local weather station. Due to air turbulence at the surface of the water, the wind velocity value is seldom less than 1 m/s (Krajewski et al., 1982).

2.3. \( H_c \), CONDUCTIVE FLUX

The heat input as a result of conduction was so negligible that it was not considered a significant variable for this study. Conduction may figure into the model if the stream under investigation was very shallow, exposed to direct sunlight and possessed a dark bottom material with a high thermal conductivity (Brown, 1969).

2.4. \( H_n \), CONVECTIVE FLUX

Convection occurs between the water and the air as the energy is transported between the two phases. The catalysts for the convective transfer of energy include the temperature gradient between the air and the water interface (°C), the atmospheric pressure, \( p \) (kPa) and the wind speed (m/s) such that,

\[ H_n = 0.0228 \cdot p \cdot W(T_a - T_w) \]  \hspace{1cm} (Krajewski, 1982).

2.5. \( H_d \), ADVECTIVE FLUX

Advective occurs when water (or any substance) at a different temperature is added to the stream. This might occur as tributary inflow, overland flow, direct precipitation or groundwater infiltration. To eliminate complexity in the modeling process, it is best to choose a reach that has no additional tributary inflow (otherwise a continuous measure of temperature, discharge, etc. is required for each tributary). The optimal period for
study is when precipitation and overland flow are negligible. This leaves groundwater flow as the primary advective input.

Groundwater discharge is a typical occurrence in Southern Ontario. If the amount of discharge and temperature of the groundwater is known, a simple temperature-dilution ratio can be used to estimate the water temperature change. In this case, the advective flux density need not be computed.

One approach taken by researchers to determine the influence of groundwater on stream temperature is to attribute the groundwater discharge to the beginning of the reach and assume complete mixing (Meisner, 1990). In this case, an inflow and groundwater discharge ratio is used to calculate a new mixed inflow temperature and discharge, the result of which is used for the temperature model. This may be appropriate for situations when the inflow to groundwater discharge ratio is high, but when the groundwater discharge begins to approach or surpass the inflow discharge, such an assumption will not give accurate results. This approach also has limitations over longer reaches (greater than 1 km).

To overcome this problem, the reach was divided into several sub-reaches, where a proportion of groundwater is attributed to the beginning of each sub-reach. The model is used to calculate the temperature change over each sub-reach with the temperature output of the previous sub-reach becoming the input to the next sub-reach (see Figure 1).

This method assumes that the rate of groundwater infiltration is distributed evenly over the length of the reach. There are limitations to this approach, but if the location and amount of groundwater is known for a specific reach, it would be a simple matter to re-allocate the true discharge proportions to each of the sub-reaches.

2.6. MODEL CONSTRUCTION AND VALIDATION

Upon observation of the various energy balance equations used to calculate stream temperature, it is evident that there are interdependencies built into the model. For example, water temperature ($T_w$) is required to solve Equations (7), (9) and (10), yet water temperature is the final state variable that the model is trying to predict. Because of this, a dynamic modeling approach is required. STELLA II (High Performance Systems), a commercial dynamic modeling software package, was used to construct the stream temperature model. Using STELLA II's feedback loop capabilities, the water temperature at the end of each sub-reach can be fed back into the equations that
require water temperature as a variable in the proceeding sub-reach. Figure 2 shows STELLA II's graphical form of the model.

The model was validated using temperature, hydrologic and meteorological data collected from Morningside Creek, just outside of Toronto, Ontario. Figure 3 shows the agreement of the modeled and actual results over a two day period in August, 1994. The standard error of estimate ($S_{r,2}$) over the two day period is 0.77°C. Generally, there was good agreement between the modeled and the actual results.

3. Sensitivity investigation

A sensitivity investigation was performed to gauge the model's sensitivity to its various parameters. The sensitivity investigation helped to: (a) identify the parameters that were sensitive and insensitive, (b) ascertain the degree to which reliable measurement was warranted, (c) validate the model's consistency with the real world system under investigation (Reckhow and Chapra, 1983).

The details of the sensitivity investigation approach can be found in LeBlanc (1994).
From the resultant descriptive statistics, a box plot was constructed to show the relative influence of each variable on stream temperature (Figure 4). The box plot shows the relative influence of each parameter on water temperature. From the sensitivity investigation, discharge was found to have the largest influence on stream temperature. Sun angle, transmissivity of vegetation and groundwater discharge also had a significant influence on the stream temperature. It is important to realize that these findings are representative of the input parameters chosen and can vary depending on how the parameters change in each geographic region.

3.1. ESTIMATING THE DEGREE OF RELIABLE MEASUREMENT

To estimate the accuracy and precision of measurements required in the model, the values of representative input parameters were raised and lowered until a stream temperature change of 0.5°C was observed. "Representative" input parameters are values that might be expected in the local region under investigation (in this case, southern Ontario, Canada). Extreme events (i.e. those events that contribute to high stream temperatures) were chosen because it is during these times when the model’s resolution would be at its greatest.

Once representative data were collected (see LeBlanc, 1994, for more details) and run through the stream temperature model, the suggested degree of reliable measurement per 2 km of reach was determined for each parameter. From these data it was apparent
R. T. LeBlanc et al.

Atmospheric pressure
Sky view factor
Relative humidity
Air temperature
Wind speed
Stream width
Groundwater discharge
Transmissivity/Shade
Sun angle
Discharge

Figure 4. Box plot of the relative sensitivities of each parameter per hour, taken from the descriptive statistics for each parameter.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Representative data</th>
<th>Degree of reliable measurement</th>
<th>Other parameters used in the model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun angle</td>
<td>70.1°</td>
<td>8.1°</td>
<td>Stream length 2 km</td>
</tr>
<tr>
<td>Sky view factor</td>
<td>0 ± 1</td>
<td>0.07</td>
<td>Groundwater temperature 8.3°C</td>
</tr>
<tr>
<td>Transmissivity</td>
<td>1</td>
<td>1.4</td>
<td>Inflow temperature 14°C</td>
</tr>
<tr>
<td>River discharge</td>
<td>8 l/s</td>
<td>14 l/s</td>
<td></td>
</tr>
<tr>
<td>Groundwater discharge</td>
<td>79 l/s</td>
<td>5.7 l/s</td>
<td></td>
</tr>
<tr>
<td>Stream width</td>
<td>2.1 m</td>
<td>0.14 m</td>
<td></td>
</tr>
<tr>
<td>Wind speed</td>
<td>4.2 m/s</td>
<td>4.8 m/s</td>
<td></td>
</tr>
<tr>
<td>Air temperature</td>
<td>26.1°C</td>
<td>3.5°C</td>
<td></td>
</tr>
<tr>
<td>Relative humidity</td>
<td>29%</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>Atmospheric pressure</td>
<td>98.68 kPa</td>
<td>70.7 kPa</td>
<td></td>
</tr>
</tbody>
</table>

*Maximum possible sun angle on 21 June, in Waterloo, Ontario, Canada.

that the sky view factor and the atmospheric pressure would require only estimated values as inputs since the degree of reliable measurement almost equalled the parameter’s representative range. The representative data and an estimate of the degree of reliable measurement are summarized in Table 1.

The values having the smallest proportional degree of reliable measurement (the largest influence on stream temperature) included sun angle, transmissivity, stream width and groundwater discharge. Three of the four sensitive parameters, transmissivity,
stream width and groundwater discharge, are susceptible to change by urbanization (sun angle is not affected by temperature). It is these three parameters that were modeled in more detail as component models. The next three sections (4, 5 and 6) describe these component models.

4. Component model 1: removal of riparian vegetation

The sensitivity investigation revealed that solar radiation was one of the most influential meteorological variables in the stream temperature model. The composition and extent of riparian vegetation, in turn, had a major influence on the radiation received and lost from the stream surface. Consequently, water temperature is very sensitive to stream shading. There is a great deal of literature describing the effects of vegetation on stream temperatures (Brown, 1969, 1970; Klein, 1979; Feller, 1981; Barton et al., 1985; Meisner, 1990).

The significance of vegetation results from a tree’s capacity to absorb short-wave radiation that would otherwise be absorbed by the stream. Vegetation also helps to moderate cooler stream temperatures at night by emitting more long-wave radiation than open sky and reducing the water’s long-wave radiation losses. In addition, the loss of roots, which provide stream bank stability, may lead to long-term cumulative temperature effects as stream geometry is altered (Beschta and Taylor, 1988).

This study limits its investigation of the effects of vegetation removal on water temperature to riparian vegetation and makes the assumption that other vegetation removed in the watershed plays a less significant role. More conclusive research needs to be done to determine the affects of groundwater heating due to interbasin vegetation removal.

Several authors have hypothesized that stream orientation affects the amount of stream shading (Geiger, 1965; Moore, 1967). Pluhowski (1970) estimated that east–west oriented streams received from 7 to 19% more sunshine than north–south streams. Advances in the ease of use of 3-D computer modeling and image analysis can provide accurate estimations of effects of stream orientation and vegetation removal on shade. To test this, a three-dimensional computer model of a typical southern Ontario stream reach was constructed. The model was oriented predominantly east–west, and had a stream width of 2 m, a stream length of 180 m (stream area = 360 m²), and was surrounded by 9 m high riparian vegetation. The model was given the coordinates of a central southern Ontario location (Waterloo, 43°27’N 80°23’W, 314 m) on an early July day (Figure 5). The simulated shade cast on the river was estimated as a percentage of the stream area using image analysis software for each hour of sunshine (using the hourly sun angles at the Waterloo location). Four simulations were run for varying amounts
of streamside vegetation (100%, 75%, 50%, and 25% vegetation) to simulate the effects of vegetation removal on shade. Once this simulation was complete, the same model was turned 90° (representing a predominantly north–south oriented stream) and the same procedure was applied. A plot of the percent shade at the stream surface for the east–west oriented stream is presented in Figure 6, while a plot of the same stream turned 90° (north–south oriented) is presented in Figure 7.

Figures 6 and 7 show the effect of stream orientation and vegetation removal on shade. For all vegetation removal scenarios, the shade in the east–west oriented stream (Figure 6) tended to decrease rapidly at sunrise, but stayed at mid range values through most of the day before ascending again at about 3:00 PM. In contrast, the shade on the north–south stream (Figure 7) seemed to stay constant until mid morning, then it dipped very rapidly until noon to below 10% shade (for all vegetation scenarios).

The hourly shade values for both stream orientations became inputs into the CrUSTe model to simulate the influence of stream orientation and vegetation removal on water temperatures for a typical day in central southern Ontario in a typical second order stream.

The diurnal thermographs are shown in Figure 8 for east–west oriented streams and Figure 9 for north–south oriented streams. Again, there were noticeable differences in the impact of stream orientation and vegetation removal on stream temperature. Vegetation removal was shown to increase stream temperatures up to 2°C. It is important to note that these thermographs are representative of a typical groundwater influenced system which tend to moderate extremes of temperatures. Vegetation removal along
Figure 7. Diurnal shade values for typical north-south oriented streams in Waterloo, Ontario during early July. % vegetation: — 100%, —— 75%, ...... 50%, ------ 25%.

Figure 8. Stream temperature scenario using data generated for typical east-west streams. % vegetation: — 100%, —— 75%, ...... 50%, ------ 25%.
streams lacking such groundwater moderation could have a much more pronounced influence on stream temperature.

In comparing the effects of stream orientation on stream temperature, the thermographs showed that while north–south oriented streams were typified by higher maximum temperatures (all other things being equal), east–west oriented streams tended to retain their maximum temperature over a longer period of time. In other words, the north–south streams had a greater maximum intensity, but the east–west streams had a longer maximum duration. Both intensity and duration of water temperatures play an important role in determining a fish's thermal tolerance. Armour (1991) presents a model for evaluating and recommending temperature regimes to protect fish.

4.1. EFFECTS OF DIFFERENT TREE TYPES ON STREAM TEMPERATURE

Canopy transmissivity, a measure of the tree's density, was also found to influence stream temperature. To visualize the impacts on stream temperature resulting from the removal of different vegetation types, two types of trees (with widely different transmissivity values) were chosen for comparison. These trees and their respective transmissivity values were *Acer saccharinum* (*T* = 0.11, McPherson, 1984) and *Gleditsia triacanthos inermis* (*T* = 0.30, McPherson, 1984). Values for the other model parameters were similar to those used in Section 4.

The results of the simulation indicated that, as more vegetation was removed, the thermographs for both transmissivity types became more similar. This trend stems from the decreasing significance of transmissivity as more and more vegetation is removed. Conversely, the largest influence on water temperature due to different transmissivity types occurred at 100% vegetation.
For the east–west oriented streams the difference in water temperatures (as a result of the two different transitivities) was most pronounced from 10:00 AM until about 3:00 PM (i.e. during the period of maximum daily water temperatures). On east–west streams the denser canopy \( T = 0.11 \) decreased the overall maximum stream temperature. The difference between the peak temperatures for the two canopy types was about 0.8°C. For the north–south oriented stream the largest difference in water temperatures occurred as the thermograph curve ascended and descended to and from the point of maximum water temperature (i.e. from 8:00 AM to 10:00 AM and 3:00 PM to 5:00 PM). Mid-morning and mid-afternoon temperature differences were from 0.5–0.7°C lower for the denser canopy. However, unlike the east–west oriented streams, the peak stream temperatures for the different canopy types on north–south oriented streams were the same (i.e. the different canopy types did not affect the maximum temperature).

5. Component model 2: channel morphology

Channel width was one of the significant CrUSTe model parameters. The greater the channel width, the greater the surface area for the exchange of radiant, evaporative and convective fluxes. Streams with high width-to-depth ratios would be expected to have greater temperature extremes than streams with low width-to-depth ratios with similar cross-sectional areas.

Researchers attribute channel morphology to discharge, velocity, sediment load, sediment size, channel slope, width and depth (Dunne and Leopold, 1978). A change in any of these characteristics causes disequilibrium and results in the re-establishment of a new channel morphology.

The size and shape of a river were found to be highly correlated with flood frequency and flood intensity. Wolman and Miller (1960) argued that very large floods were too infrequent to shape a channel, while smaller, more frequent floods did not possess the energy required to shape the channel. They concluded that bankfull discharge (the level at which the channel fills completely so that the water is level with the flood plain) is the most effective channel forming flow and that this flow had a recurrence interval of 1.5 years for most rivers.

In many instances, the width, depth, cross-sectional area and velocity can be determined from bankfull discharge (Leopold, 1968; Hammer, 1972; Dunne and Leopold, 1978; Rosgen, 1995). There is considerable consistency among regions. For a large number of basins Dunne and Leopold (1978) found that:

\[
W = aQ^{0.20} \\
A = bQ^{0.80} \\
D = cQ^{0.40} 
\]

(11)

Where \( W, A \) and \( D \) are the width (m), cross-sectional area (m²) and depth (m) respectively and \( a, b, \) and \( c \) are coefficients specific to regions. From these equations we can estimate the new stream geometry as a result of a change in bankfull discharge.

To verify these equations and to determine the coefficients and exponents for southern Ontario, a series of rural stream cross-sections dimensions were collected along with their corresponding bankfull discharge. The data were plotted on logarithmic probability paper. The equations that describe these plots are shown in Equation (12).
Figure 10. Bankfull discharge ($Q_{bf}$) as a function of drainage area for several watersheds in the Grand River Basin, Southern Ontario.

\[ W = 3.7Q^{0.52} \]
\[ A = 1.0Q^{0.84} \]
\[ D = 0.27Q^{0.32} \]  

(12)

The value of the equations above is that they describe stream reach geometry in a semi-natural condition. This gives some point of reference from which to compare urbanized streams. Researchers note that the width-to-depth ratio tends to increase with increasing urbanization (Leopold, 1968; Rantz, 1971; Hammer, 1972; Dunne and Leopold, 1978). Since the width exponent (0.52) is greater than the depth exponent (0.32), it is understandable that with increasing discharge, the width will increase faster than the depth (i.e. the width-to-depth ratio increases with increasing bankfull discharge).

"It is a characteristic of river basins that discharge of any chosen frequency of occurrence will increase less rapidly than [watershed area]" (Dunne and Leopold, 1978). A plot of the log (drainage area) versus the log (bankfull discharge) reveals a straight line. To test this for southern Ontario, 12 unregulated, semi-natural (i.e. less than 4% impervious surfaces) sub-basins were chosen from the Grand River watershed. A plot of the drainage area versus the bankfull discharge is presented in Figure 10 ($r^2=0.88$). From this graph, it is possible to estimate the "non-urbanized" bankfull discharge from the drainage area.

Using Equation (12), it is now possible to estimate the effect on the width-to-depth ratio of increasing the bankfull discharge by a given amount. The next step is to determine the increase in the bankfull discharge for urbanized streams.
During the process of urbanization the infiltration capacity of the basin is lessened (due to creation of impervious surfaces and the removal of vegetation) and storm sewers are installed. In addition, as the flood plain becomes developed, valuable peak storage space is lost. The consequence of these activities results in a more efficient transmission of the flood wave through channel. This typically causes an increase in the frequency and magnitude of the 1.5 year flood and the concomitant change in channel morphology.

Flood-prediction techniques continue to be developed and refined. Many authors, notably Rantz (1971) and Leopold (1968), have modeled the impacts of development on flood frequency and magnitude. Leopold attempted to determine the magnitude of increase in the mean annual flood (recurrence interval of 2.33 years) resulting from creation of impervious surfaces and installation of storm sewers. Building on this idea, Rantz constructed a series of nomographs which provided a simple, yet effective tool for determining increases in floods of different recurrence intervals from percent urbanization and percent area served by storm sewers. Using Rantz's nomograph at a 2-year recurrence interval (Figure 11), it was possible to estimate the increase in bankfull discharge. Since Rantz's curves were developed for use in San Francisco, California, they were first validated with empirical data for use in southern Ontario.

5.1. STREAM CHANNEL ENLARGEMENT AS A RESULT OF URBANIZATION

By determining the change in the $Q_{1.5}$ flow (1 day, 1.5 year maximum flow) as a result of urbanization, we can insert this new flow value into Equation (12) to determine the
new channel width and depth. This provides a good estimate of the new width-to-depth ratio.

It is now possible to estimate the enlargement of the width-to-depth ratio for watersheds of different sizes and varying degrees of urbanization. Table 2 presents a summary of expected channel dimensions in a natural and urbanized condition for different sized watersheds. Included in this table is the expected width enlargement factor for different degrees of urbanization (expressed as a percentage of the impervious area). Since this paper focuses on extreme events (i.e., during baseflow periods), this factor would be multiplied by the natural baseflow channel width to predict the new baseflow width at different stages of urbanization. Notice that the width enlargement factor does not change for watersheds of different sizes. As an example, if the baseflow width of a natural watershed were 2.1 m, then assuming a scenario of 40% urbanization we would expect an enlargement factor of 1.27, and a new channel width of 2.67 m.

Hammer (1972) studied channel enlargement as a result of urbanization but his research concentrated only on the enlargement of channel cross-sectional area. His approach considered the age of the development, breaking down study sites into developments from 0-4, 4-30 and greater than 30 years old, as well as a number of other urban factors. He found that there were notably lower impacts on channel enlargement after 30 years. He hypothesized that "the drainage facilities serving older residential areas may have been relatively poor, either because they were under-designed to begin with or because they had deteriorated over time" (Hammer, 1972).

Hammer’s research implied that developments must be greater than 4 years old to register a channel enlargement change. That is, the width enlargement factor would tend to take at least 4 years to reach an equilibrium after development has ceased.

5.2. STREAM TEMPERATURE CHANGE AS A RESULT OF CHANNEL ENLARGEMENT

To demonstrate the impacts of channel enlargement as a result of urbanization on stream temperature, the scenario of a 10 km² basin undergoing 60% urbanization was considered. At 60% urbanization and 30% storm sewers, one would expect the channel width to enlarge by a factor of 1.33. For a 4 m wide reach at baseflow then, we would
6. Component model 3: baseflow character and stream temperature

Inflow discharge and groundwater discharge were found to be highly significant variables in the CrUSTe model. The reason is that the conversion of the various energy fluxes to temperature is inversely proportional to the discharge. Streams or rivers with high discharges would not be expected to exhibit large diurnal temperature fluctuations since it would take a great deal of energy to raise the temperature. Streams with low discharges, on the other hand, might be expected to exhibit high temperature fluctuations. The fact that urbanization can alter the physical character of the groundwater regime has implications on baseflows and, hence, critical stream temperatures.

Urbanization is usually associated with the removal of vegetation within a watershed. Water use by vegetation impacts soil moisture content, groundwater recharge and streamflow. The initial clearing of the land tends to increase the water available for

Figure 12. Diurnal thermograph comparing a natural stream to a stream with an enlarged channel geometry as a result of urbanization. (---) non-urbanized, (—) 60% urbanized.
Table 3. \((1-C)\) values and areas for each of the soil-cover complexes in the D'Aubigny Creek watershed

<table>
<thead>
<tr>
<th>Land use</th>
<th>A</th>
<th>AB</th>
<th>B</th>
<th>BC</th>
<th>C</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>((1-C) \text{ km}^2)</td>
<td>((1-C) \text{ km}^2)</td>
<td>((1-C) \text{ km}^2)</td>
<td>((1-C) \text{ km}^2)</td>
<td>((1-C) \text{ km}^2)</td>
<td>((1-C) \text{ km}^2)</td>
</tr>
<tr>
<td>Woodlot</td>
<td>0.5</td>
<td>0.30</td>
<td>0.44</td>
<td>0.007</td>
<td>0.4</td>
<td>0.535</td>
</tr>
<tr>
<td>Long grass</td>
<td>0.45</td>
<td>0.040</td>
<td>0.4</td>
<td>0.026</td>
<td>0.35</td>
<td>0.207</td>
</tr>
<tr>
<td>Lawns</td>
<td>0.4</td>
<td>0.075</td>
<td>0.35</td>
<td>0</td>
<td>0.29</td>
<td>0.354</td>
</tr>
<tr>
<td>Pasture</td>
<td>0.42</td>
<td>0.090</td>
<td>0.38</td>
<td>0</td>
<td>0.35</td>
<td>0.982</td>
</tr>
<tr>
<td>Row crop</td>
<td>0.34</td>
<td>0.884</td>
<td>0.3</td>
<td>0.234</td>
<td>0.26</td>
<td>3.435</td>
</tr>
</tbody>
</table>

Streamflow by decreasing the rate of evapotranspiration. The effects of vegetation removal on baseflows can vary. In some cases, the reduction in evapotranspiration increases the groundwater table and increases the baseflows. In other situations where precipitation intensity is great, the rain can actually compact the soil to the point where the infiltration potential is reduced and baseflows are lessened. To model the impacts of rainfall characteristics, soil properties, vegetation and land-use on baseflows, the rational runoff method (an equation used to predict peak runoff rates) was refined. Typically, the rational equation takes the form:

\[
Q_{RF} = 0.278CA
\]

where \(Q_{RF}\) is the peak discharge in \(\text{m}^3/\text{s}\), \(C\) is the rational runoff coefficient, \(I\) is the rainfall intensity in \(\text{mm/hr}\), \(A\) is in \(\text{km}^2\).

If the annual catchment water balance is calculated it can be used with a revised form of the rational equation to estimate baseflows. Since \(C\) values are an estimation of the proportion of precipitation intercepted, then \((1-C)\) represents the proportion of precipitation infiltrating the soil. Consequently, Equation (13) can be revised as follows:

\[
Q_{BF} = 3.13 \times 10^{-3}(1-C)(I_P - (I_{ET} + I_L))A
\]

where \(Q_{BF}\) is the baseflow in \(\text{m}^3/\text{s}\), \(I_P\) is the amount of precipitation \(\text{mm/yr}\), \(I_{ET}\) is the rate of evapotranspiration \(\text{mm/yr}\), \(I_L\) is the rate of interception \(\text{mm/yr}\) and \(A\) is the area in \(\text{km}^2\). This equation assumed there are no large water bodies within the catchment, and that there are no regional aquifer recharge zones within the catchment. Regional aquifers can be factored into the water balance for use in Equation (14) if the annual groundwater storage is known.

The model was validated using the D'Aubigny Creek watershed just outside of Brantford, Ontario, Canada. D'Aubigny Creek has a watershed area of 15 \(\text{km}^2\) and is part of the larger Grand River watershed in southern Ontario. The annual rainfall rate of the larger Grand River watershed was 935 \(\text{mm/yr}\) and had an evapotranspiration rate of 554 \(\text{mm/yr}\) (Paragon Engineering Ltd, 1992) for the year being simulated. Since a detailed water balance was unavailable for D'Aubigny Creek, these values were used.

Soils and land-use maps of D'Aubigny Creek were digitized and imported into SPANS for spatial analysis. The two maps were overlaid to determine the area of the soil-cover complex. The \((1-C)\) values and respective areas for the soil-cover complexes in D'Aubigny Creek are shown in Table 3.
TABLE 4. Estimated baseflows (m³/s) as a function of landscape type and % urbanization (0.5 acre residential subdivision) for a 10 km² catchment in southern Ontario, Canada

<table>
<thead>
<tr>
<th>% Urbanized</th>
<th>Forest</th>
<th>Meadow</th>
<th>Row crop</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.083</td>
<td>0.050</td>
<td>0.030</td>
</tr>
<tr>
<td>10</td>
<td>0.077</td>
<td>0.047</td>
<td>0.029</td>
</tr>
<tr>
<td>20</td>
<td>0.070</td>
<td>0.044</td>
<td>0.027</td>
</tr>
<tr>
<td>30</td>
<td>0.064</td>
<td>0.040</td>
<td>0.026</td>
</tr>
<tr>
<td>40</td>
<td>0.057</td>
<td>0.037</td>
<td>0.025</td>
</tr>
<tr>
<td>50</td>
<td>0.051</td>
<td>0.034</td>
<td>0.024</td>
</tr>
<tr>
<td>60</td>
<td>0.044</td>
<td>0.031</td>
<td>0.023</td>
</tr>
<tr>
<td>70</td>
<td>0.038</td>
<td>0.028</td>
<td>0.021</td>
</tr>
</tbody>
</table>

The observed baseflow for June 1991 for D’Aubigny Creek was 0.060 m³/s. The predicted baseflow from the model using Equation (14) and the values in Table 4 was 0.045 m³/s. From these results, it appears that the baseflow component model has good predictive potential.

6.1. BASEFLOW ALTERATION AND STREAM TEMPERATURE CHANGE

It is impossible to generalize about the effects of urbanization on baseflow. The type of development (extent of impervious surfaces) and the location of the development (i.e. the soil/land cover types being developed over) both play an important role in determining the possible changes in baseflow.

However, in an attempt to determine the impact of land use change on certain landscapes, a methodology was developed to allow some degree of generalization. In essence, what would be useful to environmental managers and planners would be to have an estimate of how certain development types affect baseflows and stream temperatures within the catchment.

Using typical precipitation and evapotranspiration data (935 mm/yr and 554 mm/yr respectively) for the Grand River watershed and a standard watershed area of 10 km², a matrix of expected baseflows (assuming no aquifer recharge or large lakes) was created for different stages of urbanization within varying land cover classes (Table 4). Runoff curve numbers from the soil conservation service (1975) were used to determine the C values for forest, meadows, and row crop types (all cover types had a “B” type hydraulic soil group and a “good” hydraulic condition). The type of development modeled was a 0.5 acre (per lot) residential sub-division. Note that the runoff coefficients vary for different land cover types. There may also be some error resulting from assuming a constant evapotranspiration rate across landscape types.

As expected, the baseflows decrease with increasing urbanization. Baseflow reduction rates are most pronounced in forest catchments while the effects of urbanization are less pronounced on pasture and cropland.

Using these baseflow variations as inputs into the stream temperature model it is possible to see the linkages between urban development and stream temperatures as a result of baseflow alterations. The CrUSTe model parameters were set for a north–south
TABLE 5. Estimated peak stream temperatures (°C) as a function of landscape type and % urbanization for a 10 km² catchment

<table>
<thead>
<tr>
<th>% Developed</th>
<th>Forest</th>
<th>Meadow</th>
<th>Farm and crops</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18.52</td>
<td>20.84</td>
<td>20.93</td>
</tr>
<tr>
<td>10</td>
<td>18.80</td>
<td>20.86</td>
<td>20.94</td>
</tr>
<tr>
<td>20</td>
<td>19.18</td>
<td>20.88</td>
<td>20.94</td>
</tr>
<tr>
<td>30</td>
<td>19.54</td>
<td>20.89</td>
<td>20.95</td>
</tr>
<tr>
<td>40</td>
<td>20.14</td>
<td>20.90</td>
<td>20.95</td>
</tr>
<tr>
<td>50</td>
<td>20.74</td>
<td>20.91</td>
<td>20.96</td>
</tr>
<tr>
<td>60</td>
<td>20.88</td>
<td>20.93</td>
<td>20.96</td>
</tr>
<tr>
<td>70</td>
<td>20.90</td>
<td>20.94</td>
<td>20.97</td>
</tr>
</tbody>
</table>

oriented stream from 12:00 to 1:00 PM in early July, with a constant inflow of 50 l/s at 12°C and a riparian vegetation transmissivity of 15%.

As a previously forested basin becomes developed, the predicted peak hourly temperature difference between the undeveloped (0%) and 70% developed is about 2.38°C (Table 5). Similarly, for predominantly meadow basins and row crop basins the temperature differences between 0% and 70% developed was 0.10 and 0.04°C respectively. These temperature differences represent average discharge conditions. One would expect that the variation would be more pronounced during dry years when baseflows are lower.

The effect of groundwater withdrawal on the water table is variable within different regions. But due to the sensitivity of groundwater recharge on stream temperatures, any activity which compromises baseflows can have serious repercussions on freshwater stream temperatures.

7. Scenario modeling

The previous component models for vegetation removal, channel morphology change and baseflow reduction can now be aggregated to simulate the impact of urban development on stream temperatures. A step by step approach was adopted to simplify the modeling process. The scenario goes as follows:

A hypothetical rural southern Ontario town is projecting massive growth in the next 20 to 30 years. Within a watershed area of 10 km², provincial and municipal planners are projecting up to 60% urbanization (i.e. 30% impervious area). They expect up to 40% of the watershed to be serviced by storm sewers. Provincial natural resources officials reviewing the projections expressed concern over these figures since the main stream in the basin provides cold water habitat for many thermally vulnerable species including brook trout (Salvelinus fontinalis) and Atlantic salmon (Salmo salar). Their concern stems from the loss of cold water streams as a result of urbanization in a neighbouring township. In particular they are concerned with a 1 km reach at the outfall of the watershed which provides an excellent spawning ground for fish due to the abundance of cool groundwater inflows and gravel substrate. The province is
interested in predicting the impacts of the proposed development on stream temperatures in the basin.

7.1. STEP 1—BASEFLOW MODELING

Upon digitizing the existing watershed in a geographical information system and using Equation (14) with an annual precipitation rate of 922 mm/year (2-year recurrence interval) and annual evapotranspiration rate of 571 mm/year, a baseflow of 82 l/s was predicted. This figure compared well with the actual baseflow of 78 l/s.

Next, the probable location and type of the new development was digitized and a new soil/cover table was compiled to determine the change in baseflow expected. The predicted new baseflow after urbanization was 50 l/s, a decline of about 60%. The product of 60% and the actual baseflow value gives a value of 46 l/s. This number was used to represent the predicted baseflow value after urbanization. It is assumed that the stream inflow discharge at the beginning of the reach will not change considerably from rural to urban (this is a reasonable assumption for extreme events). This inflow was measured to be 25 l/s.

7.2. STEP 2—VEGETATION REMOVAL

Despite strict environmental protection along the river corridor, it is anticipated that 20% of the riparian vegetation along the 1 km reach would have to be removed due to highway construction and development. A further 5% vegetation might be expected to be lost due to bank undercutting as a result of increased flood flows as a result of urbanization.

Constructing a three-dimensional computer model of the reach, which is predominantly north–south oriented, revealed that the expected shade matched the 75% vegetation for north–south streams in Figure 7.

7.3. STEP 3—CHANGE IN CHANNEL GEOMETRY

Using Figure 10 with a watershed area of 10 km², the anticipated $Q_{10}$ is 1.4 m³/s. Assuming that the Water Survey of Canada has had a gauge on this hypothetical stream for over 20 years, a flood frequency curve of the annual, 1-day maximum discharges can be plotted. The figure of 2 m³/s was observed at the 1.5 year recurrence interval. This compares favorably with the 1.4 m³/s figure obtained from Figure 11.

The width of the stream at bankfull discharge was computed to be 4.4 m from Equation (12). Again this compared favorably with the actual width of 4 m observed from a typical cross-section of the stream. At a site visit during the baseflow period, the stream was surveyed and the wetted width was measured at 3.8 m.

At 60% urbanization and 40% storm sewers, Figure 11 predicts a $Q_{10}$ increase of 1.84 times as a result of urbanization. This gives a new $Q_{10}$ value of 2.6 m³/s. Inserting this value into the width function in Equation (12) yields a new bankfull width of 6 m, an increase of 1.36 times the original predicted stream width. Multiplying this factor by the measured baseflow width of 3.8 m yields a new wetted width at baseflow of 5.2 m.
7.4. STEP 4—TEMPERATURE MODELING RESULTS

Steps 1 to 4 have provided all of the necessary model parameters required by the CRUSTe model. The inflow temperatures illustrated in Figure 13 were assumed to be constant for both scenarios (no development and development). This assumption may be a bit misleading since the inflow temperatures in the urbanized scenario could be greater than those in the undeveloped scenario. This is one of the limitations of the model that might be overcome by networking a series of reaches. There are opportunities for further research in this area.

Since the stream velocity at baseflow was 0.27 m/s (i.e. 1 km/hr), Figure 13 need not be calibrated for travel time. From this thermograph, we can see that the model predicts an increase in temperature of almost 4°C. This increase occurred despite the fact that the stream had a large proportion of its flow supplied by groundwater. One might expect a greater temperature difference if the baseflow discharge had been lower. Note also that this modeling exercise considered baseflows at a 2-year recurrence interval. Baseflows at the 10- and 25-year recurrence interval could result in even higher temperatures.

8. Summary and conclusions

This paper has presented a model (CRUSTe) for evaluating the impacts of urbanization on stream temperature. The utility of such a model can be better understood by describing it in terms of the four categories which it encompasses.

(1) A pro-active tool for landscape planning and environmental management. The
CrUSTe model allows for the assessment of site specific development scenarios before they are constructed to determine the impacts of land-use change on the thermal regimes of streams. For streams with thermally sensitive species, the model can help planners and land managers avoid the destruction of fish habitat. Such predictive capabilities give landscape architects, planners and engineers a new tool for environmental assessment and can help guide development and design decisions. (2) A reactive tool for stream remediation and rehabilitation. The model provides a mechanism for better understanding the complex interactions between the terrestrial and the aquatic environments. For remediating streams under thermal stress or for re-creating cold water habitat, the model elaborates on the temperature sensitivity of various meteorological, hydrologic and physical parameters. It was found that the most sensitive parameters can be controlled, to some degree, by design. (3) An educational tool for understanding the historic context of a stream. The model can be used to assess the pre-developed thermal condition of the stream. Such information may be valuable for historic or educational reasons. (4) A catalyst for further research. The model opens up a variety of possibilities for understanding and predicting the state of urban streams. Since stream temperature is closely related to hydraulic oxygen concentrations, eutrophication, reaction rates, chemical uptake, etc., the model can be used in concert with other models to predict other variables characteristic of urban streams. In addition, since stream temperature affects fish habitat, it must also affect other urban wildlife in the food chain. That is, stream temperature plays an indirect role in determining the capability of the urban corridors to sustain terrestrial wildlife.

The results of this study have implications on how environmental managers, landscape architects and planners go about the planning and design of large-scale urban landscapes. The sensitivity investigation revealed that riparian vegetation, stream width and baseflow discharge (the three variables that can be controlled by design) are significant contributors to stream temperature. As such, any activity (planned or unplanned) that alters these parameters would be expected to alter stream temperature.

References


SUSTAINABLE YIELD OF GROUNDWATER

Victor M. Ponce

May 2007

EXECUTIVE SUMMARY

All groundwater pumping comes from capture; the greater the intensity of pumping, the greater capture comes from decreases in natural discharge and increases in recharge. Natural discharge, riparian, wetland, and other groundwater-dependent ecosystems, as well as the baseflow of stream capture depends on usage, and it is not related to size or hydrogeological characteristics of the aquifer.
natural recharge. The traditional concept of safe yield, which equates safe yield with natural recharge, has been widely discredited. It has now been replaced with sustainable yield.

Sustainable yield depends on the amount of capture, and whether this amount can be accepted as a compromise between a policy of little or no use, on one extreme, and the sequestration of all natural deep percolation on the other extreme. A reasonably conservative estimate of sustainable yield would take all or suitable deep percolation. On a global basis, deep percolation is about 2% of precipitation.

Sustainable yield may also be expressed as a percentage of recharge. Limited experience suggests that these percentages may be around 40%, with the least conservative around 70%, and reasonably conservative 10%. Sustainability may be fostered by enlightened management which seeks to capture rejects encourage clean artificial recharge, and limit negative artificial recharge.

A holistic approach to groundwater sustainability considers the hydrological, ecological, sociotechnological, cultural, institutional and legal aspects of groundwater utilization, seeking to establish a compromise between conflicting interests. Communities are beginning to consider baseflow conservations standard against which to measure groundwater sustainability. In the end, sustainability reflects conservation policy; the more conservative a policy, the more sustainable it is likely to be.

1. INTRODUCTION

Water occurs both on the surface and under the surface of the Earth. The water on the surface is called "surface water" and the water under the surface is called "ground water" (Fig. 1). These two are part of the hydrologic cycle, which is the continuous circulatory movement of the water on Earth (Fig. 2). In nature, surface water and ground water are related. Surface water can become ground water through infiltration, while ground water can become surface water through discharge. Therefore, surface water and ground water are inextricably connected; one cannot be properly evaluated without regard to the other.

While being part of the hydrologic cycle, the similarities between surface water and ground water appear to end there. They can be shown to differ in two important ways:

1. Surface water is completely renewable, usually within days or weeks, while ground water is not completely renewable, since it may take decades, centuries, or even longer time to recharge.

2. Fresh surface water is scarce, particularly when compared with the large volumes of ground water which are known to exist below the surface.
With development pressures taxing the surface waters in many regions of the world, the notion of using groundwater to help resolve perceived surface water scarcities has gained prominence. Without the need to store water in areas with sufficient rainfall, communities have turned toward groundwater mining. Thus, the sustainability has become an important consideration. It is not just about ensuring that the water is available, but also about ensuring that it is used efficiently and sustainably. To what extent can groundwater resources be exploited without compromising the principle of sustainable development?

This study examines the historical development of groundwater use and the limits placed on it throughout the years. The concepts of safe yield and sustainable yield are reviewed. The concept of safe yield, which equates safe yield to annual recharge, is shown to be flawed because it only takes into account the water balance. Sustainable yield extends beyond the conventional boundaries of hydrology and encompasses surface water hydrology, ecology, and other related topics. The study considers a more holistic approach to the sustainable yield of groundwater.

2. BACKGROUND

Excessive groundwater pumping can lead to groundwater depletion, and this may have environmental and economic consequences. Attempts to limit groundwater pumping have been common in areas where the annual amount of water withdrawn by pumping is close to or exceeds the amount of water stored in the aquifer. The concept of a safe yield, defined as the attainment and maintenance of a long-term balance between the annual amount of water withdrawn by pumping and the annual amount of water stored in the aquifer, has been widely used. However, the traditional definition is too narrow because it does not take into account the rights of groundwater users (springs and baseflow) and groundwater-dependent ecosystems (wetlands and vegetation) (Sophocleous, 1997).

http://ponce.sdsu.edu/groundwater_sustainable_yield.html
Recently, the emphasis has shifted to sustainable yield (Alley and Leake, 2004; Ma Seward et al., 2006). Sustainable yield reserves a fraction of safe yield for the benefit of waters. There is currently a lack of consensus as to what percentage of safe yield should be sustainable yield. The issue is complicated by the fact that knowledge of several sciences is required for a correct assessment of sustainable yield. Additionally, there are economic, and legal implications which have a definite bearing on the analysis.

At the outset, a distinction is necessary between pristine and non-pristine groundwater reservoirs. Pristine reservoirs are those that have not been subject to human intervention; consequently, they have a history of pumping. Average annual recharge is normally the long-term average value. In pristine reservoirs, average natural recharge, which is precipitation, is equal to average natural discharge, which feeds springs, streams, wetlands, and groundwater-dependent ecosystems. Thus, net recharge, i.e., average annual recharge minus average annual discharge, is zero (Theis, 1940).

---

In pristine reservoirs, natural recharge is equal to natural discharge; thus, net recharge is zero.

---

Natural discharge constitutes the baseflow of streams and rivers, and, in shallow reservoirs, it is the water that sustains certain types of vegetation, such as the hygrophytes, and phreatophytes (see Glossary). Most groundwater is continuously flowing, gravitational forces, eventually to join the surface waters (Fig. 3), or, ultimately, the ocean (Fig. 10). It is only a matter of time before the natural recharge shows up as natural discharge, evapotranspiration or surface water, somewhere downstream.
Three groundwater scenarios are possible:

1. A **pristine groundwater system**, in equilibrium or steady state, in the absence of

2. A **developed groundwater system**, in equilibrium or steady state, with moderate fixed depth; and

3. A **depleted groundwater system**, in nonequilibrium or unsteady state, with heavy an ever increasing depth.

In the pristine groundwater system (Fig. 4 a), average natural recharge is equal to average discharge, net recharge is zero, and pumping is zero. Thus, natural recharge (the blue left) equals natural discharge (the blue block on the right) (Fig. 4 a).

In the developed groundwater system (Fig. 4 b), captured recharge (the brown block on increase in recharge induced by pumping. Likewise, captured discharge (the brown block is the decrease in discharge induced by pumping. Then, residual discharge (the blue right) is equal to natural recharge (the blue block on the left) minus captured discharge. is equal to the sum of captured recharge plus captured discharge. Net recharge is the intensity of pumping; the greater the intensity of pumping, the greater the net recharge the developed groundwater system is equal to net recharge, i.e., capture (Fig. 4 b).
In addition to captured recharge and captured discharge, the depleted groundwater sys
to features captured storage (the dark brown block on the left). Net recharge is equi
recharge plus captured discharge. Pumping in the depleted groundwater system is 
recharge plus captured storage (Fig. 4 c).

The greater the level of development, the greater the amounts of captured recharge
discharge, and, in the case of a depleted system, captured storage. The greater 
discharge, the smaller the residual discharge. Since all aquifer discharge feeds surfa 
evapotranspiration, it follows that intensive groundwater development can substantial
subregional, or regional groundwater-fed surface water bodies and groundwat
ecosystems.

(a) PRISTINE GW SYSTEM

(b) DEVELOPED GW SYSTEM

(c) DEPLETED GW SYSTEM

Fig. 4  Recharge and discharge in groundwater systems: 
(a) pristine, (b) developed, (c) depleted.

3. PERSPECTIVE

Lee (1915) defined safe yield as the limit to the quantity of water which can be withdr
and permanently without dangerous depletion of the storage reserve. He noted 
permanently extracted from an underground reservoir reduces by an equal quantity t
water passing from the basin by way of natural channels, i.e., the natural discharge. To 
existence of this natural discharge, Lee observed that heavy pumping would commonly 
drying up of springs and wetlands. Thus, he distinguished between a theoretical safe y
the natural recharge, and a practical safe yield, a lower value which takes into accour
maintain a residual discharge (Fig. 4 b). According to Lee, the residual discharge must b
and deducted from the theoretical safe yield in order to obtain the practical safe yield.

Theis (1940) recognized that all ground water of economic importance is in consta
through a porous rock stratum, from a place of recharge to a place of discharge (Fig. 5).
that under pristine conditions, aquifers are in a state of approximate dynamic equilibrium by pumping is a new discharge superimposed on a previously stable system; consequently balanced by:

a. an increase in natural recharge;

b. a decrease in natural discharge;

c. a loss of storage in the aquifer; or

d. a combination thereof.

All groundwater of economic importance is in constant movement through a porous rock stratum, from a place of recharge to a place of discharge.

Significantly, Theis (1940) distinguished between natural recharge and available recharge. Recharge is the sum of unrejected and rejected recharge (Fig. 5). The unrejected recharge is the portion of available recharge rejected by the aquifer on account of being full (at least part of the time). To assure maximum utilizable supply, Theis argued that groundwater development should tap primarily the rejected recharge, secondarily, the evapotranspiration by non-productive vegetation. Thus, he defined potential yield as equal to the amount of rejected recharge plus the fraction of natural discharge feasible to utilize. According to Theis (1940), where rejected recharge is zero, the only way the well discharge is by artificial recharge. The latter is the increase in recharge induced by pumping.
Kazmann (1956) argued that the concept of safe yield, when taken independent of con
regional hydrology, is a fallacious one, because it cannot be reconciled with the leg:
appropriation. All water coming from the ground must be replaced by water coming 1
surface in order for a perennial groundwater supply to be obtained. When all surface
area overlying an aquifer has been appropriated, a perennial supply cannot be obtain
ground without encroaching on established rights. Echoing Theis (1940), Kazmann
recharge as an effective technological fix to the safe yield quandary.

Artificial recharge is useful where water is being lost by runoff in the same areas where
supplies are being depleted. The need for recharge is apparent when springs start to dry
lifts increase, or shallow wells go dry. If these conditions persist for a reasonably k
ground water is probably being mined. In artificial recharge, the excess runoff is retaine
where there are possibilities for increased underground storage. The usual methods of
the improvement of natural openings and the impoundment of storm runoff for slower
benefits can be local or regional (Soil Conservation Service, 1967).

Todd (1959) defined safe yield as the maximum quantity of water which can be extra
underground reservoir, yet still maintain the supply unimpaired. Pumping in excess of sa
to overdraft, which is a serious problem in certain groundwater basins in the Unite
elsewhere. He argued that until overdrafts are reduced to safe yields, permanen
depletion of the ground water supplies is to be expected. He referred to permanent
mining of groundwater because of its analogy to the mining of ores and petroleum.

The concept of sustainable development emerged in the late 1980s, forcing a reconsid
yield practices. Sustainable development must meet the needs of the present without o
the ability of future generations to meet their own needs (World Commission on Env
Development, 1987). Implicit within this definition is the realization that natural resou
exploited in an unsustainable fashion, i.e., in a way that future generations will find i
difficult to avail themselves of similar quantities of the same resources. Thus, the inter
ethical dilemma.

Sustainability refers to renewable natural resources; therefore, sustainability implies
Since groundwater is neither completely renewable nor completely nonrenewable, qu
question of how much groundwater pumping is sustainable. In principle, sustainable u
which is in agreement with sustainable development. This definition is clear; however
application requires the understanding of complex interdisciplinary relationships, which
recently been examined.

Alley et al. (1999) defined groundwater sustainability as the development and use of gr
a manner that can be maintained for an indefinite time without causing unacceptable e
economic, or social consequences. The definition of "unacceptable" is largely subjective	on the individual situation. For instance, what may be established as an accept
groundwater withdrawal with respect to changes in groundwater level, may reduce the
sustainable yield of groundwater, safe yield, groundwater recharge, groundwater utilization...

...surface water, locally or regionally, to an unacceptable level. According to Alley et al. (1999), safe yield should be used with respect to specific effects of pumping, such as water level reduction and streamflow. Thus, safe yield is the maximum pumpage for which the consequence is considered acceptable.

---

**Groundwater sustainability is defined as the use of ground water in a manner that can be maintained for an indefinite time without causing unacceptable consequences.**

Alley et al. (1999) focused on the High Plains aquifer as a field example of how groundwater change in response to pumping and its relation to sustainability. The southern part of the aquifer (in New Mexico and Texas) slopes gently from west to east, and it is cut-off by sources of water upstream and downstream by river-carved escarpments (Fig. 1). Development, discharge along the eastern escarpment equaled recharge (Fig. 6B). Development, recharge increased from 24 to 510 million cubic feet per day, discharge decreased from 24 to 10 million cubic feet per day, and storage decreased 330 million cubic feet per year (right). The southern High Plains aquifer is perhaps the best known example of nonequilibrium overdraft of a regional groundwater system in the United States.
Sophocleous (2000a) pointed out that the traditional concept of safe yield ignores the fact that the long term, natural recharge is balanced by discharge from the aquifers by evaporation and/or exfiltration into streams, springs, and seeps. Consequently, if pumping equals or exceeds natural recharge, streams, marshes, and springs may dry up. Additionally, continued pumping may eventually deplete the aquifer. To illustrate, Sophocleous presented maps showing the decreased length of streams within the High Plains aquifer (Fig. 7). The map shows a marked decrease in total length of stream flows in the western third of the state, within period (1961-1994), showing the impact of groundwater depletion on surface-water resources.
Loucks (2000) observed that the assessment of sustainability must involve professional disciplines. Sustainability studies will require a balance of the entire hydrologic system, aquifer. A careful accounting of the fate of all water is essential for effective management. Aboveground consumption is the key to sustainable management, and not necessarily which groundwater is pumped (Kendy, 2003).

Alley and Leake (2004) recognized the dependence of yield on the amount of capture, which tends to be a constant for a given basin, capture is a function of development, the greater the pumping, the greater the capture. Thus, capture is impossible in all cases. There is concern about the long-term effects of groundwater development on the health of springs, wetlands, lakes, streams, and estuaries. Sustainability is a process, encompassing, addressing issues across the disciplines.

Maimone (2004) argued that if sustainable yield must be all-inclusive, the idea that a single, correct number representing sustainable yield must be repealed. Instead, he proposed a working definition, coupled with an adaptive management approach, based on these components:

1. Understand the local, subregional, and regional effects, and interactions thereof.
2. Develop a comprehensive conceptual water budget, including surface water and groundwater, consumptive vs non-consumptive use.
3. Understand the boundaries and rate of replenishment of the system.
4. Understand human water needs and their changing nature.
5. Consider the temporal aspects of yield, including droughts and floods.
6. Consider the effects of new technology and changes in societal perceptions.
7. Work with stakeholders to understand tradeoffs and develop consensus.
8. Recognize the interdisciplinary nature of the impacts of groundwater utilization.

http://ponce.sdsu.edu/groundwater_sustainable_yield.html

7/23/2015
Seward et al. (2006) found serious problems with the simplistic assumption that sustainable yield should equal recharge. In many cases, sustainable yield will be considerably less than annual recharge; therefore, the general statement that sustainable or "safe" yield equal recharge is incorrect. Natural recharge does not determine sustainable yield; rather, the latter is determined by the amount of capture that it is permissible to abstract without causing undesirable or harmful consequences.

4. ANALYSIS

The historical perspective confirms that safe yield and sustainable yield are indeed evolving concepts. Lee (1915) was the first to recognize the need to reserve a fraction of the recharge for the surface waters and related ecosystems. Theis (1940) thought it advisable to rely on rejected recharge, if any, to guarantee a portion of the safe yield. Kazmann (1956) considered the idea that all recharge was already part of the surface waters, an effective way of ensuring a supply of groundwater was to rely on artificial recharge. Alley et al. (1999) and Seward et al. focused instead on whether the effects of groundwater pumping were socially acceptable. Assessing groundwater sustainability, issues of surface water hydrology, ecology, and resources technology are seen to be intertwined with the issue of social license.

The concepts may be summarized as follows:

- All groundwater of economic importance is in transit from a place of recharge to a discharge point. Therefore, any water extracted from the ground would have to be replaced by a corresponding withdrawal from surface water. If the latter is appropriated without proper compensation, a conflict arises (Kazmann, 1956). Where rejected recharge is pre-existing, it may be used as groundwater, provided there is no prior claim to it.

- A perennial safe yield may be assured as long as capture abstracts only reject recharge. Thus, in humid and other areas where the water table is near the surface, a modicum of capture may be self-sustaining if it can count on abstracting rejected recharge (Timmerman, 1956).

- Determinations of safe or sustainable yield must subtract from recharge the fraction shown to fulfill the needs of surface water and related ecosystems (Lee, 1915). Controversy remains as to whether vegetation can be classified as beneficial or not. Furthermore, the effects of groundwater development on neighboring springs requires a thorough hydrological assessment.

- Artificial recharge constitutes essentially free groundwater and, therefore, encourages development in areas where ground water is being actively developed or depleted. Effective when it uses rain or snowmelt water in rural areas (Soil Conservation Service, 1952), artificial recharge with wastewater may represent a threat to public health and subject to regulation. Municipal wastewater should receive at least secondary treatment before being used. In any case, the cross-contamination of potable water supplies with

http://ponce.sdsu.edu/groundwater_sustainable_yield.html

7/23/2015
originating in artificial recharge should be avoided. Agricultural wastewater wastewaters, and stormwater runoff from industrial areas are generally not suite recharge (National Research Council, 1994).

- Certain socioeconomic activities such as deforestation, overgrazing, overcultivative development should be limited or regulated to the extent possible, because tendency to reduce recharge, amounting to "negative" artificial recharge.

- Assessments of sustainable yield must reach beyond hydrogeology, to ent interdisiplinary synthesis of surface water hydrology, ecology, geology, and climato a few. In addition, since ground water is a resource held in common, sust assessments must consider the socioeconomic context (Hardin, 1968). In gen communities will have different perceptions of what constitutes an accept groundwater withdrawal, and these perceptions may vary over time.

- The crux of the sustainable yield dilemma is how to reconcile ecology and econ context of groundwater utilization. Ecology is represented by the established righ water bodies and ecosystems to all or a portion of the exfiltrating ground waters ( 2000a). The economics is encapsulated in the following question: How can comm not to use such precious resource? There is a pressing need to consider the soc and legal aspects of the decision to pump a certain amount of groundwater. The question, for which there is no simple answer, is: Who owns the groundwater?

The solution is to focus on a water balance that considers both surface water and precipitation, the source of all ground water, separates into several components (paths):

1. Return to the atmosphere via evaporation;
2. Return to the atmosphere via evapotranspiration;
3. Return to the ocean through direct runoff;
4. Return to the ocean through baseflow and, subsequently, streamflow;
5. Return to the ocean through deep percolation.

Of the five components of precipitation, only the third (direct runoff) is totally independent water. Fractions of evaporation and evapotranspiration may [originate and] be part of given All baseflow originates and is part of ground water. All deep percolation is part of ground not part of streamflow.

The components vary with climate, scale, and local/regional geologic/hydrogeologic or the sake of reference, on a global annual basis, evaporation and evapotranspiration of precipitation, streamflow is 40% (direct runoff is 28% and baseflow 12%), and deep percol (Fig. 8) (World Water Balance, 1978; L'vovich, 1979; Ponce, 2006).
Like precipitation, natural recharge separates into several components (paths) as follows:

1. Return to the atmosphere via evaporation from bare soil;
2. Return to the atmosphere via evaporation from bodies of water;
3. Return to the atmosphere via evapotranspiration from vegetation, both natural and human induced (agriculture);
4. Return to the ocean through the baseflow of streams and rivers; and
5. Return to the ocean through deep percolation.

Of the five components of natural recharge, only No. 5 (deep percolation) is totally indep of surface waters; therefore, it may be a potential candidate for capture by groundwater s on a global annual basis, up to 2% of precipitation may be potentially tapped by groundv with minimum encroachment on established [surface water] rights. In practice, specific v percolation would have to be established on a local, subregional, or regional basis. For basins lying in close proximity to the ocean, the capture of all or fractions of deep perco be examined carefully because of the possibility of salt-water intrusion.

Of the remaining four components (Nos. 1 to 4), it may be readily argued that all or frac (evaporation from bare soil) may be also a candidate for capture by groundwater syst difficult to argue in favor of capturing all or fractions of No. 2 (evaporation from water bo
3 (evapotranspiration from vegetation). It is most difficult to argue in favor of capturing a fraction of No. 4 (baseflow).

It is most difficult to argue in favor of capturing all or fractions of baseflow.

In general, a detailed water balance and related interdisciplinary studies are required to determine the acceptability of set values of sustainable yield, not on component No. 5 (deep percolation), but also appropriate fractions of components 1, 2, 3, and 4. Essentially the goal is to be able to determine an appropriate yield-to-recharge percent to determine if this percentage be accepted as a reasonable compromise between conflicting interests.

What are typical values of the yield-to-recharge percentage? In this connection, it is informative to examine examples of usage-to-recharge percentages. Solley et al. (1988) have estimated that the average annual usage of water in the United States in 1995 was approximately 77 billion gallons per day, which is 8.6% of the estimated more than 891 billion gallons per day of natural recharge per day to natural recharge. Nation’s groundwater systems (Nace, 1960; Alley et al., 1999). Limited experience indicates that the maximum sustainable yield-to-recharge percentages are likely to be somewhat higher (Miles and Chisholm, 1996; Hahn et al., 1997).

5. CASES

This section describes several case studies of groundwater utilization. The objective is to provide a practical view of evolving issues in groundwater management.

Recent experience in Nevada suggests that groundwater modeling can be effectively used to assess the long-term assessment of the water balance (Prudic and Herman, 1996). Paradise valley watershed, which is part of the Humboldt river, in Humboldt County, Nevada. According to a recent report, the steady-state groundwater model, the natural inflow to, and outflow from, the Paradise valley groundwater system was 91 m³/yr. Approximately 88% of the inflow (recharge) occurred through evapotranspiration; the rest occurred through leakage along mountain fronts and other natural inflows. About 96% of the discharge occurred through evapotranspiration; the rest occurred through leakage to streams and other outflows. A simulation of 300 years of pumping was performed to determine the magnitude and distribution of pumping of 1982, followed by 300 years with no pumping. The simulated pumping rate was 44 m³/yr, which is almost half the natural inflow (a yield-to-recharge of 48%).

The results of the simulation show the long-term consequences of groundwater withdrawal. The pumped water came mostly from storage; with time, however, storage changes direction and the source of water from a reduction in evapotranspiration and a stream to ground. After 300 years, 72% of the pumped water was coming from the sustainable yield of groundwater, safe yield, groundwater recharge, groundwater utilization...
evapotranspiration, 24% from reductions in streamflow, and only 4% from aquifer storage (Herman, 1996). This study shows that under long-term equilibrium conditions, groundwater is likely to come, first, from reductions in evapotranspiration, and second, from reductions in baseflow. Miles and Chambet (1995) assumed that there is an acceptable level to which aquifer baseflow (baseflow) may be allowed to fall, following groundwater pumping. The acceptable baseflow reduction is then linked to the expected drought duration and used to calculate recharge ratio. The latter is expressed as a function of dimensionless aquifer diffusivity, $v = \frac{t}{T}$, which $t$ = time, $T$ = transmissivity, $S$ = coefficient of storage, and $L$ = aquifer length. The applied to the catchment of the River Worfe, in Wales, Great Britain, and its underlying Sandstone aquifer. Calculated yield-to-recharge percentages, when baseflow was reduced, which is clearly the least conservative assumption, varied in the range 50-78%.

In Cheju Island, Korea, the mean annual precipitation is 1872 mm, which amounts to 3.5 m in this volume, 19% (0.64 km³ yr⁻¹) constitutes direct runoff, 37% (1.26 km³ yr⁻¹) is evapotranspiration, 44% (1.49 km³ yr⁻¹) is recharge to aquifers (Hahn et al., 1997). Sustainable yield was defined as the average rate of pumping that can be maintained without endangering either the quantity or quality of pumped water. The estimate assumed that sustainable yield is a suitable percentage of recharge, the percentage varying with local hydrogeologic conditions. Significantly, sustainable yield was established in this way is independent of the size or hydrogeologic parameters of the aquifer. Sustainable yield was estimated for the whole island at 0.62 km³ yr⁻¹, which amounts to a recharge percentage of 42%. For the sake of comparison, in 1993, the total amount of water developed in Cheju Island was 0.20 km³, which is about 14% of the annual recharge; however, there is some indication that the actual usage is only a fraction of that figure (Hahn et al., 1997).

Sustainable yield was estimated as a percentage of annual recharge, the percentage varying with local hydrogeologic conditions.

Sophocleous (2000a, 2000b) has provided extensive documentation on the Kansas aquifer, focusing on groundwater management. In 1972, the Kansas Legislature passed the Kansas Groundwater Act that authorized the formation of local groundwater management districts (GMDs) to help manage the development and use of groundwater resources. In the mid-1970s, the Central Kansas GMDs 2 and 5 adopted a safe yield policy of balancing groundwater pumping with annual recharge (Fig. 9). This policy slowed down the rate of groundwater depletion, including the establishment of the safe yield policy, both GMD 2 and GMD 5 groundwater level declines of more than 6 m in parts of their districts. In some areas, these declines amounted to 60 m, reducing by more than 50% the saturated aquifer thickness. As a result, streamflows in Western and Central Kansas have been decreasing and riparian area has been progressively degrading, with numerous dead cottonwood and poplar trees and a countryside. In response to these streamflow declines, in 1984 the Kansas Legislature enacted a minimum instream flow law, which requires that minimum desirable streamflows (MDS) be maintained in different streams in Kansas (Sophocleous, 2000a).

http://ponce.sdsu.edu/groundwater_sustainable_yield.html 7/23/2015
sustainable yield of groundwater, safe yield, groundwater recharge, groundwater utiliza...  Page 17 of 32

Fig. 9 Kansas Groundwater Management Districts.

With continuing declines in groundwater levels and streamflow during the 1980s, the Ka- and 5 moved in 1994 toward the conjunctive management of stream-aquifer systems, their safe yield policies to include baseflow when evaluating a groundwater applicator estimated as the streamflow that is exceeded 90% of the time on a monthly basis. Signi- GMD 2 continues to refer to their evolving policies with the term safe yield, GMD 5 has instead the term sustainable yield (Sophocleous, 2000b).

Alley et al. (1999) and Maimone (2004) have described the case of Nassau County, N. tradeoff between groundwater quality and surface-water quantity. Nassau County has about 500 km² and a population of 1.3 million people, and is a moderately dense suburban county. In the 1970s and 1980s, with nitrate concentrations in ground water in to on-lot septic systems, a decision was made to install sewer lines in 90% of the count outfalls used for treated sewage disposal. As a result, the consumptive use of ground pumped and not returned through recharge) rose to 250 hm³yr⁻¹. The response of the a to extensive sewering was a loss of storage and a reduction in the natural discharge (ba amount almost equal to the increased consumptive use. A 14-ft drop in average groun was documented shortly after the sewerage project was completed (Fig. 10). Thus, a been made to allow for some streams to dry up in exchange for improved groundwater q
In Suffolk County, New York, with an area somewhat greater than 2000 km² and a population greater than Nassau County, sewerage accounts for less than 70 hm³yr⁻¹ of consumptive use. As a result, most streams in Suffolk County still have relatively undiminished baseflow. U.S. Geological Survey maps show that Suffolk County chose to protect its streams and wetlands, and to monitor changes in groundwater levels and the wetland areas very closely. Although Suffolk County has not adopted a definition of sustainable yield, the acceptable impact to streams has been defined as 5% of the average annual recharge rates in order to control salt water intrusion (Maimone, 2004).

The Chester County Water Resource Authority, in Pennsylvania, has recently adopted a comprehensive water resources plan for its watersheds (Chester County Water Resources Authority, 2002). Significantly, stream baseflow was selected as the standard against which...
groundwater pumping. Accordingly, to protect stream baseflow, the regulation limited withdrawals in the following categories:

- For first order streams: To maintain the net volume of cumulative groundwater below 50% of the volume of the 1 in 25-year average annual baseflow, unless an hydrogeologic analysis or instream flow study determines that a larger volume of water can be safely extracted and still sustain the ground water and instream resources of that drainage area.

- For baseflow-sensitive subbasins: To maintain the net volume of cumulative withdrawals below 50% of the volume of the 1 in 25-year average annual baseflow of the basin that contains streams designated as Exceptional Value and High Quality waters, and state-designated instream fish and wildlife. More detailed hydrogeologic analysis or instream flow study determines that a larger volume of water can be safely extracted and still sustain the ground water and instream resources of that subbasin.

- For all other areas: To maintain the net volume of cumulative groundwater withdrawals below 100% of the volume of the 1 in 25-year average annual baseflow.

The more conservative lower management target of 50%, applicable to first order streams and designated baseflow-sensitive subbasins, would protect half of the baseflow [low flow] instantaneously once every 25 years. [In practice, this means using up to 50% of the low flow]. The less conservative upper target of 100%, applicable to all other areas, would protect the baseflow that recurs once every 25 years.

The more conservative lower management target of 50% would protect half of the baseflow that recurs instantaneously once every 25 years.

In Kings County, New York, total groundwater withdrawals in 1903 were about 30 million gallons per day, with no obvious cone of depression observed at that time (Fig. 11). Total pumpage increased to early 1940s at a maximum pumping rate of about 75 million gallons per day. In the 1950s, table levels were near or below sea level throughout Kings County, and the cone of depression extended into southwestern Queens County. In 1947, public-supply pumpage ceased in Queens County. The source of water for public supply changed to the upstate surface-water supplies New York City through water tunnels. Furthermore, legislation was implemented during this period that required wastewater from some industrial/commercial uses, including all water, to be recharged to the aquifer system. In this case, the focus was on recovering water tables rather than on preserving the quality of the ground water. Concurrently, as a result of these changes, industrial pumping declined to a long term stable rate of slightly less than a million gallons per day. In 1965, heads have risen throughout Kings County (Fig. 11) and have maps show a small but continuing recovery of the water table (Alley et al., 1999).
The cases described above enable the following conclusions:

1. Developed or depleted groundwater systems invariably lead to the degradation of wetland ecosystems.

2. Continued development or depletion of ground water leads to substantial baseflow.

3. Over the long term, capture in developed groundwater systems must come reductions in natural discharge.

4. Many communities are choosing to regulate groundwater use in order to protect ecosystems, wetlands, and baseflow.

5. A decrease in groundwater pumping can recover depleted water tables and foster more natural conditions.

6. Sustainable yield is unrelated to the size or hydrogeologic parameters of the aquifer.

7. Sustainable yield can no longer be equated with natural recharge.

Fig. 11 Temporal evolution of the water table, from 1903 to 1965, in Kings County, New York.
8. Sustainable yield estimates are commonly based on a percentage of natural re
though there appears to be no relation between them. Lower percentages con
servative, while higher percentages will be less conservative.

9. Sustainable yield estimates span several disciplines, from hydrogeology, to hydrol
economics, water resources technology, and the legal aspects of groundwater utili

10. Baseflow conservation is emerging as the standard against which to measure
pumping and sustainable yield.

11. Sustainable yield is, for all practical purposes, a moving target; no single approach
at all times and in all cases.

12. Enlightened sustainable yield assessments require an adaptive management appr

6. SYNTHESIS

All groundwater reservoirs of economic importance are temporarily holding water in 1
place of recharge to a place of discharge (Theis, 1940). Any amount of water extra
ground by mechanical means (through pumping) would have to be eventually replaced
amount coming from the surface waters. A pristine groundwater reservoir is in steady
inflows equal to outflows. When a groundwater reservoir is full, it rejects all water, which I
but to augment the surface waters. Conversely, when a groundwater reservoir is not fi
more water, but it will discharge more water too, through natural discharge. The natu
supports riparian, wetland, and other groundwater-dependent ecosystems, as well as th
streams and rivers.

All pumping comes from capture, and all capture is due to pumping (Seward et al., 2006
the intensity of pumping, the greater the capture. Capture comes from decreases in nat
and increases in recharge, the latter coming either from increased ground surface ree
the surrounding areas. In depletion cases, capture is augmented with decreased storag
permanent lowering of the water table.

The water that seeps below the ground surface can follow one of three paths:

1. Return to the atmosphere via evaporation and evapotranspiration;

2. Return to the ocean via baseflow and subsequent streamflow; or
3. Return to the ocean through deep percolation.

Of these three, only deep percolation is completely independent of the surface waters. It is the only component of precipitation (or recharge) that may be potentially subject to (capture) by pumping. Studies are needed on a local, subregional, and regional basis deep percolation as a percentage of precipitation, or alternatively, as a percentage of groundwater basins in close proximity to the ocean, the possibility of salt-water intrusion examined carefully.

A groundwater reservoir is essentially a leaky, porous natural geologic container (Fig. 1). Precipitation P separates into direct runoff Q, evaporation and evapotranspiration ET recharge NR. All natural recharge eventually flows out as either natural discharge percolation DP, at various spatial scales, from small to large watersheds. Natural discharge return to the atmosphere via evaporation and evapotranspiration ET, or to the ocean via The deeper the ground water, the larger the spatial scale of natural discharge, from the regional scale (Fig. 3).

Fig. 12 Geometric model of a groundwater reservoir.

The portion of natural discharge that returns to the atmosphere via evaporation evapotranspiration is mostly already committed. Only a small fraction of it (the water that directly from the ground) may be subject to capture, if deemed necessary to satisfy su
needs. The case for the sequestration of the other two fractions (the evaporation from bare ground and the evapotranspiration from vegetation) is usually less defensible.

Not all water pumped is lost to the groundwater system; only the water consumed and not returned to the aquifer. Thus, a precise water balance, which takes into account all uses, is needed to determine sustainability (Kendy, 2003).

Sustainable yield does not depend on the size, depth, or hydrogeologic characteristics of the aquifer. Current practice notwithstanding, sustainable yield does not depend on the aquifer's natural recharge because the natural recharge has already been appropriated by the natural discharge (Lamberti, 2000a). Sustainable yield depends on the amount of capture, and whether this amount is acceptable as a reasonable compromise between little or no use, on one extreme, and the capture of all natural discharge, on the other extreme. Sustainable yield is seen to be a moving target determined after a judicious study and appraisal of all issues regarding groundwater utilisation. These issues include hydrogeology, hydrology, ecology, climatology, social and economic development, and related institutional and legal aspects, to name the most relevant.

In practice, sustainable yield may be taken as a suitable percentage of precipitation. A conservative estimate would take up to the deep percolation amount as sustainable yield, so that it does not lead to excessive salt-water intrusion. On a global basis, deep percolation accounts for about 2% of precipitation. In the absence of basin-specific studies, this figure may be used for purposes of planning to base sustainable yield assessments. A fraction of evaporative loss (evapotranspiration ET) is seen to be part of discharge (ND), which originates in recharge from precipitation, so that the fractions of deep percolation, evaporation, evapotranspiration and baseflow, the latter being the candidates for capture, can be ascertained.

Sustainable yield can also be expressed as a percentage of recharge. Globally, if recharge is assumed to be approximately 20% of precipitation, then deep percolation would be about 4% of recharge. Thus, a reasonably conservative estimate of sustainable yield would be 10%. Limited experience indicates that average values of this percentage may be around 40%. However, in some instances, conservative percentages may exceed 70% (Miles and Chambet, 1995; Hahn et al., 1995). The current concept of sustainable yield represents a compromise between theory and practice. A reasonable estimate of sustainable yield would be about 10% of recharge. Values higher than 10% may reflect the need to consider other factors besides conservation.

Communities are beginning to consider baseflow conservation as the standard means of assuring sustainable yield (Maimone, 2004). Baseflow may be defined as the minimum low flows of selected frequency. Ultimately, baseflow conservation may be the only practical way of assuring reasonable regulation of groundwater capture, and, therefore, does not end up sequestering the entire natural discharge.

---

Baseflow conservation may be the only practical way of assuring that groundwater capture is regulated and, therefore, does not end up sequestering the entire natural discharge.

---

http://ponce.sdsu.edu/groundwater_sustainable_yield.html 7/23/2015
A fundamental question remains: Who owns the groundwater? The answer is straightforward. In theory, whoever owns the natural discharge owns the groundwater. This natural discharge can be shown to provide natural and social services. Natural services are associated with the maintenance of terrestrial and aquatic ecosystems that rely on the natural discharge. These ecosystems may comprise, for example, wetland species, and minimum instream flows to sustain fisheries and wildlife. Socioeconomic services associated with water rights that may have been already appropriated to individual or organizational entities. In practice, it does not appear viable, in all cases, to disallow groundwater use that the entire natural discharge may have been already appropriated.

Experience shows that reasonable compromises may be established on a case-by-case context, it is extremely important to strive toward a holistic approach to sustainability. It considers the hydrogeological, hydrological, ecological, socioeconomic, technological, institutional and legal aspects, in a seamless fashion, seeking to establish a reasonable compromise between conflicting interests. For the most part, groundwater depletion may be unacceptable, but a reasonable amount of steady capture may be acceptable if a consensus is achieved as to its size, with full recognition and, consequently a thorough evaluation, of the impacts.

Groundwater sustainability may be enhanced by increasing recharge in three ways:

1. Capturing rejected recharge;
2. Encouraging clean artificial recharge; and
3. Limiting negative artificial recharge.

The greater the amount of capture coming from rejected recharge, the more sustainable (Theis, 1940). Likewise, the greater the clean artificial discharge, the more sustainable (Kazmann, 1956). Limiting negative artificial recharge, to the extent possible, would go toward assuring sustainability.

7. CONCLUSIONS

The issue of how much to pump in a sustainable context is shown to have no simple traditional concept of safe yield, which equates safe yield with natural recharge, is widely discredited. Since 1987, the concept of sustainable yield has emerged. It provides a reasonable compromise between the rights of established ground water users, rights of downstream ecosystems and surface water users to the natural discharge which by that ground water. The ideal solution appears to be to conserve all ground water, extract suitable fractions of deep percolation, for the benefit of the surface waters. However, may prove to be too harsh, and probably socioeconomically not viable in places where usage has become, over the years, a way of life.
It is clear that sustainable yield can no longer be taken as equal to natural recharge. Compromise may be to consider sustainable yield as a fraction of natural recharge, thorough evaluation is made of the tradeoffs, including the hydrological and ecologic groundwater development. Baseflow, more properly baseflow conservation, is emerging as a standard against which groundwater pumping will be increasingly measured in the future.

In the absence of detailed holistic studies, a reference value of sustainable yield may be set at or a suitable fraction of, the global average for deep percolation, estimated as 2% of local availability. Detailed local and regional studies will determine whether this value may be increased or decreased on a case-by-case basis to reflect one or more of the following:

a. An improved understanding of the components of the water balance;
b. A workable compromise between conflicting socioeconomic interests; or
c. The choice of a less conservative approach to resource management.

Sustainability goes hand-in-hand with conservation; the more conservative the proposed policy, the more sustainable it will be. Sustainable yield is seen to be a moving target in adaptive management.

8. SUMMARY

- All groundwater reservoirs are temporarily holding water in transit from a place of origin to a place of discharge.
- A pristine groundwater reservoir is in steady state, with inflows equal to outflows.
- All pumping comes from capture; the greater the intensity of pumping, the greater the potential for rapid depletion.
- Capture comes from decreases in natural discharge and increases in aquifer recharge.
- Natural discharge supports riparian, wetland, and other groundwater-dependent systems and the baseflow of streams and rivers.
- The traditional concept of safe yield, which equates safe yield with natural recharge, and has been widely discredited.
- Sustainable yield depends on the amount of capture, and whether this amount can be accepted as a reasonable compromise between a policy of little or no use, on one hand, and the sequestration of all natural discharge, on the other extreme.
- Capture depends on usage and it is not related to size or hydrogeological character of the aquifer, or to the natural recharge.

http://ponce.sdsu.edu/groundwater_sustainable_yield.html

7/23/2015
sustainable yield of groundwater, safe yield, groundwater recharge, groundwater utiliza... Page 26 of 32

- Sustainable yield is a moving target, to be determined after a judicious study and issues regarding groundwater utilization.

- A reasonably conservative estimate of sustainable yield takes up all or suitable fracture percolation as sustainable yield. On a global average basis, deep percolation is precipitation.

- Sustainable yield may also be expressed as a percentage of recharge. Limite suggests that average values may be around 40%, with the least conservative around 10%.

- Interdisciplinary studies are needed to develop more experience in yield percentages applicable on a local, subregional, and regional basis.

- Sustainability may be fostered by enlightened management seeking to cap recharge, encourage clean artificial recharge, and limit negative artificial recharge.

- Artificial recharge is most effective when it uses rain or snowmelt water. Municipal should receive at least secondary treatment prior to being used for artificial recharge. In case, the cross contamination of potable water supplies with wastewaters originating from recharge should be avoided.

- A holistic approach to groundwater sustainability considers the hydrogeological, ecological, socioeconomic, technological, cultural, institutional and legal aspects of utilization, in a seamless fashion, seeking to establish a reasonable compromise among conflicting interests.

- Communities are beginning to consider baseflow conservation as the standard measure groundwater sustainability.

- In the end, sustainability reflects resource conservation policy; the more conservatively it is likely to be.

ACKNOWLEDGEMENTS

This study was made possible with the support of the people of the communities of B Campo, in East San Diego County, California.

REFERENCES

http://ponce.sdsu.edu/groundwater_sustainable_yield.html 7/23/2015


http://ponce.sdsu.edu/groundwater_sustainable_yield.html 7/23/2015


GLOSSARY

aquifer = a saturated permeable geologic formation which can yield significant quantities of water to wells and springs (Fig. 13).

aquifer length [L] = the dimension of an aquifer in a direction perpendicular to the drainage plane.

aquitard = the less permeable beds in a stratigraphic sequence, not permeable enough to yield significant water to wells and springs (Fig. 13).

artificial recharge = the increase in groundwater recharge induced by human design.

available recharge = the sum of unrejected recharge and rejected recharge, if the latter is nonzero.

baseflow = the fraction of streamflow that originates in groundwater.

baseflow conservation = the policy of protecting baseflow to minimize the risk of capture by ground water.

http://ponce.sdsu.edu/groundwater_sustainable_yield.html
sustainable yield of groundwater, safe yield, groundwater recharge, groundwater utiliza... Page 29 of 32

\textbf{bog} = a type of wetland that accumulates acidic peat, a deposit of dead plant material (Fig. 13).

\textbf{capture} = the amount withdrawn by pumping.

\textbf{captured discharge} = the decrease in discharge induced by pumping.

\textbf{captured recharge} = the increase in recharge induced by pumping.

\textbf{captured storage} = the decrease in groundwater storage induced by non-equilibrium pumping.

\textbf{coefficient of storage [S]} = the ratio of free-draining water volume to aquifer volume.

\textbf{confined aquifer} = an aquifer whose upper boundary is below the water table (Fig. 1).

\textbf{conservation} = a careful protection of something of intrinsic value, such as a finite natural resource.

\textbf{consumptive use} = the amount of groundwater use that is consumed, therefore, not returned through aq

\textbf{deep percolation} = the fraction of precipitation (or recharge) that returns directly into the ocean, bypass waters.

\textbf{depleted system} = a groundwater system in non-equilibrium or unsteady state, with heavy pumping at depths.

\textbf{developed system} = a groundwater system in equilibrium or steady state, with moderate pumping at a fb

\textbf{dimensionless aquifer diffusivity} = a dimensionless expression of aquifer diffusivity, based on the relai

which \( t = \) time, \( T = \) transmissivity, \( S = \) storage coefficient, and \( L = \) length.

\textbf{direct runoff} = the runoff that flows directly on the surface, without infiltrating under the ground.

\textbf{ecosystem} = a natural system consisting of a collection of biotic and abiotic components, including flora, forms, water and soil, governed by a set of natural laws.

\textbf{evaporation} = the process by which atoms or molecules in a liquid state gain sufficient energy to en state.

\textbf{evapotranspiration} = the sum of evaporation and plant transpiration, which is the movement of water the subsequent loss of water as vapor through stomata in its leaves.

\textbf{exfiltration} = the water that percolates into the ground surface from below.

\textbf{gravitational force} = the force that drives all mass from a place of higher potential to a place of lower pot

\textbf{ground water} = the water flowing by gravity, below the surface of the Earth, and which fills the por alluvium, soil, or rock formation.

\textbf{groundwater mining} = the depletion of a groundwater reservoir beyond its capacity to replenish naturally

\textbf{holistic} = concerned with the whole, rather than with the parts; seamless.

\textbf{hydraulic conductivity [K]} = the [mean] velocity of flow through soil or rock formation [aquifer].

\textbf{hydrologic cycle} = the continuous recirculatory movement of the waters of the Earth.

\textbf{hydrophyte} = a plant adapted to live in water or waterlogged soil.

\textbf{hygrophyte} = a plant that thrives in very wet soil and/or is more or less restricted to moist sites.

http://ponce.sdsu.edu/groundwater_sustainable_yield.html

7/23/2015
infiltration = the water that percolates downward, below the ground surface, through the upper soil layer.

natural discharge = discharge to the surface waters [exfiltration], which feeds springs, streams and lakes, and groundwater-dependent ecosystems.

natural recharge = groundwater replenishment from runoff or snowmelt, through seepage from the surface.

negative artificial recharge = the decrease in recharge induced by socioeconomic activities, such as overgrazing, overcultivation, and urban development.

net recharge = the difference between recharge and discharge.

non-pristine reservoir = a reservoir that has a history of pumping.

nonrenewable = not replenishable, or unrecoverable, within a period less than a human lifespan.

phreatophyte = rooted plant that obtains water from a permanent ground supply or from the water table, above it.

primary treatment = the wastewater treatment level that removes settleable organic and inorganic sedimentation, and floating materials by skimming.

pristine reservoir = a reservoir that has not been subject to human intervention.

recharge = any addition to the groundwater system by percolation through the land surface (Fig. 13).

recharge basin = a reservoir or basin which has the purpose of recharging surface water into the ground water.

rejected recharge = the amount of available recharge rejected by an aquifer on account of being full (at time).

residual discharge = the difference between natural discharge and captured discharge.

renewable = replenishable or recoverable within a period less than a human lifespan.

riparian vegetation = vegetation that satisfies its physiological needs for water by extracting it from the overlying unsaturated (vadose) zone.

runoff = the water flowing on the surface of the Earth, from rain, snowmelt, and other sources.

safe yield (traditional) = the attainment and maintenance of a long-term balance between the groundwater withdrawn and the annual amount of recharge.

secondary treatment = the wastewater treatment level that removes soluble and colloidal biodegradable and suspended solids and, in some cases, nitrogen and phosphorus.

simulation = the imitation or modeling of a prototype or system, by describing its properties, using a seeking answers to "what if" questions.

spring = a natural feature (location) where concentrated groundwater flow exfiltrates into the land surface.

stream = natural feature whose purpose is to return a fraction of precipitated water, and the solids it carries thus closing the hydrologic cycle.

streamflow = the sum of direct runoff and baseflow.

surface runoff = the runoff flowing on the surface of the Earth directly into streams (Fig. 13).
surface water = the water flowing on the surface of the Earth, including overland flow and streamflow.

sustainable development = the development that meets the needs of the present without compromis future generations to meet their own needs.

sustainable yield = the groundwater yield which is in agreement with the principle of sustainable develop

tertiary treatment = any physical, chemical, or biological treatment process used to accomplish a degree treatment greater than that achieved by secondary treatment.

traditional safe yield = the attainment and maintenance of a long-term balance between the an groundwater withdrawn and the annual amount of recharge.

transmissivity [T] = the product of hydraulic conductivity K and aquifer thickness b, i.e., T = Kb; a volume of water that moves through an aquifer.

unconfined aquifer = an aquifer whose upper boundary is the water table (Fig. 1).

undeveloped reservoir = a reservoir that has not been subject to human intervention.

unrejected recharge = the amount of available recharge taken by an aquifer on account of not being full.

vadose zone = the unsaturated zone extending between the ground surface and the water table.

water balance = an accounting of the various components of the hydrologic cycle.

water table = the upper surface of the zone of saturation in an unconfined aquifer.

water quality = the description of the chemical, physical, and biological characteristics of water, usually suitability for a particular purpose or use.

watershed = the land area from which water drains into a point, either a stream, river, or reservoir.

wetland = a terrestrial ecosystem where the water table is regularly at or close to the ground surface.
Fig. 13  The groundwater system of the Ottawa valley, Canada.


Documents in Portable Document Format (PDF) require Adobe Acrobat Reader 5.0 or higher
download Adobe Acrobat Reader.
Ground-Water Depletion Across the Nation

U.S Geological Survey Fact Sheet 103-03
November 2003

This fact sheet is available as a pdf.

Ground-water use has many societal benefits. It is the source of drinking water for about half the nation and nearly all of the rural population, and it provides over 50 billion gallons per day in support of the Nation’s agricultural economy. Ground-water depletion, a term often defined as long-term water-level declines caused by sustained ground-water pumping, is a key issue associated with ground-water use. Many areas of the United States are experiencing ground-water depletion.

An aquifer can be compared to a bank account, and ground water occurring in an aquifer is analogous to the money in the account. Hydrologists refer to this type of accounting as a water budget. Ground water can be recharged (deposited) by infiltration from precipitation, surface water, or applied irrigation water; it can be kept in storage (saved); and it can be discharged naturally to streams, springs, or seeps, or transpired by plants (withdrawn). In a ground-water system prior to development, the system is in longterm equilibrium—discharge is equal to recharge, and the volume of water in storage remains relatively constant. Ground-water levels fluctuate in time over a relatively small, natural range. Once pumping begins, however, this equilibrium is changed and ground-water levels decline. Just as a bank account must be balanced, withdrawals from an aquifer by pumping must be balanced by some combination of increased recharge, decreased discharge, and removal from storage (or depletion). An inventory of ground-water levels in wells reflects the volume of water stored (or occurring) in the aquifer, and is analogous to a financial statement.

---

An aquifer can be compared to a bank account, and ground water occurring in an aquifer is analogous to the money in the account.

---

The volume of ground water in storage is decreasing in many areas of the United States in response to pumping. Ground-water depletion is primarily caused by sustained ground-water pumping. Some of the negative effects of ground-water depletion include increased pumping costs, deterioration of water quality, reduction of water in streams and lakes, or land subsidence. Such effects, while variable, happen to some degree with
any ground-water use. As with other natural resources, society must weigh the benefits against the consequences of such use. In order to provide the scientific information needed for informed decisions, these effects must be observed over time to determine their impact.

**What Are Some Effects Of Ground-Water Depletion?**

If intensive pumping from an aquifer continues, then adverse effects may occur.

*Water-well problems* Declining ground-water levels have three main effects on water wells. First, as the depth to water increases, the water must be lifted higher to reach the land surface. As the lift distance increases, so does the energy required to drive the pump. Thus, power costs increase as ground-water levels decline. Depending on the use of the water and the energy costs, it may no longer be economically feasible to use water for a given purpose. Second, ground-water levels may decline below the bottom of existing pumps, necessitating the expense of lowering the pump, deepening the well, or drilling a deeper replacement well. Third, the yield of the well may decline below usable rates.

Ground-water budgets before and after development of the Gulf Coastal Plain aquifer system (all flows in cubic feet per second). The large withdrawals from the aquifers have been balanced by increases in recharge to the aquifer system and decreases in storage and discharge from the aquifer system (modified from Williamson and Grubb, 2001).

---

A hydrograph showing ground-water-level declines in the Buckman well field, which supplies water for Santa Fe,
Reduced surface-water flows In most areas, the surface- and ground-water systems are intimately linked. Ground-water pumping can alter how water moves between an aquifer and a stream, lake, or wetland by either intercepting ground-water flow that discharges into the surface-water body under natural conditions, or by increasing the rate of water movement from the surface-water body into an aquifer. In either case, the net result is a reduction of flow to surface water, though the full effect may take many years to develop.

A related effect of ground-water pumping is the lowering of ground-water levels below the depth that streamside or wetland vegetation needs to survive. The overall effect is a loss of riparian vegetation and wildlife habitat.

Subsidence Land subsidence is "a gradual settling or sudden sinking of the Earth's surface owing to subsurface movement of earth materials." Though several different earth processes can cause subsidence, more than 80 percent of the subsidence in the United States is related to the withdrawal of ground water (Galloway and others, 1999).

Deterioration of water quality Coastal aquifers tend to have wedgeshaped zones of saltwater underlyng the potable freshwater. Under natural conditions the boundary between the freshwater and saltwater tends to be relatively stable, but pumping can cause saltwater to migrate inland, resulting in saltwater contamination of the water supply. Inland aquifers can experience similar problems where withdrawal of good-quality water from the upper parts of inland aquifers can allow underlying saline water to move upward and degrade water quality. Additionally, where ground water is pumped from an aquifer, surface water of poor or differing quality may be drawn into the aquifer. This can degrade the water quality of the aquifer directly or mobilize naturally occurring contaminants in the aquifer.
Where Does Ground-Water Depletion Occur In The United States?

Ground-water depletion has been a concern in the Southwest and High Plains for many years, but increased demands on our ground-water resources have overstressed aquifers in many areas of the Nation, not just in arid regions. In addition, ground-water depletion occurs at scales ranging from a single well to aquifer systems underlying several states. The extents of the resulting effects depend on several factors including pumpage and natural discharge rates, physical properties of the aquifer, and natural and human-induced recharge rates. Some examples from east to west across the Nation are given below.

A 1942 photograph (top) of a reach of the Santa Cruz River south of Tucson, Arizona, shows stands of mesquite and cottonwood trees along the river. A photograph (bottom) of the same site in 1989 shows that the riparian trees have largely disappeared, as a result of lowered ground-water levels. Photos: Robert H. Webb, USGS.

Atlantic Coastal Plain In Nassau and Suffolk Counties, Long Island, New York, water pumped for domestic supply is used and sent to a wastewater treatment plant and then discharged into the surrounding saltwater bodies. As a result of these actions, the water table has been lowered, the base flow of streams has been reduced or eliminated, the length of perennial streams has been decreased, and saline ground water has moved inland.

Many other locations on the Atlantic coast are experiencing similar effects related to ground-water depletion. Surface-water flows have been reduced due to ground-water development in the Ipswich River basin, Massachusetts. Saltwater intrusion is occurring in coastal counties in New Jersey; Hilton Head Island, South Carolina; Brunswick and Savannah, Georgia; and Jacksonville and Miami, Florida (Barlow, 2003).
**West-central Florida** Ground-water development in the Tampa-St. Petersburg area has led to saltwater intrusion and subsidence in the form of sinkhole development and concern about surface-water depletion from lakes in the area. In order to reduce its dependence on ground water, Tampa has constructed a desalination plant to treat seawater for municipal supply.

**Gulf Coastal Plain** Several areas in the Gulf Coastal Plain are experiencing effects related to ground-water depletion:

Ground-water pumping by Baton Rouge, Louisiana, increased more than tenfold between the 1930s and 1970, resulting in ground-water-level declines of approximately 200 feet. Baton Rouge is underlain by a series of aquifers, and pumping has shifted among them with time. The large water-level declines have resulted in saltwater encroaching from the Gulf of Mexico into several of the aquifers (Taylor and Alley, 2001).

---

Decline in ground-water levels in the sandstone aquifer, Chicago and Milwaukee areas, 1864-1980 (Alley and others, 1999).

---

In the Houston, Texas, area, extensive ground-water pumping to support economic and population growth has caused water-level declines of approximately 400 feet, resulting in extensive land-surface subsidence of up to 10 feet. Among other issues,
subsidence is responsible for increased susceptibility to flooding and the permanent inundation of some areas.

Continued pumping since the 1920s by many industrial and municipal users from the underlying Sparta aquifer have caused significant water-level declines in Arkansas, Louisiana, Mississippi, and Tennessee. Such declines have caused concerns about the Sparta's sustainability resulting in the aquifer being declared "critical" in Arkansas. The Memphis, Tennessee, and West Memphis, Arkansas, area is one of the largest metropolitan areas in the world that relies exclusively on ground water for municipal supply. These large withdrawals have caused regional water-level declines of up to 70 feet, and have resulted in interstate concerns over continued and increased pumping in the Memphis area.

**High Plains** The High Plains aquifer (which includes the Ogallala aquifer) underlies parts of eight States and has been intensively developed for irrigation. Since predevelopment, water levels have declined more than 100 feet in some areas and the saturated thickness has been reduced by more than half in others. Water levels are recovering in some areas due to management by State and local agencies, improved irrigation efficiency, low crop prices, and agricultural programs (McGuire and others, 2003).

**Chicago-Milwaukee** area Since the first documented water well was completed in the Chicago area in 1864, ground water has been the sole source of drinking water for about 8.2 million people in the Great Lakes watershed. This long-term pumping has lowered ground-water levels by as much as 900 feet in the sandstone aquifer underlying the Chicago area and eastern Wisconsin. Concern over how such pumping affected surface water in the Great Lakes region led to the reduction of ground-water withdrawals in much of the area. Water levels are recovering in some areas, however, declines continue in others (Grannemann and others, 2000).

**Pacific Northwest** Ground-water development of the Columbia River Basalt aquifer of Washington and Oregon for irrigation, public-supply, and industrial uses has caused water-level declines of more than 100 feet in several areas; management efforts to reduce withdrawals have reversed some of the declines. The Snake River Plain aquifer in Idaho provides water for extensive irrigation as well as much of the flow of the Snake River through springs. Since 1950, water levels and spring discharge have decreased due to intensive use of ground water for agriculture (Burns, 1997).

**Desert Southwest** Increased ground-water pumping to support population growth in south-central Arizona (including the Tucson and Phoenix areas) has resulted in water-level declines of between 300 and 500 feet in much of the area. Land subsidence was first noticed in the 1940s and subsequently as much as 12.5 feet of subsidence has been measured. Additionally, lowering of the water table has resulted in the loss of streamside vegetation as documented by historical photographs.

In 1999, Las Vegas, Nevada, was the fastest growing municipal area in the United States. In places, ground-water levels have declined 300 feet since the first flowing artesian well was drilled in 1907. These water-level declines have resulted in as much as 6 feet of subsidence since 1935, as well as having caused springs to dry up and artesian wells to stop flowing (Pavelko and others, 1999).

Locations in the basins of southern California, Nevada, Utah, Arizona, and New Mexico where substantial ground-water level declines have been measured. In some areas, water levels have recovered in response to reduction in pumping and increased recharge efforts (Leake and others, 2000).
This earth fissure formed on Rogers Lake at Edwards Air Force Base, California, in January 1991, and forced the closure of one of the space shuttle's alternative runways. The fissure has been attributed to land subsidence related to ground-water pumping in the Antelope Valley area (Galloway and others, 2003).
In Antelope Valley, on the western edge of the Mojave Desert in southern California, water-level declines have exceeded 300 feet in some areas since the early 1900s. As a result, measured land subsidence exceeded 6 feet locally between 1930-92. The land surface is continuing to subside, resulting in damage to roads, buildings, and other structures (Galloway and others, 2003).

What Information Do We Need To Monitor The Nation’s Ground-Water Depletion And Its Effects?

About 140 million residents (about 50 percent of the population) in all 50 States depend on ground water for their direct needs. Ground water provides about 40 percent of the Nation’s public-water supply and much of the water used for irrigation. This reliance on ground water necessitates long-term monitoring of ground-water levels to track ground-water depletion. Though water-level monitoring takes place for many aquifer systems within individual States, coordinated water-level monitoring generally has not been done for aquifers that cross State boundaries (the High Plains aquifer is an exception). No comprehensive national ground-water-level network exists with uniform coverage of major aquifers, climate zones, or land uses.

Data on ground-water levels and rates of change are "not adequate for national reporting."

The State of the Nation’s Ecosystems
H. John Heinz Center for Science, Economics, and Environment, 2002

Long-term ground-water-level data from individual wells provide the information needed to monitor ground-water depletion locally. Periodic assessments of changes in ground-water storage could be made by measuring more wells over larger areas at 5- to 10-year intervals. Such changes could be documented for major aquifers and then compiled into regional and national assessments. A major task at the beginning of such an assessment would be the analysis of ground-water withdrawals and changes in storage that occurred during the 20th century (U.S. Geological Survey, 2002).

In order to preserve and optimize the use of our critical ground-water resources, science can provide the information necessary to make informed choices on issues that have long-term environmental and ecological effects. For many aquifers in the United States, the basic data needed for such assessments are not available, and hence our knowledge of the water budget for them is limited. In about 1950, in Albuquerque, New Mexico, several supply wells were pumped dry, leading C.V. Theis, one of the major scientists in the field of hydrogeology, to comment, "What happened was that the city got a notice from its bank that its account was overdrawn and when it complained that no one could have foreseen this, only said in effect that it had no bookkeeping system" (Theis, 1953).

—J.R. Bartolino and W.L. Cunningham

REFERENCES

For more information on ground-water-resource issues, please contact:

Chief, Office of Ground Water
U.S. Geological Survey
411 National Center
12201 Sunrise Valley Drive
Reston, VA 20192
(703) 648-5001
http://water.usgs.gov/ogw

This report is available online in Portable Document Format (PDF). If you do not have the Adobe Acrobat PDF Reader, it is available for free download from Adobe Systems Incorporated.
Issue Paper:
Ground Water Recharge Area Protection (Water Quality)

1. Introduction And Background

1.1. Purpose And Scope
This issue paper examines various approaches for protecting aquifer recharge areas from a water quality perspective and recommends an approach for protecting Kitsap County ground water resources. WAC 365-190-080 states "Counties and cities shall classify recharge areas for aquifers according to the vulnerability of the aquifer. Vulnerability is the combined effect of hydrogeological susceptibility to contamination and the contamination loading potential." Other issue papers address factors that affect aquifer recharge from a quantity viewpoint. This paper proposes an approach to aquifer protection that is responsive to the unique circumstances of Kitsap County. The paper will also address well head protection and in particular the State's developing Wellhead Protection Program.

1.2. Background
Ground water aquifers hold nearly 50 times the volume of the Nation's surface waters, constitute approximately 96% of all the fresh water in the United States, and serve as the primary drinking water source for half of the population (nearly 117 million people). In Kitsap County, over 80% of potable water comes from ground water supplies. Every state has documented cases of ground water contamination. Once ground water is contaminated it is difficult, costly, and sometimes impossible to clean up. Preventing ground water contamination avoids the unnecessary costs of remediation and the potential damage to human health and the environment. Unfortunately, ground water contamination has occurred in Kitsap County.

The Kitsap County Historical Record of Ground Water Contamination contains many incidents of contamination. The number of designated and proposed National Priority List hazardous waste sites (NPL sites, frequently called superfund sites) is growing. There are currently seven NPL sites in Kitsap County. The Washington State Department of Ecology (Ecology) records document information on 98 leaking underground storage tanks. The Bremerton-Kitsap County Health District (BKCHD) has records on 46 investigations of significant pollution (Affected Media Contaminants Reports). The above information sources combined suggest the total number of contaminated sites is greater than 120, of which 36 have resulted in confirmed ground water contamination problems. The County's NPL sites, rapid development, and increased knowledge of potential sources of contamination, have caused Kitsap County citizens to be very concerned about protecting aquifer recharge areas. An on-going monitoring program carried out by Kitsap Public Utility District (KPUD), with input from the major water purveyors, has thus far not revealed contamination of the known principal aquifers of the County.
1.3. Approaches to Protecting Ground Water

Communities have used various approaches to protect ground water and associated recharge areas. One approach is to implement land-use controls in areas designated as aquifer recharge areas. Another tactic involves systematically ranking and controlling existing and potential threats to ground water. Other systems use computer generated, groundwater models to evaluate the effects various land use management alternatives and other activities could have on ground water resources. Each approach has inherent benefits and disadvantages that limit whether they are appropriate for a particular area. The following is a review of some of the different approaches used to protect ground water.

This paper draws extensively on "Ground Water Resource Protection, A Handbook for Local Planners and Decision Makers in Washington State," prepared by the King County Planning Division and Washington State Department of Ecology.

1.3.1. Aquifer Recharge Areas

An aquifer recharge area is defined as the surface area which receives rain and passes a portion downward where it replenishes ground water within an aquifer. The primary aquifer recharge area of a specific aquifer, in particular deep aquifers, may or may not correspond with the surficial area directly above the aquifer. Permeable soils, in particular, provide the potential for precipitation on an area to become ground water recharge. More generally, it is the surficial features, existing land use and ground cover, as well as soil permeability and overlying geologic material which are used to evaluate aquifer recharge areas.

Ground water flow systems in aquifers can be analogous to surface water drainage patterns and, like them, contain smaller local flow systems within larger regional flow systems. Localized flow systems are influenced by aquifer recharge areas. Local flow systems are generally shallower than regional systems. Pollutants introduced to regional flow regimes may travel greater distances, thus contaminating greater volumes of ground water. Regional flow systems can be quite extensive and can encompass many square miles.

Both local and regional flow systems may be present below a given site. Each can have its own recharge area or have combined recharge areas. The recharge areas of shallow aquifers may be relatively large in aerial extent and often have direct surface exposure, thus making them directly susceptible to surficial contaminants. Recharge to deep aquifers is from overlying shallow aquifers or through windows in overlying aquitards. Recharge to deep aquifers can be complicated when intervening aquifers and confining layers exist.

1.3.2. Environmentally Sensitive Areas

Community land use policies and practices include a broad definition of environmentally sensitive areas. The land use definition of ESA incorporates a significant measure of interest in socio-economic matters such as loss of property or
life as the result of utilizing unsafe construction sites (e.g., unstable slopes) in addition to concern for resources (e.g., water, wildlife).

When designating aquifers as being sensitive, it is essential to consider the recharge areas associated with them as being environmentally sensitive areas (ESAs). One of the underlying problems of discussing environmentally sensitive areas is in their definition. Geographically identifying the boundaries of an ESA can be difficult even when the definition is clear.

The following comments from the Bainbridge Island Subarea Plan are informative:

"Environmental concerns and land use are closely related. When development occurs without careful examination of effects on its surroundings, several undesirable outcomes are possible. Hazards to that development or adjoining properties may be created or increased. Natural resources may be damaged. Governmental costs from environmental degradation may be incurred in the future which a developer may not consider during his / her one-time contact with a project."

"ESAs designations are intended to flag concerns in the review process and to make applicants aware of potential hazards or natural resources which may be damaged by unsound development decisions. The designations are not intended, however, to eliminate all development. Compatible development will be allowed which either avoids designated ESAs or mitigates potential problems through engineering, siting, design or other techniques. Proposals are examined on a case-by-case basis to allow for creative solutions (although some mitigative techniques are suggested in the discussions below) and to assure that the special combinations of factors in a particular case are addressed."  

1.3.3. Aquifer Recharge Areas as ESAs

Designating an area as an aquifer recharge area can be valuable in protection ground water supplies. In the context of aquifer recharge area protection, ESAs are those areas that have a potentially critical influence on maintaining the quality of water in the aquifer. Jaffe and Dinovo, in applying the ESA concept to ground water suggested that a sensitive area is an area in which ground water can be easily contaminated. They proposed two commonly used approaches to define sensitive areas within a hydrogeologic study area. One approach identifies recharge areas where flow has a strong downward component and may potentially carry contaminants into the aquifer. These areas are frequently characterized by very permeable soils or a shallow water table.

The other approach focuses on ground water use, particularly drilled wells. Wells draw water from the surrounding part of the aquifer, called the area of influence, whose boundary depends on the hydraulic conductivity, thickness, and lateral
Columbia Water Center

Columbia Water Center White Paper

Assessment of trends in groundwater levels across the United States

March 2014

Tess Russo
Upmanu Lall
Hui Wen
Mary Williams
Table of Contents

Key Points ........................................................................................................................................... 3

1. Introduction ...................................................................................................................................... 3

2. Data source and description .............................................................................................................. 4

3. Methods .......................................................................................................................................... 5

  3.1. Analysis of Groundwater Elevation Trends over Time ................................................................. 5

  3.2. Correlation between Groundwater Elevation and Groundwater Extraction ............................. 6

  3.3. Correlation between Groundwater Elevation and Climate .......................................................... 6

4. Results and Discussion ...................................................................................................................... 7

  4.1. Groundwater Level Trends ....................................................................................................... 8

  4.2. Relationship between Groundwater Elevation and Pumping ................................................... 12

  4.3. Correlation between Groundwater Elevation and Climate .......................................................... 14

5. Comparison to other US Groundwater Studies .............................................................................. 16

6. Summary ....................................................................................................................................... 18

References ........................................................................................................................................... 18
Key Points
1. Groundwater levels have declined across much of the United States
2. An increase in pumping rates corresponds with declining groundwater levels in most counties
3. On average, long-term climate trends appear to correspond to groundwater level changes, and there is not as much sensitivity to annual precipitation variability

1. Introduction
The objective of this study is to address the following questions:
(1) How have groundwater levels changed across the US between 1949 and 2009?
(2) How do groundwater level trends correlate with extraction rates?
(3) How do groundwater level trends correlate with precipitation and climate trends?
This study addresses these questions by analyzing historical groundwater records across the continental USA, and comparing observed trends to climate and groundwater use records.

Groundwater constitutes a critical component of our water resources. In the United States, groundwater accounts for 60% of irrigation and provides drinking water for more than 40% of the population. It serves as a valuable resource in regions lacking access to surface water and, in most areas, provides an essential buffer during dry periods. If human use upsets the balance between recharge, capture, and natural outflows, aquifer equilibrium is lost until a new balance is reached (Alley and Leake 2004).

As a critical national resource, it is important that water users and managers are aware of groundwater level trends and the dominant controls on availability. Understanding aquifer response to extraction and climate should serve as the basis for water use policies to avoid irreversible negative consequences. Sustainable planning and proper management of groundwater extraction must also account for climate change and climate variability. As precipitation patterns change across the US (e.g. Karl & Knight, 1998), so will recharge rates, potentially exacerbating water stressed regions (Döll 2009).

In arid and semi-arid areas where low precipitation results in low or zero natural recharge, mining of fossil groundwater from aquifers depletes a non-renewable resource. With less water to support the aquifer structure, compaction can cause land subsidence and permanent reduction in storage capacity. Groundwater extraction rates have shown to be more influential than climate change in the Edwards Aquifer in Texas (Loáiciga 2003). In the High Plains Aquifer system, including the Ogallala, fossil water is extracted at nearly 10 times the rate of recharge, resulting in the largest groundwater depletion in the country (Scanlon et al. 2012; Konikow 2013).

Correlations between groundwater levels and precipitation can help assess aquifer vulnerability to climate change (Ng et al. 2010). Deep groundwater production wells can show high water level variability over time, sometimes with a variable lag in response times to climate ranging from a few seconds to millions of years (Sophocleous 2012). Several studies have investigated groundwater...
levels and climate correlations in US aquifers. Long term climate cycles have the most observable effects on groundwater levels, generally due to the relatively long recharge and aquifer response time. In Southern California, the Pacific Decadal Oscillation (PDO) and El Niño Southern Oscillation (ENSO) have been shown to cause the largest variations of groundwater levels (Hanson and Dettinger 2005). In the High Plains Aquifer system, groundwater levels were most highly correlated with PDO (Gurdak et al. 2007). Groundwater response to climate also depends on local geology (Chen, Grasby, and Osadetz 2004), land use and land cover (Scanlon et al. 2005), and other factors affecting infiltration and recharge rates.

2. Data source and description

Groundwater level and usage data for this study were obtained from the US Geological Survey (USGS-NWIS). Depth to groundwater values were downloaded for 24,532 wells having >30 records from 1949 to 2009 and a screen depth > 30 m (100 ft) (Figure 1). To preclude wells with short records unlikely to span multiple years, we set a minimum threshold of 30 observations. Groundwater level measurements were rarely recorded at regular intervals; the number and frequency of measurements taken each year varies by site.

![Figure 1](image)

**Figure 1** Groundwater well data retrieved from the US Geological Survey, (A) Histogram of well depths, and (B) Density of monitoring wells deeper than 30 m (100 ft).

We obtained water use data from the USGS Estimated Use of Water reports (USGS-NWIS). State-scale water use data including surface and groundwater use is available every 5 years from 1960 to 2005 (2010 will be released later this year, 2014). Higher resolution data is available at the county-scale from 1985 to 2005 (e.g. Figure 2). This study used only the more recent county-scale data.
Figure 2. Area normalized fresh groundwater extraction in 2005.

Annual average precipitation for each county from 1949 to 2009 was obtained from Maurer et al. (2002). Data for the long term climate cycle analysis, including the PDO indices and the North Atlantic Oscillation (NAO) indices were obtained from the National Oceanic and Atmospheric Administration (NOAA) National Weather Service group.

3. Methods

3.1. Analysis of Groundwater Elevation Trends over Time

Groundwater level depths were analyzed for each well over the study time period, 1949 to 2009. The analyses in this paper use annual averages based on the calendar year. For every well record, the presence and statistical significance of groundwater elevation trends over the 61 year period were evaluated using the Kendall’s tau-b tests. We consider groundwater records with a p-value < 0.1 in support of the null hypothesis of no trend to be significant. There are 16,410 wells with significant (p<0.1) trends in groundwater level. For these wells, the slope of the time trend of groundwater level was calculated using the Theil-Sen method. This is a robust trend estimator that is not affected as much by a few outliers in the data. The average of the slopes for a given grid area are presented in the results.

We used a similar method for calculating the 5-year slopes with data centered at 1950, 1955, ..., 2005. This produced twelve 5-yr slope values per well with a 61 year record, and several 5-year slope values for shorter total records. The trend anomaly of each 5-year slope is presented as a ratio with the long term trend \( \frac{s_{5\text{year}}}{s_{\text{long-term}}} \), so that the short term departure that may be due to climate or other factors can be easily identified. For example, a well that shows an overall declining 61-year trend may experience a groundwater level increase at some point, resulting in a positive 5-year trend anomaly for this period. The 5-year analysis period data were also compared to the 5-yr water use data, described in the next section.
To identify groups of wells whose behavior in time is similar, we performed a k-means cluster analysis on a subset of the wells that have continuous records for annual average depth to groundwater. The groundwater depth time series values were first normalized by dividing by average depth over the period of record. This analysis was done for wells with continuous records between 1965 and 2005, a shorter period than the full study time period. This allowed us to analyze a larger number of locations.

3.2. Correlation between Groundwater Elevation and Groundwater Extraction
County-level water use data are available every 5 years from 1985 to 2005, and they include both groundwater and surface water consumption. The trend of groundwater extraction was calculated for each county over the period of record, 1985 to 2005, using the Thell-Sen slope. Positive slopes indicate increases in groundwater extraction, while negative slopes indicate declines. The anomaly at each year of record (1985, 1990, ..., 2005) was calculated to illustrate the variation from the long-term trend of extraction over time.

3.3. Correlation between Groundwater Elevation and Climate
Preliminary studies showed that groundwater level and annual precipitation were weakly correlated for medium and deep wells (>100 ft, 30 m). For shallower wells, rainfall and groundwater levels appeared to correlate at diurnal to seasonal time scales. In this report, we focus on the deeper wells and the longer time scales.

The time variations in groundwater level records and annual precipitation in the county where the well is located were compared using a cross wavelet analysis (Torrence and Compo 1998; Grinsted, Moore, and Jevrejeva 2004). A Morlet wavelet was used for the wavelet analysis. Wells with continuous annual average records between 1949 and 2009 were selected for analysis. The cross wavelet analysis allows one to identify periods where there may be synchronous variations in two time series that correspond to a “signal” in one of them. Here, a signal is something that recurs with a certain period. For example, precipitation might have a signal corresponding to climate patterns including ENSO, PDO and NAO.

Five-year mean values of the NAO and PDO indices were compared to groundwater elevation trends using the 5-yr periods described in Section 3.1. Annual precipitation at the county level was combined with county area to calculate the area-averaged precipitation over the US. The corresponding precipitation anomaly throughout the study time period is the difference of the 5-yr trend and the long-term trend (Figure 3). We also illustrate the relationship between climate patterns (NAO and PDO), precipitation anomaly, and groundwater trend anomaly.
Figure 3 Average climate and precipitation trends: (A) Annual average PDO index (B) Annual average NAO index, and (C) Area averaged precipitation anomaly over the continental US (annual anomaly in black, 5-yr running mean in red). Note the overall increasing trend that is punctuated by two continental scale droughts in the mid-1980s, and late-1990s.

4. Results and Discussion

A majority of deep wells in the USGS dataset with trends show declines in groundwater over the study period. The relationship between groundwater pumping, climate, and groundwater level is complicated and spatially variable. In this report, we illustrate some of the dominant patterns observed, and potential correlations between resource use and availability. On average, we see that dry periods in the climate record correlate with increased groundwater decline, while wet periods correlate with groundwater recovery. During a wet period, groundwater pumping may decrease while aquifer recharge increases, both reducing stress on groundwater resources. The opposite conditions and results occur during a dry period. This relationship is captured in the 5-year average groundwater trends, when compared to the average precipitation over the US (Figure 4). Further relationships between groundwater and climate are discussed in Section 4.3.

Though the median trend for every 5-yr period is negative, the distributions of slope anomalies are relatively large, spanning both negative and positive values (Figure 4B). Almost all regions of the country have both rising and declining groundwater levels in different wells. There are 11,483 wells with significant (p<0.1) long-term declining trends, and 4,839 wells with significant long-term rising trends. Wells with differing trends can be located close together in space because wells may be drilled within a large depth range (Figure 1A), potentially reaching different aquifers. These layered aquifers can have different head level trends based on their own physical parameters, extraction rates, and recharge rates.
4.1. Groundwater Level Trends

Long term groundwater level trends vary in magnitude and direction across the country during the study period, 1949 – 2009 (Figure 5). Large regions with generally contiguous groundwater declines include the central and southern Ogallala aquifer (western Nebraska, Kansas, Oklahoma, and northwest Texas), the lower Mississippi (Arkansas, Mississippi, and Louisiana), the Southwest and central western United States (Southern California, Nevada, Arizona, Utah, and southern Idaho), and the central Atlantic Coastal Plain (Maryland, eastern Virginia, North Carolina).
Figure 5 Average slope from wells with statistically significant trends at the 10% level observed between 1949 and 2009. Negative (red/orange) indicates decline in groundwater level, while positive (blue) indicates a rise in groundwater level. Wells with insignificant trends shown in gray.

Despite extensive negative trends across the US, groundwater trend anomalies vary significantly by region during the study period (Figure 6 and Figure 7). In the 1970s, central and coastal California experienced their highest rates of decline during the study period. Similarly at this time, the entire Ogallala Aquifer saw groundwater declines, as did much of the mid-west including North Dakota, South Dakota, Wisconsin, Iowa, and Michigan. The lower Mississippi and regions of the Atlantic Coastal Plain were also experiencing relatively high groundwater declines during this period.

The 1990s brought a period of groundwater recovery for several regions in the western US, including central California, southern Idaho, and northern Utah. Widespread groundwater rise was seen in North Dakota and the northern Ogallala (central Nebraska). There were still groundwater declines in the south, and southwestern US, though smaller than average in many areas. In the 2000s, trend anomalies indicate groundwater declines in the northern Midwest and northern Ogallala. Groundwater declines in the central and southern Ogallala, the lower Mississippi, the southeast, and the Atlantic Coastal Plain all generally had negative anomalies from the 1970s through 2000s, indicating continuous declines through this period.
Figure 6 Five-year trends anomalies (1965, 1975, 1995 and 2005) for wells with long-term declining records. The median trend anomaly is shown at each point. Positive (blue) indicates groundwater level increases, while negative (orange to red) indicates groundwater level declines.

Figure 7 Five-year trends anomalies (1965, 1975, 1995 and 2005) for wells with long-term rising records. The median trend anomaly is shown at each point. Positive (blue) indicates groundwater level increases, while negative (orange to red) indicates groundwater level declines.
The wells with long-term rising trends (Figure 7) provide additional information about groundwater behavior, and combined with Figure 6 illustrate the spatial proximity of declining and rising wells across the country. The anomalies are often the same sign for both long-term declining and rising wells, during the most extreme periods of decline or recovery. For example, the wells in the California Central Valley which experienced long-term rises, have short periods of groundwater level decline during the 1970s (Figure 7), the period of time when long-term declining wells experienced their largest declines (Figure 6). Wells with rising trends in the northern Ogallala experienced their greatest increases during the 1990s, when long-term declining wells also experienced increases. The rises observed in the 1990s in the northern Ogallala were followed by declines in the 2000s, again for both wells with long-term rising and declining trends. The results discussed here do not account for groundwater well depth and aquifer properties, which would provide further insight and possibly explain the observed spatial variability.

The similarity of trends between wells across the country can be illustrated using a cluster analysis. Continuous groundwater level records between 1965 and 2005 can be grouped into four clusters (Figure 8). Each cluster includes wells located across the US, with relatively low regional grouping, indicating groundwater levels are changing by similar amounts in many regions of the country (Figure 8B). The distribution of well depth varies across clusters (Figure 8C), suggesting there may be a relationship between aquifer depth and groundwater trend in multiple US aquifers.

Cluster 1 wells have a small rising trend in groundwater levels overall (Figure 8A) and include the shallowest wells ranging from 30 to 100m (Figure 8C). Overall, the clusters with declining trends (2 and 3) have the deepest wells and the largest spread in well depth. Of these the shallower wells have a smaller decline. Cluster 2 wells show large, consistent declines in groundwater level. Cluster 3 wells show declines as well, though approximately 1/3 smaller than in Cluster 2. Clusters 2 and 3 have the deepest well distributions. Deep aquifers and wells are often used for water supply, however they may take the longest to recharge. The middle and southern Ogallala contains wells with groundwater level declines seen in Clusters 2 and 3. Cluster 4 well depths vary around the mean, but do not have a consistent rising or declining trend. The depth distribution is similar to Cluster 3, though with fewer deep wells. The northern Ogallala contains wells in Clusters 1 and 4, indicating small to no change, and slight rising groundwater levels over time (Figure 8B). Other regions, including the Atlantic Coastal Plain, the lower Mississippi, and southern Idaho/eastern Utah, contain wells from all four clusters, suggesting that there is considerable heterogeneity in the wells in that region and local extraction patterns vary.
Figure 8 Cluster analysis of wells with continuous records from 1965 to 2005. (A) GW elevation normalized by the long-term mean for each of the four k-means clusters. Individual well records shown in color, mean shown in black. Negative slopes represent declining water levels. (B) Location of wells identified by color for each cluster. (C) Distribution of well depths for each cluster.

4.2. Relationship between Groundwater Elevation and Pumping
The median normalized groundwater extraction rates show a linear increase between 1985 and 2005, with the exception of a notably larger increase in pumping in 2000 (Figure 9A and 9B).

Figure 9 (A) Boxplot of groundwater use anomalies for all counties in each of the 5 years with water use data, (B) Detail of Figure 9A close to the mean.
We do not see a strong direct correlation between the area normalized groundwater extraction trend and the groundwater level trend for the period 1985 to 2005. A majority of counties do agree on the sign of the trends; groundwater extraction increases correspond with groundwater level declines. However, in some counties the relationship between pumping trend and groundwater trend is an inverse relationship. This indicates groundwater elevation changes cannot be explained exclusively by changes in pumping. To illustrate the spatial variability of the relationship between groundwater extraction trend and groundwater elevation trend, we overlay the two plots in map-view (Figure 10).

A majority of the counties with data have declining groundwater levels and increasing extraction rates, signified by yellow to red fill and horizontal hatching, respectively. These trends are most notable along the Mississippi River in Arkansas, Mississippi, and Louisiana, the southwest US, and the coasts of Virginia and North Carolina.

The groundwater extraction rate does not always correlate with groundwater level trends. Some counties with declining groundwater extraction still see declining groundwater levels. This combination of pumping and groundwater behavior is most notable in southern California, northern Nevada, southern Idaho, and some counties in northern Texas. Though the extraction rate is declining, its magnitude may still be high relative to the safe yield or sustainable extraction rate of the aquifer. Conversely, counties experiencing groundwater level rise in light of increasing groundwater extraction rates may be experiencing the opposite, where the relative magnitude of extraction rates are still within the sustainable limits, despite increases. This combination is most notable in the northern and southern regions of the Atlantic Coastal Plain. These counties should evaluate their groundwater balance and rates of use in more detail to determine where aquifer recharge or capture is coming from, and how long extraction can increase before it is no longer sustainable.
4.3. Correlation between Groundwater Elevation and Climate

There are two common relationships between deep groundwater wells and local precipitation. Some wells have constant trends with minimal to no correlation with either local precipitation or long term climate (e.g. Figure 11A,B,C). Other deep wells wavelet spectra show an increase in power between the 8 to 16 year period band (e.g. Figure 11D) which often corresponds to oscillations seen in the precipitation spectrum (Figure 11E,F). This supports the general conclusion...
that these deeper wells are either not affected by precipitation patterns, or respond to longer term climate cycles, rather than inter-annual rainfall variability.

Figure 11. Wavelet and cross wavelet power spectra results for two groundwater level records with the local county precipitation records. (A) A 87 m deep well in eastern LA County, (B) spectrum for precipitation for LA County suggesting some power in the 8 to 16 year band post 1970, (C) cross wavelet power spectrum for groundwater level and precipitation indicating some commonality in the 8 to 16 year band post 1985, but his cannot be assessed reliably due to edge effects, (D) A 70 m deep well in southern Idaho (Minidoka County), near the Snake River showing strong periodic oscillations between 8 and 16 years, (E) spectrum for precipitation with a 4 to 8 year signal in the 1970 to 1990 period and a 12 to 16 year band active over the period of record, (F) cross wavelet power spectrum for groundwater level and precipitation in Minidoka County, indicating agreement both frequency bands identified for precipitation.

Further assessment is required to determine whether the dominant relationships between groundwater and climate can be broadly categorized across the country based on aquifer properties, well depth, pumping, and other parameters.

We find that the 5-yr groundwater trend anomaly correlates positively with the average PDO and NAO indices (Figure 12). Positive PDO and NAO generally result in more precipitation in the western and eastern US, respectively. Average precipitation over each 5-yr record suggests that nationally wetter years (positive precipitation anomaly), generally associated with a positive PDO index, result in smaller magnitude groundwater declines (Figure 12). The relationship between average precipitation anomaly and NAO is not as clear as for PDO. We see a stronger correlation between PDO and groundwater elevation trends, where five out of six wet periods correspond to positive indices, and the smallest groundwater declines of the study period.
Figure 12 Five year average (A) PDO and (B) NAO Index values plotted against the 5-yr average groundwater elevation trend anomaly (the long-term average is removed). Point color indicates 5-yr precipitation anomaly averaged over US (red = lower precip, blue = higher precip).

The average NAO indices are negative for a majority of the study period, with positive values occurring in the 1980s and 1990s (Figure 3). The PDO cycling is similar, with negative indices on average until 1980, followed by positive indices for the remainder of the study time period except for the early 2000s. The positive PDO indices in the 1980s and 1990s may explain the groundwater elevation recovery through much of the west during wet conditions (Figure 6). Wet conditions appear to correspond to smaller groundwater declines, possibly due to increased groundwater recharge and/or less pumping for irrigation and other uses. Similarly, positive NAO indices and wet conditions in the eastern US may contribute to the groundwater elevation recovery along the northern Atlantic Coast, and the increase in rate of groundwater rise for wells with long-term rising trends (Figure 7).

5. Comparison to other US Groundwater Studies
We compare our results to previous studies of groundwater depletion and groundwater stress. Groundwater depletion calculated by Wada et al. (2010) for the year 2000 only exceeds 2mm (0.08 in) west of 95°W, including the Ogallala, select regions in the southwest and northwest, and California (Figure 13A). Our study of the historic groundwater levels suggests groundwater depletion covers a much greater area of the United States, including much of the southeastern US and Atlantic Coastal Plains (Figure 13B). Further analysis of aquifer storativity and thickness is needed to determine whether depletion volume in these regions is comparable to the areas in the western US.
**Figure 13.** (A) Groundwater depletion for the year 2000 in mm/yr (modified from Wada et al. (2010)), (B) Average groundwater elevation declines between 1998 and 2002. N.b. groundwater depletion rate is not directly comparable to changes in groundwater level trend without accounting for aquifer storativity, which is not included in this study.

Groundwater depletion estimates were assembled by Konikow (2013), using a synthesis of studies for the major US aquifers spanning between 1900 and 2008 (**Figure 14**). The results highlight the depletion in the California central valley and the Ogallala aquifers. This paper also captures the depletion along the lower Mississippi, though suggests relatively minimal depletion along the Atlantic Coastal Plain. The results contrast the present study primarily in the amount of recharge occurring in the Northwest volcanic systems (Columbia River, WA and Snake River, ID), and possibly the Atlantic Coastal Plain. The latter areas show high groundwater trend variability in both space and time, with no clear indication of significant recharge (**Figure 6, Figure 13B**). The storativity of the aquifers is needed to quantitatively compare the rates of groundwater level change to the depletion volumes.

**Figure 14.** Groundwater depletion estimates, modified from Konikow (2013).
6. Summary

Analysis of historical groundwater level records indicates groundwater levels declined between 1949 and 2009 throughout much of the continental US. Most notably, groundwater level declines in the southern and eastern US are comparable to declines in areas of the often-discussed water stressed areas of the Ogallala and southwest US. Results suggest significant downward trends in water storage over several decades, however rates of groundwater depletion cannot be inferred from groundwater level changes without knowing aquifer storativity. As the groundwater levels decline, impacts may include increased energy consumption for pumping, requirement of deeper wells, and irreversible consequences including permanent aquifer compaction and land subsidence.

The causes of groundwater level change are multifaceted, varying in time and space across the US. In this study we found correlations between pumping rate and groundwater level in a majority of counties. However, there are a number of counties with contrasting changes in pumping rates and groundwater trends, for example increasing pumping rates and rising groundwater levels in the northern and southern extents of the Atlantic Coastal Plain. Climate is a clear controlling factor on groundwater levels. Due to the focus on deep wells, we found minimal correlation with inter-annual precipitation patterns, though on average the wells correlated with the long-term climate patterns, and specifically with the PDO.

Groundwater is increasingly relied on as a critical resource for meeting everyday water demands and buffering variability in surface water supply. Overall, the US is extracting groundwater at unsustainable rates, causing long-term groundwater level declines and loss of groundwater storage. The dynamics of extraction, recharge, and changes in the surface water – groundwater balance are complicated and variable across the US. A national scale study or model of these dynamics would be time and computationally prohibitive. Results from this study can be used to identify regions within the US where a more detailed aquifer or sub-aquifer scale analysis is required to improve groundwater management.

References


Assessment of trends in groundwater levels across the United States


Critical Aquifer Recharge Areas

Guidance Document

Prepared by:

Laurie Morgan
Washington State Department of Ecology
Water Quality Program

January 2005
Publication Number 05-10-028

Printed on Recycled Paper
For additional copies of this document contact:

Department of Ecology
Publications Distribution Center
P.O. Box 47600
Olympia, WA 98504-7600

Telephone: (360) 407-7472

Headquarters (Lacey) 360-407-6000
If you are speech or hearing impaired, call 711 or 1-800-833-6388 for TTY

If you need this information in an alternate format, please contact us at 360-407-6404. If you are a person with a speech or hearing impairment, call 711 for relay service or 800-833-6388 for TTY.
# Table of Contents

List of Figures and Tables............................................................................................................. ii

Acknowledgements....................................................................................................................... iii

Section 1 Introduction...................................................................................................................... 1

The Growth Management Act and Critical Areas ........................................................................ 2

Critical Aquifer Recharge Areas................................................................................................... 2

Section 2 Basic Concepts................................................................................................................. 5

Section 3 Protecting the Functions and Values of Critical Aquifer Recharge Areas ............ 9

Step 1: Identify where groundwater resources are located..................................................... 10

Step 2: Analyze the susceptibility of the natural setting where ground water occurs.............. 20

Step 3: Inventory existing potential sources of groundwater contamination.................... 21

Step 4: Classify the relative vulnerability of ground water to contamination events ........... 22

Step 5: Designate areas that are most at risk to contamination events................................. 23

Step 6: Protect by minimizing activities and conditions that pose contamination risks.......... 23

Step 7: Ensure that contamination prevention plans and best management practices are followed......................................................... 24

Step 8: Manage groundwater withdrawals and recharge....................................................... 24

Section 4 Best Available Science ................................................................................................. 25

Section 5 Working with State and Federal Laws and Rules...................................................... 29

Section 6 Adapting to Local Conditions and Settings............................................................... 33

Section 7 Adaptive Management – Change Happens................................................................. 34

Section 8 References.................................................................................................................... 35

Appendix A U.S. EPA Potential Sources of Drinking Water Contamination Index .................. 37

Appendix B Where to Get More Information............................................................................ 43

Appendix C Selected GMA Hearings Board Decisions.............................................................. 51

Appendix D Example Costs and Consequences of Ground Water Contamination .......... 55

Appendix E Example County Fact Sheets for Pollution Prevention........................................ 57
List of Figures and Tables

Figure 1: The Hydrologic Cycle ................................................................. 5
Figure 2: The water table lowers when discharge is greater than recharge .......... 6
Figure 3: Location, extent, and uses of a drinking water supply aquifer .......... 11
Figure 4: Township, range, section, quarter-quarter, and well location ........ 13
Figure 5: Representation of an aquifer system (Jones, 1999) ......................... 14
Figure 6: Well logs include observations about aquifers and overlying earth materials 15
Figure 7: This topographic map shows hilly bedrock next to a flatter river valley .... 16
Figure 8: Hydrogeologic map of the Chimacum Basin (Simonds, 2004) ........ 18
Figure 9: Hydrogeologic cross-sections of the Chimacum Basin (Simonds, 2004) 19
Figure 10: Contaminant path, vadose zone, and aquifer schematic drawing .... 20
Table 1: Laws, rules, and guidance for groundwater protection ..................... 31
Acknowledgements

Many people have commented on this document and have provided helpful suggestions. Others have been involved with Critical Aquifer Recharge Areas and this document has benefited greatly from their knowledge and perspective. I would like to thank the following people for their help and guidance: Stephen Swope, Pacific Ground Water Group; John Sonnen, Thurston County; Ken Johnson, King County; Thomas Barry, city of Redmond; Richard Hoiland, city of Vancouver; Jalyn Cummings, Snohomish County; Doug Kelly, Island County; Catherine Weisman, Evergreen Rural Water of Washington; Susan Braley, John Stormon, Doug Wood, Bari Schreiner, Gerry Kunkel, Diane Dent, and Ann Kahler, Department of Ecology; Doug Peters and Chris Parsons, Department of Community, Trade and Economic Development; David Jennings, Department of Health; Jennifer Parker, U.S. Environmental Protection Agency; Kirk Cook, Department of Agriculture; and the Interagency Ground Water Committee.

I would also like to express deep appreciation for all of the counties and cities that are working on their Critical Aquifer Recharge Area ordinances and planning. I would particularly like to mention eastern Washington jurisdictions working on groundwater protection from which I have gained knowledge and perspective including Ferry County, Spokane County, the Columbia Basin Ground Water Management Area, and others.
Section 1
Introduction

This guidance document helps local jurisdictions and the public understand what is required for the protection of local groundwater resources under the Growth Management Act. It includes guidance for planning, ordinances, and for including the Best Available Science (BAS) as these relate to Critical Aquifer Recharge Areas.

This guidance will also explain how the laws and rules of the state of Washington for water quality, pollution prevention, and water resources relate to Critical Aquifer Recharge Area protection.

We are revising the guidance to improve usability and clarity, and to provide additional explanation.

When the public drinking water supply is compromised, the community faces risk and great expense. Contaminated water can cause illness and ingestion of toxic chemicals or other harmful substances. Remediation of contaminated ground water is overwhelmingly expensive. A contamination event can cause city wells to be shut down, result in expenses for new wells, and incur costs for cleaning up contaminated soil and ground water.

Prevention of groundwater contamination is far less expensive than cleanup. EPA studies have shown that investing funds for groundwater protection is cost-effective compared to groundwater cleanup at a ratio that runs anywhere from 1:5 to 1:200 (U.S. EPA, 1995).

The Growth Management Act requires protection of public groundwater drinking supplies so that tragic contamination events and their associated costs can be prevented. In addition, public drinking water supply depends on groundwater availability. Without replenishment, the amount of water in aquifers can be diminished or even depleted.

A good groundwater protection program involves:

- Identifying groundwater resources at risk,
- Identifying threats to ground water, and
- Monitoring to make sure a condition that could cause an unacceptable risk is not occurring and taking action when necessary.
The Growth Management Act and Critical Areas

The Growth Management Act (Chapter 36.70A Revised Code of Washington) (GMA) requires comprehensive land use planning by counties and cities. The act, commonly known as the GMA, specifies 13 overall planning goals. These goals include urban growth, transportation, economic development, natural resource industries, public facilities, open space and recreation, historic preservation, environmental planning, and others.

The environmental planning goal is to “protect the environment and enhance the state’s high quality of life, including air and water quality, and the availability of water” (RCW 36.70A.020).

The GMA requires the designation and protection of “Critical Areas” to prevent harm to the community from natural hazards and to protect natural resources.

- **Natural hazards** are frequently flooded areas and geologically hazardous areas.
- **Natural resources** are wetlands, fish and wildlife habitat conservation areas, and “areas with a critical recharging effect on aquifers used for potable water,” which are called Critical Aquifer Recharge Areas.

The goal of establishing Critical Aquifer Recharge Areas is to protect the **functions and values** of a community’s drinking water by preventing pollution and maintaining supply.

**Critical Aquifer Recharge Areas**

A Critical Aquifer Recharge Area (CARA) is defined by the GMA as “areas with a critical recharging effect on aquifers used for potable water.”

The Washington Administrative Code (WAC) Chapter 365-190 uses the following definition:

“**Areas with a critical recharging effect on aquifers used for potable water are areas where an aquifer that is a source of drinking water is vulnerable to contamination that would affect the potability of the water.”**

Identifying “areas with a critical recharging effect on aquifers used for potable water,” depends on understanding aquifer recharge and what is meant by “a critical recharging effect.”

**Aquifer recharge** occurs where rainfall, snowmelt, infiltration from lakes, wetlands and streams, or irrigation water infiltrates into the ground and adds to the water underground that can supply a well. On the other hand, **discharge areas** are where ground water is headed toward the ground surface and ultimately flows out from a spring, wetland, stream, lake, estuary, or ocean shore. Wells can also serve as discharge areas, especially larger volume wells, such as those used by municipalities.
Most of a watershed is typically a recharge area, with discharge areas occurring to a more limited extent in topographically lower areas. Recharge areas and discharge areas can be mapped using hydrogeologic techniques to determine where ground water is and where it is flowing.

**Aquifers used for potable water** are identified by looking at existing and future planned uses. Existing wells and their protection areas, sole source aquifers, and aquifers otherwise identified as important supplies, are examples of “aquifers used for potable water.”

**Setting priorities for** the most critical supplies helps jurisdictions make decisions about where to focus their efforts. Areas may be categorized to reflect these priorities. An example would be to apply more strict regulations and monitoring within the one-year time of travel of a city well, as opposed to more sparsely developed areas of the county. More strict regulation may be applied in an area where the aquifer is shallow and vulnerable to contamination more than an aquifer that is deep and protected.

**Ground Water and Other Critical Areas**

Ground water is inextricably linked with all of the critical areas including wetlands, fish and wildlife habitat, critical aquifer recharge areas, frequently flooded areas, and geologically hazardous areas.

- Ground water is a source of water to streams, lakes, estuaries, wetlands, and springs; and therefore serves a critical function for wildlife and fish habitat. Some plants that provide habitat, like willows, depend on shallow ground water.

- Ground water is often a key factor in flooding and geologic hazards.

The GMA also requires that local jurisdictions give special consideration to conservation or protection measures necessary to preserve or enhance anadromous fisheries. Since ground water is an important component of stream flow, it is necessary to maintain the groundwater supply to streams where needed to protect salmon and other anadromous species.

**Qualified Professional Assistance**

Professional hydrogeologic work for the establishment of Critical Aquifer Recharge Areas should be performed by a hydrogeologist licensed in the state of Washington (RCW 18.220 and WAC 308-15). In particular, the delineation and characterization of aquifers and the analysis of environmental fate and transport of potential contaminants through the ground should be performed by a qualified licensed professional.
Many activities associated with Critical Aquifer Recharge Areas may be done by others (who are not licensed professional hydrogeologists) such as planning, pollution prevention, education and outreach, ordinance enforcement, and other activities associated with city and county programs.
Section 2
Basic Concepts

This section lists basic concepts that help with understanding the occurrence and movement of ground water.

Where Ground Water Comes From

Recharge is water that is added to ground water, whether it is from rainfall that infiltrates through the ground, snowmelt, or some other source. Recharge can come from quite a distance through the ground over a long period, or it can come from relatively local and more recent sources.

![Figure 1: The Hydrologic Cycle](image)

The Hydrologic Cycle

The hydrologic cycle is how water evaporates from the oceans, gathers in clouds, and rains or snows onto the land. After it rains or the snow melts, the water then either evaporates, is used up by plants, runs off to streams, lakes, or the ocean; or infiltrates into the soil. Some of this infiltration will reach the underground water table and will recharge the aquifer.

Recharge can also carry contaminants into ground water from the land surface. Therefore, recharge is at the center of preventing pollution and maintaining supply both for drinking water and for freshwater habitats.
Where Ground Water Goes

Ground water flows through the ground from where it is recharged to where it is discharged. **Discharge** is where water moves from underground to the land surface. Springs are a familiar example. Ground water also discharges to lakes and streams. In fact, in Washington, ground water can make up a majority of stream flow, especially in late summer and early fall (Pitz, Sinclair, 1999). This is why groundwater discharge is such an important aspect of maintaining or restoring freshwater habitat.

The Water Table

The water table occurs where the underground is saturated with water. Discharge of ground water, whether by pumping or by seeping into streams and springs, can lower the water table if the recharge does not keep up. The effect can be to pull the water down below a well or to dry up a stream. Sometimes the water table rises above the land surface. This can fill lakes and streams, or even cause flooding when the water has nowhere to drain.

People can cause the water table level to lower both by removing ground water from wells and by reducing the quantity of recharge, as happens where there is too much paved or impervious surface and ground water cannot infiltrate where it formerly did. Figure 2 shows the effects of a declining water table (modified from USGS illustration at http://ga.water.usgs.gov/edu/earthgwaquifer.html).

The Importance of Recharge...

![Diagram](image)

Figure 2: If the groundwater table drops to the lower dashed line, both the stream and the well go dry. The water table lowers when discharge (water out) is greater than recharge (water in).

Hydrogeologists can map recharge and discharge zones by measuring many water levels.

The **hydrogeologic setting** is the framework that controls groundwater occurrence and movement. Where ground water flows, the rate at which it flows, where it recharges or discharges, and how deep it occurs are all functions of what the land is like – the soil,
sediments, and rocks that ground water moves through. The hydrogeologic setting also includes the topography and the weather patterns that control recharge.

Knowledge of hydrogeologic settings is useful for establishing critical aquifer recharge areas. Prioritization of critical aquifer recharge areas can be based on the susceptibility of those settings to contamination or water quantity impacts.

Susceptibility refers to what the ground is like. When water can move readily through the ground, it can carry contaminants to ground water more quickly. Sandy, shallow aquifers are more susceptible than deep aquifers that are overlain by clay.

Vulnerability refers to the risk of contamination from chemical use combined with the risk from the susceptibility of aquifers.

Note: Sometimes, the terms vulnerability and susceptibility are used interchangeably, so you should be sure of the author's meaning when you encounter these terms.

Susceptibility factors:

- **The vadose zone** consists of the unsaturated earth materials above an aquifer. Depth to water is the distance through the vadose zone a contaminant would travel to reach the water table. The deeper the water table, the longer the travel time.

- **Permeability** is a scientific measurement of the rate of infiltration in inches of water per hour. Infiltration rate is a measure of how fast water and pollutants can move downwards through the earth materials of the vadose zone. The more permeable the ground is, the faster water moves down through it, the more the underlying ground water is susceptible to contamination. Coarse sands and gravels allow water to pass through much more quickly than fine silts and clays.

- **Chemical retardation** is a measurement of how clays and organic matter react with some chemicals to slow their passage or change them chemically.

- **Adsorption** is a measurement of the tendency of ions dissolved in water to stick to particles of silt or clay. The particle size and the amount of organic matter affect the adsorption. A sand with no organic matter may not adsorb at all, while an organic silt or clay may adsorb well. In short, a contaminant can be captured or slowed down by sticking to clay.

- **Low permeability layers**, such as clay or glacial till, may occur between the land surface and an aquifer, either within the vadose zone or within an aquifer system. These layers would restrict downward migration of contaminants and would provide a measure of protection to the aquifer.

Note: **Care should be taken with presuming a confining layer is protective, because layers may not be laterally extensive and may have some feature that allows leakage.**
• **Hydraulic conductivity** is a measure of how fast a quantity of water can move through an aquifer (for a given gradient through a unit area). The higher the hydraulic conductivity, the faster the flow.

• **Gradient** is the result of differences in elevation between two locations of the water table or the differences in pressure between locations in a confined aquifer. The higher the gradient, the faster the flow.

  *Just as a ball rolls downhill, water flows downhill – from higher water table elevations to lower water table elevations. Water also flows in the direction that pressure is moving it. Just as you can push a ball uphill, high-pressure conditions can push water upwards. Both pressure differences and elevation differences create gradients.*

• **Groundwater flow direction** is determined by gradients, which in turn are influenced by pumping, discharge to surface water, topography, and geologic setting.

• **Groundwater flow rate** depends on the nature of the geologic materials water flows through along with the pressure on the water. Coarser materials allow faster flow, and higher pressures induce faster flow.

**Preventing pollution** depends on controlling land use activities to prevent contaminant spills and leaks. Critical aquifer recharge areas are designated so that greater control can occur where land use activities are a high-risk for polluting sensitive aquifers.

**Prioritization** of Critical Aquifer Recharge Areas can be accomplished by identifying where high-value water resources are located in highly susceptible areas (King County, 2004).

**Critical aquifer recharge area maps** are delineations of where a community’s groundwater supply meets criteria such as susceptibility, potential for contamination, and priority.

**Wellhead protection zones** are areas around wells where contamination would result in polluting the water supply well within a specific time period. Time periods used by the Department of Health Drinking Water Program are six months, one year, five years, and ten years.

**Aquifers** are created when water saturates, or fills, the underground where the ground is permeable enough to yield useable quantities of water to a well. Layers that are not permeable enough to yield useable quantities of water to a well are called *aquitards.* Common types of aquifers are sand and gravel, fractured bedrock, and *karst* (limestone).

In the Puget Sound region, the landscape that defines aquifers is made up mainly of glacial deposits. In eastern Washington, there are several types of geologic settings that contain aquifers. One major type of aquifer in eastern Washington is the Columbia Flood
basalts. In the Columbia Basin, irrigation has created aquifers by filling the sands and gravels over the Columbia Flood basalts.

There may be a whole system of multiple confined aquifers and a water-table aquifer in an area. Sometimes the water table aquifer and confined aquifers beneath are connected and water from one aquifer flows into another.

A confined aquifer is an aquifer that lies beneath a confining layer, such as a silt or clay layer. This condition can cause the water to be under pressure, resulting in an artesian well. Sometimes this pressure is great enough to cause the well to flow out at the surface. Ground water in confined aquifers flows from the direction of the highest pressure to the lowest pressure.

A water-table aquifer is water under normal atmospheric pressure. This aquifer is not capped by a layer of clay or fine silt. Water-table aquifers flow generally in accordance with the topography towards rivers, streams, lakes, and springs.

Safe yield (Fetter, 1980) is the amount of naturally occurring ground water that can be economically and legally withdrawn from an aquifer on a sustained basis without impairing the native groundwater quality or creating an undesirable effect such as environmental damage. It cannot exceed the increase in recharge or leakage from adjacent strata plus the reduction in discharge caused by pumping.

Section 3
Protecting the Functions and Values of Critical Aquifer Recharge Areas

The functions and values of Critical Aquifer Recharge Areas are to provide the public with clean, safe, and available drinking water. In order to accomplish this goal, information is needed about the location and extent of aquifers that supply public drinking water, the susceptibility of these supplies to contamination, and potential contamination risks. In addition, planning, programs, and ordinances are needed to prevent contamination from occurring.

The following steps characterize where groundwater resources are important to the community and how to protect them.

- Identify where groundwater resources are located.
- Analyze the susceptibility of the natural setting where ground water occurs.
- Inventory existing potential sources of groundwater contamination.
- Classify the relative vulnerability of ground water to contamination events.
- Designate areas that are most at risk to contamination events.
- Protect by minimizing activities and conditions that pose contamination risks.
• **Ensure** that contamination prevention plans and best management practices are followed.

• **Manage** groundwater withdrawals and recharge impacts to:
  - **Maintain availability** for drinking water sources.
  - **Maintain stream base flow** from ground water to support in-stream flows, especially for salmon-bearing streams.

The following section provides more details about each one of these steps

**Step 1: Identify where groundwater resources are located**

The GMA discusses the use of both mapping and performance standards to identify critical areas.

**Maps** are highly useful for Critical Aquifer Recharge Areas because they can show the location of public water supply wells, private wells, and aquifer boundaries. They can also be used to show the location of areas that have been rated for susceptibility. Maps can be used to see where pollution prevention is most needed and to help plan development. Known Critical Aquifer Recharge Areas should be mapped.

**Performance standards** are the criteria for designation of a critical area. A performance standard is applied when reviewing development projects to determine what category of Critical Aquifer Recharge Area the proposal is in and what the applicable site conditions are. Policies, planning, ordinances, and programs are applied based on the outcome of the evaluation of the proposal using performance standards.

The use of performance standards is recommended for ... circumstances where critical areas cannot be specifically identified [WAC 365-190-040(1)]. The purpose of a performance standard is to have an objective standard for comparison (WWGMHB, 1997).

To use performance standards, local jurisdictions need sufficient information to:

• Make an informed determination as to whether or not critical areas are present on the site.

• Determine whether or not the proposed activity will impact those critical areas.

The **Critical Areas Handbook** (Washington Dept. of Community Trade and Economic Development, 2003) states that:

"**Critical areas may be designated by adopting specific performance standards, delineating specific geographic areas, or both. Generally, performance standards are preferred, as any attempt to comprehensively map wetlands, for example, throughout a jurisdiction would likely be too inexact for regulatory purposes. Even so, mapping critical areas for information purposes is advisable. All areas meeting the definition of one or more critical area type, regardless of any formal identification, are required to be designated critical areas.**"
Identifying the Location and Extent of Drinking Water Supply Aquifers

Mapping drinking water supply aquifers makes use of well location and well log information as well as the location and characteristics of aquifers.

Well locations are important to identify to help prioritize risk and guide local ordinances and planning near active public wells.

Aquifer locations are important to identify to give the jurisdiction information about where groundwater resources are. When new wells are needed, knowledge of where aquifers may supply water is critical. This knowledge is used in water system planning and is a vital consideration for long-term planning.

The following illustration shows:

- Public water supply wells (Group A) and their protection zones
- Smaller public water supply wells (Group B)
- Wells that serve one or two households
- The location and the extent of a local aquifer

![Diagram showing location, extent, and uses of a drinking water supply aquifer.](image)

Figure 3: Location, extent, and uses of a drinking water supply aquifer.

*Note: The private wells on this map appear at the nearest quarter-quarter section, NOT where they are actually located on the ground. This is because well logs report locations...*
this way, and that is what we have to use for mapping. Private wells are included in this illustration to give an idea of the use of the aquifer.

Public Water Supply Wells

Public drinking water supply systems are regulated by the Department of Health under the Safe Drinking Water Act (SDWA). The state regulates systems with 15 or more connections, and the local health jurisdiction regulates systems with 3 to 14 connections.

The SDWA also includes the Source Water Protection Program. Under this program, wellhead protection zones are defined and the susceptibility of the well to contamination is rated. Potential contamination sources within the protection zones are also inventoried.

The wellhead protection zones are defined by the areas where a spill incident could result in contamination of the well within a specified time period. The time periods are six months, one year, five years, and ten years. Zones based on these time periods are known as time-of-travel zones. Methods of delineating wellhead protection zones vary from modeling to drawing a circle around the well at a fixed radius (least accurate method). These mapped wellhead protection zones may be designated as a category of Critical Aquifer Recharge Area. A jurisdiction may have stricter requirements closer to the well. For example, some uses may be prohibited within the one-year time-of-travel zone that is allowed with mitigation in the ten-year time-of-travel zone.

Maps of public water supply wells and their protection zones are available on the internet.


Note: It should be kept in mind that any information system may have missing or inaccurate information.

Domestic Wells

Residences that are located too far from a public water supply system must rely on individual wells, springs, or surface water. Individual domestic wells are an important and widespread source of drinking water supply in Washington.

Maps of domestic well locations together with well logs help with identifying the location, extent and use of drinking water supply aquifers.

To find information about domestic wells, contact the Department of Ecology Water Resources Program.
State law requires that a well log be filed with the Department of Ecology when a well is constructed. Well log information includes location by address and township/range/section/quarter-quarter.

The Department of Ecology map, which is derived from well logs, locates wells in the center of the Township, Section, Range, Quarter-quarter square. The actual well location may be anywhere within the 40-acre square.


Figure 4: Township, range, section, quarter-quarter, and well location

Well logs are available at the Department of Ecology Regional Offices in Lacey, Yakima, Spokane, and Bellevue. Many counties also maintain copies. The Department of Ecology Well Log Viewer internet site has downloadable well logs, well records, and maps of well locations at [http://apps.ecy.wa.gov/welllog/](http://apps.ecy.wa.gov/welllog/).

The well locations were created by placing a point at the center of the township/range/section/quarter/quarter-quarter square. The following should be kept in mind when using these well locations.
• There are many wells for which well logs have not been submitted, and therefore do not appear on this map.

• Sometimes the location information written on the well log is incorrect, and so the location shown for the well on the map is inaccurate.

  *It is up to the well driller to provide accurate information on the well log. The well owner should make sure the location information is correct.*

• The point that represents the well is placed in the center of the township, range, section, quarter-quarter square. The actual location of the well is anywhere within the 40-acre square.

  For example, if the center of the square is in a lake, and the actual well location is on shore, the map will plot the well in the lake. The well IS NOT in the lake. There are thousands of well logs, and the locations have not been adjusted individually.

**Well Identification Using Parcel Maps**

Another way of identifying private wells is to look at a map of parcels with existing residences that are outside of public water supply service areas. This will give an indication of areas within the jurisdiction that rely on private wells.

**Aquifers**

This section looks at what tools are used to identify and characterize aquifers. Well logs, maps, testing, and field reconnaissance are some of the tools used to identify aquifers.

![Diagram of an aquifer system](image_url)

*Figure 5: Representation of an aquifer system (Jones, 1999)*
WATER WELL REPORT

STATE OF WASHINGTON

OWNER: CITY OF LACEY
Address: P.O. Box 3400, 420 College St., Lacey, WA 98509-3400

LOCATION OF WELL: Thurston

STREET ADDRESS OF WELL: MADRONA PARK SUBDIVISION

PROPOSED USE: Domestic ☐ Irrigation ☐ Industrial ☐ Municipal ☐ Driveway ☐ Test Well ☐ Other ☐

TYPE OF WORK: Owner's number of well (if more than one)
Abandoned ☐ New well XX Method of Construction: Dug ☐ Bored ☐ Reinforced ☐ None ☐

DIMENSIONS: Diameter of well 16 inches. Depth of completed well 333 ft.

CONSTRUCTION DETAILS:
Casing installed: 16' slim. from 12' to 265'.

Perforations: Yes ☐ No ☐
Type of perforator used
Size of perforations

Screens: Yes ☐ No ☐
Manufacturer's Name: Westco
Type: Stainless steel
Model No.:
Inside 14' Drug 150/120
From 265' to 382'
Drug 14' From 265' to 308'

Gravel packing: Yes ☐ No ☐
Size of gravel

Perforations from

Surfaces sealed: Yes ☐ No ☐
To what depth?

Material used in seal

Depth of screen

PUMP:
Manufacturer's Name

WATER LEVELS:
State level 249.53 if below top of well Date 5/4/97
Artesian pressure
Artesian water level

WELL TESTS:
Drawdown in amount water level after lower static level
Was a pump test made? Yes ☐ No ☐ If yes, by whom HOKKAIDO
Yield 305 gal/min. with 2.01 ft. drawdown after 1 hr.

Recovery data taken when pump turned off (water level measured from 10' top to water level)

Date of test 6/4/97

Initial level

Water Level

Temperature of water

Was a chemical analysis made? Yes ☐ No ☐

WELL CONSTRUCTION CERTIFICATION:
I, the undersigned, declare that the foregoing is true and correct and that the well was constructed in accordance with the provisions of the Code of Washington as amended.

NAME: HOKKAIDO DRILLING & DEVELOPING CORP.
Address: P.O. BOX 100, GILBERT, WA 98338-0100
License No.: 1146

Contractor's Registration No.: HOKKADO17803 Date: JUNE 24, 1997
(USE ADDITIONAL SHEETS IF NECESSARY)

EcoDry is an Equal Opportunity and Affirmative Action employer. For special accommodation needs, contact the Water Resources Program at (206) 407-8800. The TDD number is (206) 407-8900.

Figure 6: Well logs include observations about aquifers and the earth materials that overlie the aquifers.
Well logs contain information about aquifers:

- Location of the well
- The kinds and depths of underground materials (sand, gravel, silt, clay, bedrock, etc.)
- Water level at the time of drilling
- Where the aquifers are and how far they extend. Many well logs are needed for this analysis.
- An estimate of the amount of water that can be pumped from a well.

Maps are used to help define the boundaries of aquifers.

Figure 7: This topographic map shows hilly bedrock next to a flatter river valley. The boundary of the aquifer is likely to be where the hills slope up from the valley.

Topographic maps show landscape changes that are often associated with aquifer boundaries. (For example, the boundary for a river valley aquifer may be where the bedrock slopes up from the valley floor.)

Surficial geology maps show where geologic materials are located that are likely to contain aquifers, such as alluvial deposits.
Testing methods help hydrogeologists to identify and characterize aquifers. For example, aquifer tests involve pumping water out of a well at a known rate and measuring the effect in other wells over time. These tests show how much water can be pumped from a well, how far away other wells can be affected. They may also show to what extent water from one aquifer may leak into another.

Geophysical methods are used to determine characteristics such as the nature and geometry of geologic materials, the extent of aquifers, depth to water, and water quality.

Modeling takes all of the available information and observations that a hydrogeologist has and uses the computer to account for known conditions. It allows a hydrogeologist to model different (what-if) scenarios and to find out what may happen when various choices are made. Example questions that modeling can address are:

- What would the effect of pumping from a well field be on stream flow?
- If a spill occurred here, how long would it be before the contaminants reach the well?
- How would a drought affect water table levels and stream flows?

Hydrogeology studies look at all the available resources to map and describe aquifers. Consultants, the state, academic studies, the U.S. Geological Survey (USGS) and other agencies are sources of this type of information. These studies can be used to support the identification and characterization of Critical Aquifer Recharge Areas. Figures 8 and 9 show a hydrogeologic map and cross-section from a USGS study in Jefferson County (Simonds, 2004).
Figure 8: Hydrogeologic map of the Chimacum Basin (Simonds, 2004)
Figure 9: Hydrogeologic cross-sections of the Chimacum Basin (Simonds, 2004)

Critical Aquifer Recharge Area Guidance
Step 2: Analyze the susceptibility of the natural setting where groundwater occurs.

How do contaminants get to a well? Contaminants be spilled onto the ground or may leak from an underground tank and travel downward to the aquifer. After reaching the aquifer, contaminants may be carried along with the ground water flow to a well.

How fast and how far a contaminant travels depends on both the natural setting and the chemical and physical characteristics of the contaminant. There are many different chemicals with varied characteristics and this makes assessing all of the possible environmental fate scenarios difficult.

It is much more feasible to start with characterizing susceptibility. This involves determining the physical characteristics of the ground above the aquifer, called the vadose zone, and the characteristics of the aquifer. With susceptibility assessed, the nature of the contaminants can be taken into account by limiting or prohibiting the use of chemicals that are high-risk contaminants within high priority susceptible areas.

Susceptibility of the ground (vadose zone)

Along with the characteristics of the contaminant, the characteristics of the vadose zone determines how easily a spill of a contaminant could get down to the water table. Characteristics important for susceptibility assessment typically may include depth to water, infiltration rate, permeability, chemical retardation factors, adsorption, and the presence or absence of a impermeable layer.
Susceptibility of the aquifer

The characteristics of the aquifer control how fast a contaminant reaches a well once it has entered the aquifer. Factors typically considered in assessing the susceptibility of aquifers include hydraulic conductivity, vertical and horizontal gradients, and ground water flow direction and rate.

Hydrogeologic studies contain information about aquifers useful for understanding aquifer characteristics and relative susceptibility. Where aquifer studies have not yet been undertaken, well logs and maps of surficial geology and soils will begin to provide an idea of relative susceptibility to contamination. A qualified professional knows how to use this information along with other methods to describe the overall hydrogeologic setting and its relative susceptibility.

Hydrogeologists use this information along with contaminant characteristics such as solubility, sorption, concentration, and other chemical and physical properties to answer questions such as:

- How likely is it that a contaminant spilled in a certain location would reach a well?
- How fast would a contaminant spill get to a water well?
- How concentrated would the contaminant still be when it got there?

Source water protection susceptibility rating

The Department of Health evaluates and assigns a susceptibility rating for each public water supply well, based on a number of factors, including whether or not there is a protective confining layer above the aquifer. This rating and information is useful in support of a susceptibility assessment, along with wellhead protection plans, which include information about how fast a contaminant could move toward the well based on time-of-travel estimates.

Step 3: Inventory existing potential sources of groundwater contamination.

Anywhere that a potential pollutant is used, handled, transferred, or stored is a potential source of groundwater contamination. Examples are facilities for transferring chemicals from trucks to tanks, drycleaners, machinery manufacturers, and many more. The U.S. EPA Potential Sources of Drinking Water Contamination Index is in Appendix A.

Many of these facilities may be constructed, maintained, and operated in a way that prevents spills from getting to the ground as much as is feasible. Some operations, however, are inherently more risky for pollution than others. These would include facilities that handle a large quantity of toxic materials, especially where these toxic materials are transferred or handled, increasing the possibility of an incident leading to a spill.
Source water protection contaminant inventories

Public water supply systems with 15 or more connections are regulated under the federal Safe Drinking Water Act. They must inventory potential contamination sources around the wells. The Department of Health works with the Department of Ecology to provide web-based maps of potential contamination sources along with locations of wellhead protection zones. The Facility-Site atlas can be accessed from the Department of Ecology geographic information system (GIS) applications website:
http://www.ecy.wa.gov/services/gis/apps/apps.htm

The GIS cover of facilities and sites regulated by the Department of Ecology can be accessed from the GIS Data website (click on Facility Site):
http://www.ecy.wa.gov/services/gis/data/data.htm

Step 4: Classify the relative vulnerability of ground water to contamination events.

All ground water is vulnerable; some areas where strategic public groundwater resources are located are more vulnerable than other areas. The concept of using criteria to create classifications or categories of vulnerability helps local jurisdictions apply the appropriate measures for the risks involved.

Susceptibility refers to natural conditions, and vulnerability refers to the total contamination risk from both the natural conditions and potential contaminant sources. The base classification of Critical Aquifer Recharge Areas can be based on susceptibility, and an overlay of existing contamination sources used to give the community an idea of where its strategic groundwater supplies may be most at risk under current land use conditions.

For new development, classification based on natural conditions allows a jurisdiction to make decisions about the type of land uses that should or should not be allowed, or which may be allowed with conditions.

There is more than one way to classify Critical Aquifer Recharge Areas. Here are three methods and some illustrations:

- Categories based on susceptibility
  - Water table sand and gravel aquifers
  - Deeper less susceptible aquifers
  - Confined aquifers

- Categories based on set priorities and risk
  - Large public water supply systems one-year time of travel protection zone
  - Densely populated areas that rely on ground water
- Medium public water supply systems protection zones
- Rural areas with a high dependence on ground water
- Discontinuous local drinking water aquifers of limited extent
- Sole Source Aquifers

- Categories based on areas that have the same policies, plans, ordinances, and programs that will be applied.

The examples are not meant to be exhaustive. The categories depend on local hydrogeologic settings, use of the drinking water aquifers, and the actions that a local jurisdiction needs to set in place to protect the public potable groundwater resource.

**Step 5: Designate areas that are most at risk to contamination events.**

The next step in establishing Critical Aquifer Recharge Areas is to designate areas where the public drinking water supply has been determined to be at risk for contamination.

So that local planning and regulation can be guided appropriately, designation makes it clear.

- Where these areas are located (map) and what the performance standards are (criteria).
- Why they are at risk (susceptibility and potential contaminant sources).
- What the importance of this area is to the public drinking water supply (prioritization).

**Step 6: Protect by minimizing activities and conditions that pose contamination risks.**

Anywhere chemicals are stored, handled, transferred, or used is a potential spill or leak risk.

There are all too many examples of groundwater contamination here in Washington. Municipal water supplies have been contaminated by industrial or commercial use of chemicals. The city of Tumwater, the city of Vancouver, and the city of Lakewood all have had contaminated wells. In Eastern Washington, well water turned yellow from dinoseb, a pesticide spilled at Alexander Farms. These events have been expensive and distressing.

Local jurisdictions need authority to require pollution prevention and to obtain compliance before a situation contaminates the local drinking water supply. Ordinances can be specific to the jurisdiction, or a jurisdiction may choose to adopt state or federal laws or rules by reference. Often, county or city hazardous waste pollution prevention programs with associated regulations are operated to prevent local land use activities from creating major cleanup sites.
Step 7: Ensure that contamination prevention plans and best management practices are followed.

The best plans and practices cannot prevent contamination if they are not used. The ability to inspect, obtain compliance and enforce is needed to make sure that the county or city can stop a threat to ground water when the land user is negligent or uncooperative.

Step 8: Manage groundwater withdrawals and recharge.

- Maintain availability for drinking water sources.
- Maintain stream-base flow from ground water to support instream flows, especially for salmon-bearing streams.

Recharge

Development has a profound effect on the hydrology of an area. The increase in impervious surfaces and disturbance of natural vegetation result in increasing runoff and decreasing recharge. Local jurisdictions can improve recharge by encouraging methods that increase recharge, such as low impact development and rain gardens.

The Puget Sound Action Team is a helpful resource for information and assistance with low impact development at [http://www.psat.wa.gov/Programs/LID.htm](http://www.psat.wa.gov/Programs/LID.htm).

Water supply planning

The Watershed Planning Act (Chapter 90.82 RCW) provides for water supply planning by local entities within a Water Resources Inventory Area (WRIA), including at least the counties, the largest city or town within the WRIA, and the water utility that uses the most water. Many of the WRias are engaged in watershed planning. For the current status of watershed planning, see the website at [http://www.ecy.wa.gov/watershed/index.html](http://www.ecy.wa.gov/watershed/index.html).

Ground Water Management Areas (GMA) may be established by either the state or local government under RCW 90.44.400. Criteria for identifying potential Ground Water Management Areas include (among others): Aquifer systems that are declining due to restricted recharge or over-utilization and aquifers identified as the primary source of supply for public water supply systems.

Large water systems regulated by the Department of Health are required to have a water system plan. This plan includes analyses of future water demand and supply. Smaller water systems are required to have a small water system management program (see [http://www.doh.wa.gov/ehp/dw/Programs/water_sys_plan.htm](http://www.doh.wa.gov/ehp/dw/Programs/water_sys_plan.htm)).

Local governments also include water planning in their comprehensive plans and must meet water supply planning requirements under the Growth Management Act. The Washington Department of Community, Trade, and Economic Development has written a fact sheet called Watershed Planning that explains the link between growth management, and watershed planning at http://www.cted.wa.gov/DesktopModules/CTEDPublications/CTEDPublicationsView.aspx?tabID=0&alias=CTED&lang=en&ItemID=897&MId=944&wversion=Staging.

Section 4
Best Available Science

Best available science is required by the Growth Management Act and is defined by the Washington Administrative Code. Best available science guidance has been published by the Department of Community, Trade, and Economic Development. These sources should be consulted to obtain a good knowledge of how the concept of best available science functions within the Growth Management Act.

The main sources of information for requirements of the Growth Management Act and related rules for best available science and critical areas are:

- **Chapter 36.70A.172 RCW**
  Critical areas, designation and protection, best available science to be used.

  In designating and protecting critical areas under this chapter, counties and cities shall include the best available science in developing policies and development regulations to protect the functions and values of critical areas. In addition, counties and cities shall give special consideration to conservation or protection measures necessary to preserve or enhance anadromous fisheries.

- **Chapter 365-195-905 through 925 WAC**
  Chapter 365-195-905 WAC discusses the characteristics of a valid scientific process.

  In the context of critical areas protection, a valid scientific process is one that produces reliable information useful in understanding the consequences of a local government’s regulatory decisions and in developing critical areas policies and development regulations that will be effective in protecting the functions and values of critical areas.

  The objective of including science is “to protect the functions and values of critical areas.” Science plays a central role in delineating critical areas,
identifying functions and values, and recommending strategies to protect their functions and values (OCD, 2003).

The rule goes on to list the characteristics of a valid scientific process, including peer review, methods, logical conclusions and reasonable inferences, quantitative analysis, context, and references. It then lists sources, including research, monitoring, inventory, survey, modeling, assessment, synthesis, and expert opinion. This section of the WAC is particularly applicable to Critical Aquifer Recharge Areas.


See Appendix B – Resources for more information about how to obtain or access these sources.

**How best available science applies to Critical Aquifer Recharge Areas**

Science for **Critical Aquifer Recharge Areas** involves knowledge about the occurrence and movement of ground water.

- Identifying where “areas with a critical recharging effect on potable aquifers” are located.
- Analyzing their physical characteristics.
- Assessing the risk for contamination.
- Evaluating effective best management practices for preventing contamination.
- Assessing the potential impacts on drinking water sources and stream flow from groundwater withdrawals and changes in recharge.

*The GMA requires best available science to be used for special consideration of anadromous fish species. Science is used to establish where ground water affects streams and other surface water habitats, and what the effects are.*

Best available science and the functions and values of Critical Aquifer Recharge Areas. The Growth Management Act requires protection of **water quality and quantity**:

- Planning goals include water quality and availability.

  RCW 36.70A.020 – Planning goals

  *Protect the environment and enhance the state's high quality of life, including air and water quality, and the availability of water.*
• Comprehensive plans should address groundwater quality and quantity protection in the land use element.

RCW 36.70A.070 Comprehensive plans – Mandatory Elements

_The land use element shall provide for protection of the quality and quantity of ground water used for public water supplies._

Best available science for Critical Aquifer Recharge Areas, therefore, should address both quality and quantity.

**When Should Best Available Science Be Applied?**

1. **Upfront, during the planning process.**

Two main benefits of applying best available science upfront in the planning process (Washington Department of Community Trade and Economic Development, 2003) are:

• It enables understanding of where critical areas are located and how they naturally function. This guides how best to regulate land uses that may impact critical areas.

• Upfront planning and adoption of scientifically defensible development standards enables decisions to be made with information that is on-hand instead of needing to be developed for each project. This should lessen the expense and time needed to make decisions.

2. **At the time of application.**

Project review may entail that the applicant provide the county or city with information that is supported by best available science. An example would be a hydrogeologic report. This information is especially important to evaluate projects against performance-based standards.

**Sources for Best Available Science for Critical Aquifer Recharge Areas**

Groundwater scientists rely on a number of standard methods for characterizing the occurrence and movement of ground water. These methods involve everything from topographic maps, aerial photos, on-the-ground mapping, use of existing maps for soils and geology, well log analysis, aquifer tests, geophysics, water quality testing, water level measurements, monitoring well installations, testing for seepage of ground water into streams (or from streams into ground water), and modeling. There are also dozens of approaches to assessing groundwater vulnerability or susceptibility to contamination in the professional literature. Pollution prevention and best management practices for preventing contamination are widely published.

These methods have **standards of practice**. Some examples, just to name a few, are:
• Quality assurance standards for water quality sampling
• Standard methods for measuring water levels
• Aquifer test methods and standards
• Field methods

Existing Sources of Information

Local government can use information that local, state or federal natural resource agencies have determined represents the best available science. They can also use information provided by a qualified scientific expert or team of qualified scientific experts.

Sources that provide scientifically valid information useful for Critical Aquifer Recharge Areas include:

Public water supply data and source water protection information

• Well head protection zone plans
• Contaminant inventories
• Aquifer characterization/susceptibility rating
• Well information
• Water quality sampling data
• System size and location
• Water system plans
• Studies completed for public water supply systems

State, federal, local, academic, and consultant studies

• USGS studies
• Water supply papers
• State studies
• Consultant studies for local government
• Consultant studies for a state-regulated facility
• Academic studies

Smaller jurisdictions can rely on the information generated by public water supply systems, state, and federal required studies for facilities located within their jurisdiction, and other studies as listed above. A literature review helps to document best available science for the record. Asking for volunteers in the community, technical assistance from the state, and applying for grants are ways to augment local resources. (See WAC 365-195-910 (2)).
What are the potential consequences if best available science is not applied?

Failure to apply best available science for critical areas under the Growth Management Act may be appealed to the Growth Management Hearings Board. When the board finds a county or city in noncompliance with the Growth Management Act, the board issues a Compliance Order. Failure to comply with a board order can result in state sanctions and loss of funding. See Appendix B for where to find more information about the Growth Management Hearings Board.

Section 5
Working with State and Federal Laws and Rules

The Washington State Growth Management Act and rules refer to how local authorities should coordinate with other government authorities in several places. Three of the concepts contained in the GMA rules follow.

- Local government should consider and coordinate with state, federal, and other authority’s laws, rules, and permits (WAC 365-195-735 – State and Regional Authorities).
- Local plans and policies may in some respects be adequately implemented by adopting the provisions of such other programs as part of the local regulations (WAC 365-195-825 – Regulations specifically required by the act).
- Projects may be approved based on compliance with other local, state or federal rules or laws, providing environmental concerns are mitigated (RCW 43.21C.240 – Project review under the growth management act).

The GMA allows jurisdictions to avoid duplication of effort by making use of what is already being done by others. The functions and values of Critical Aquifer Recharge Areas should still be protected. Success, then, depends on identifying potential contamination sources, identifying other laws, rules, and planning efforts that are relevant to Critical Aquifer Recharge Areas and identifying where local action is needed to ensure protection.

Potential Contamination Sources

EPA has listed common potential sources of contamination along with likely contaminants. The U.S. EPA Potential Sources of Drinking Water Contamination Index Listed in Appendix A is the table copied from their web page, which also may be accessed at http://www.epa.gov/safewater/swp/sources1.html

This chart lists some potential facilities and activities where one might find the contaminants referred to as primary and secondary drinking water standards. The listing of a contaminant does not mean that it will always occur at the associated source, nor
does it encompass all contaminants that may be present. Sources are divided into four major categories.

- Commercial/industrial
- Residential municipal
- Agricultural/rural
- Miscellaneous (underground injection control/naturally occurring)

This list is intended as a resource guide for creating an inventory list. A state or local community may have different sources of concern from the list in Appendix A based on local variability such as existing industrial activity, and known contaminant occurrence information.

**Existing Regulation of Potential Contamination Sources**

The GMA lists laws, rules, permits, and planning processes to consider in WAC 365-195-710 – Identification of other laws.

The following table (after Cook, 2000) lists many of the activities relevant to groundwater protection along with the citation.
### Table 1: Laws, rules, and guidance for groundwater protection

<table>
<thead>
<tr>
<th>Activity</th>
<th>Statute - Regulation - Guidance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Above Ground Storage Tanks</td>
<td>Chapter 173-303-640 WAC</td>
</tr>
<tr>
<td>Animal Feedlots</td>
<td>Chapter 173-216 WAC, Chapter 173-220 WAC</td>
</tr>
<tr>
<td>Below Ground Storage Tanks</td>
<td>Chapter 173-360 WAC</td>
</tr>
<tr>
<td>Dangerous Waste Regulations</td>
<td>Chapter 173-303 WAC</td>
</tr>
<tr>
<td>- Siting of Chemical Treatment Storage and Disposal Facilities;</td>
<td>Chapter 173-303-282 WAC</td>
</tr>
<tr>
<td>- Hazardous Waste Generators (Boat Repair Shops, Biological Research Facility, Dry Cleaners, Furniture Stripping, Motor Vehicle Service Garages, Photographic Processing, Printing and Publishing Shops, etc.)</td>
<td>Chapter 173-303-170 WAC</td>
</tr>
<tr>
<td>- Spills and discharges into the environment</td>
<td>Chapter WAC 173-303-145</td>
</tr>
<tr>
<td>Injection Wells (Dry Wells)</td>
<td>Federal 40 CFR Part 144—Underground Injection Control Program and Part 146—Underground Injection Control Program: Criteria And Standards; Chapter 173-218 WAC</td>
</tr>
<tr>
<td>Junk Yards and Salvage Yards</td>
<td>Chapter 173-304 WAC, Best Management Practices to Prevent Stormwater Pollution at Vehicles Recycler Facilities (WDOE 94-146)</td>
</tr>
<tr>
<td>Oil and Gas Drilling</td>
<td>Chapter 332-12-450 WAC, Chapter 173-218 WAC</td>
</tr>
<tr>
<td>On-Site Sewage Systems (Large Scale)</td>
<td>Chapter 173-240 WAC</td>
</tr>
<tr>
<td>On-Site Sewage Systems &lt; 14,500 gal/day</td>
<td>Chapter 246-272 WAC, Local Health Ordinances</td>
</tr>
<tr>
<td>Pesticide Storage and Use</td>
<td>Chapter 15.54 RCW, Chapter 17.21 RCW</td>
</tr>
<tr>
<td>Solid Waste Handling and Recycling Facilities</td>
<td>Chapter 173-304 WAC</td>
</tr>
<tr>
<td>Surface Mining</td>
<td>Chapter 332-18 WAC</td>
</tr>
</tbody>
</table>

### Identifying Gaps in Protection

Federal and state laws and rules do not replace local planning, ordinances, and programs. Local jurisdictions should maintain the ability to protect ground water under their own authority. Local government can focus on local conditions in a way that the state cannot.

The Department of Ecology through RCWs, WACs, and permits, sets minimum operating standards for many types of potentially polluting facilities. If a permitted facility is poorly managed or experiences some sort of engineering failure (which may happen even with good management), contamination may be released into the environment.

Local government planning can influence the types of future developments that occur in various areas and may be able to encourage potentially contaminating facilities to locate in areas where the aquifer has a lower susceptibility if contaminants are released. In this
way, the potential for aquifer pollution is lowered and the public is protected. Land use planning at the local level is the most effective way to influence where facilities choose to locate.

- **Counties and cities.**
  - Regulate land use through comprehensive planning, zoning, and ordinances.
  - Have authority to ensure a landowner does not pollute the public drinking water supply.
  - Are more able to track conditions and adapt to local concerns much more readily than the state.

- **Federal and state laws, rules, and programs are often targeted toward larger facilities.** For example, pollution prevention plans are required by the state if a facility generates 2,640 pounds of hazardous waste a year. A much smaller quantity of hazardous chemicals can cause contamination, especially if improper disposal into a septic system or a dry well occurs. The local jurisdiction should consider requiring pollution prevention plans where needed and not already required.

- **Compliance depends on state resources to enforce.** The state covers a large area and a large number of facilities, and therefore illegal activities may occur that are not detected by the state until contamination has occurred. Local attention can prevent the creation of new cleanup sites.

### Prohibited and conditioned uses

Some land use activities, such as landfills, have been found to be a high-risk for groundwater contamination. Although a high-risk use may be regulated by other authorities, local jurisdictions should consider prohibiting these uses from being located within high-risk high-priority Critical Aquifer Recharge Areas. Where these uses are already sited, they should be closely monitored and strict pollution prevention requirements followed.

Examples of uses that should be considered for prohibition in Critical Aquifer Recharge Areas are landfills, wood treatment facilities, chrome platers, tank farms, and facilities that treat, store, or dispose of hazardous waste. Chemical facilities that transfer or use large amounts of chemicals should also be considered to be a risk for ground water contamination.

Some uses that have a moderate to low risk for contamination can be allowed within Critical Aquifer Recharge Areas conditionally on meeting certain requirements for approval. These are typically pollution prevention measures such as secondary containment for chemical storage areas, spill prevention measures, and contingency plans for emergencies.

Here are some questions the local jurisdiction should consider when coordinating their planning and ordinances with federal and state laws, rules, and programs.
• Does the jurisdiction know where potentially polluting activities are located?
• Are effective protective requirements for potentially polluting activities in place?
• Is there provision for compliance monitoring?
• Is there a means to obtain compliance if there is a violation?
• Does the jurisdiction have a plan for ensuring that existing land uses are protective of ground water?

Section 6
Adapting to Local Conditions and Settings

The Growth Management Act allows for differences in regional or local conditions.

See WAC 365-195-060 Regional and Local Variations

Washington has varied landscapes and populations, from sparsely populated rural areas to large cities, from dry desert to rain forest.

Ferry County has a population of 7300 (in 2003). Republic, the county seat, has a population of 975 people. Ferry County is located in the mountainous Okanogan region where ponderosa pines flourish in the dry climate.

King County has both populous and rural areas and has varied landscapes, from the Puget Sound to the high plateau in the shadow of Mount Rainier. The total population of King County was 1,779,300 in 2003.

The settings in which ground water occurs, the resources for programs, and the resources at risk vary in different parts of the state. This means that a program that protects the functions and values of Critical Aquifer Recharge Areas in one part of the state will not necessarily look like a program in another.

The Western Washington GMA Hearings Board (WWGMHB) states:

The GMA does not require a “one size fits all” approach. A GMHB is to be guided by a common sense appreciation of the size and resources of a local jurisdiction and the magnitude of the problems to be addressed. MCCDC v. Shelton 96-2-0014 (FDO 11-14-96)

The fundamental requirement of the Growth Management Act is that the functions and values of the critical area should be protected. For Critical Aquifer Recharge Areas, that means that public drinking water quality and quantity should be addressed in planning and ordinances.
A good groundwater program:

- Identifies threats to ground water.
- Identifies groundwater resources at risk.
- Monitors to make sure a condition that could cause an unacceptable risk is not occurring.
- Educates and informs people so that they can do their best to protect ground water
- Takes action when necessary!

**Section 7**

**Adaptive Management – Change Happens**

The GMA requires periodic review and update of plans and ordinances for critical areas. In addition, when the scientific information for addressing critical areas is inadequate, it requires that adaptive management be used in order to determine the impacts on the critical areas from development regulations, and to reduce those impacts to protect the functions and values of the critical areas. ([WAC 365-195-920](http://www.leg.wa.gov/WAC/index.cfm?section=365-195-920&fuseaction=section))

Adaptive management involves strategic testing of hypotheses and related monitoring to see how well plans, ordinances, and programs are protecting Critical Aquifer Recharge Areas. Changes are then made as more is known or as early hypotheses are proven incorrect, as conditions change, or to improve or correct a method of protection as needed. Monitoring data results can also lead to changes in how monitoring is done and what is monitored. The comprehensive plan and development regulations should include an iterative process for amendments as new information becomes available.

Examples of new information are hydrogeologic studies that provide more information about the boundaries and characteristics of aquifers, significant land use changes and the associated groundwater contamination risks associated with population increases, and the results of the evaluation of voluntary and regulatory programs. A fundamental component of adaptive management is the commitment to change based upon the outcome of testing hypotheses through strategic monitoring.
Section 8

References


# Appendix A

## U.S. EPA Potential Sources of Drinking Water Contamination Index

<table>
<thead>
<tr>
<th>Commercial / Industrial</th>
<th>CONTAMINANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Above-ground storage tanks</td>
<td>Arsenic, Barium, Benzene, Cadmium, 1,4-Dichlorobenzene or P-Dichlorobenzene, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Lead, Trichloroethylene (TCE), Tetrachloroethylene or Perchloroethylene (Perc)</td>
</tr>
<tr>
<td>Automobile, body shops/repair shops</td>
<td>Arsenic, Barium, Benzene, Cadmium, Chlorobenzene, Copper, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, 1,4-Dichlorobenzene or P-Dichlorobenzene, Lead, Fluoride, 1,1,1-Trichloroethane or Methyl Chloroform, Dichloromethane or Methylene Chloride, Tetrachloroethylene or Perchloroethylene (Perc), Trichloroethylene (TCE), Xylene (Mixed Isomers)</td>
</tr>
<tr>
<td>Boat repair/refinishing/marinas</td>
<td>Benzene, Cadmium, cis 1,2-Dichloroethylene, Coliform, Cryptosporidium, Dichloromethane or Methylene Chloride, <em>Giardia Lamblia</em>, Lead, Mercury, Nitrate, Nitrite, trans 1,2-Dichloroethylene, Tetrachloroethylene or Perchloroethylene (Perc), Trichloroethylene (TCE), Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>Cement/concrete plants</td>
<td>Barium, Benzene, Dichloromethane or Methylene Chloride, Ethylbenzene, Lead, Styrene, Tetrachloroethylene or Perchloroethylene (Perc), Toluene, Xylene (Mixed Isomers)</td>
</tr>
<tr>
<td>Chemical/petroleum processing</td>
<td>Acrylamide, Arsenic, Atrazine, Alachlor, Aluminum (Fume or Dust), Barium, Benzene, Cadmium, Carbofuran, Carbon Tetrachloride, Chlorobenzene, Copper, Cyanide, 2,4-D, 1,2-Dibromoethane or Ethylene Dibromide (EDB), 1,2-Dichlorobenzene or O-Dichlorobenzene, 1,4-Dichlorobenzene or P-Dichlorobenzene, 1,1-Dichloroethylene or Vinylidene Chloride, cis 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Di(2-ethylhexyl) adipate, Di(2-ethylhexyl) phthalate, 1,2-Dichloroethane or Ethylene Dichloride, Dioxin, Endrin, Epichlorohydrin, Ethylbenzene, Hexachlorobenzene, Hexachlorocyclopentadiene, Lead, Mercury, Methoxychlor, Polychlorinated Biphenyls, Selenium, Styrene, Sulfate, Tetrachloroethylene or Perchloroethylene (Perc), Toluene, 1,2,4-Trichlorobenzene, 1,1,1-Trichloroethane or Methyl Chloroform, Trichloroethylene (TCE), Vinyl Chloride, Xylene (Mixed Isomers), Zinc (Fume or Dust)</td>
</tr>
<tr>
<td>Construction/demolition</td>
<td>Arsenic, Asbestos, Benzene, Cadmium, Chloride, Copper, Cyanide, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Fluorides, Lead, Selenium, Tetrachloroethylene or Perchloroethylene (Perc), 1,1,1-Trichloroethane or Methyl Chloroform, Trichloroethylene (TCE), Turbidity, Xylene (Mixed Isomers), Zinc (Fume or Dust)</td>
</tr>
<tr>
<td>Dry cleaners/dry cleaning</td>
<td>Tetrachloroethylene or Perchloroethylene (Perc), 1,1,1-Trichloroethane or Methyl Chloroform, 1,1,2-Trichloroethane</td>
</tr>
<tr>
<td>Dry goods manufacturing</td>
<td>Barium, Benzene, Cadmium, Copper, Dichloromethane or Methylene Chloride, Di(2-ethylhexyl) phthalate, Lead, 1,1,1-Trichloroethane or Methyl Chloroform, Polychlorinated Biphenyls, Tetrachloroethylene or Perchloroethylene (Perc), Toluene, Tetrachloroethylene (TCE), Xylene (Mixed Isomers)</td>
</tr>
<tr>
<td>Electrical/electronic manufacturing</td>
<td>Aluminum (Fume or Dust), Antimony, Arsenic, Barium, Benzene, Cadmium, Chlorobenzene, Copper, Cyanide, Carbon Tetrachloride, 1,2-Dichlorobenzene or O-Dichlorobenzene, 1,2-Dichloroethane or Ethylene Dichloride, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Di(2-ethylhexyl) phthalate, Ethylbenzene, Lead, Mercury, Polychlorinated Biphenyls, Selenium, Styrene, Sulfate, Tetrachloroethylene or Perchloroethylene (Perc), 1,1,1-Trichloroethane or Methyl Chloroform, 1,1,2-Trichloroethane, Trichloroethylene (TCE), Thallium, Toluene, Vinyl Chloride, Xylene (Mixed Isomers), Zinc (Fume or Dust)</td>
</tr>
<tr>
<td>POTENTIAL SOURCE</td>
<td>CONTAMINANT</td>
</tr>
<tr>
<td>-----------------</td>
<td>------------</td>
</tr>
<tr>
<td><strong>Commercial / Industrial continued</strong></td>
<td></td>
</tr>
<tr>
<td>Fleet/trucking / bus terminals</td>
<td>Arsenic, Acrylamide, Barium, Benzene, Benzo(a)pyrene, Cadmium, Chlorobenzene, Cyanide, Carbon Tetrachloride, 2,4-D, 1,2-Dichlorobenzene or O-Dichlorobenzene, 1,4-Dichlorobenzene or P-Dichlorobenzene, 1,2-Dichloroethane or Ethylene Dichloride, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Di(2-ethylhexyl) phthalate, Epichlorohydrin, Heptachlor (and Epoxide), Lead, Mercury, Methoxychlor, Pentachlorophenol, Propylene Dichloride or 1,2-Dichloropropane, Selenium, Styrene, Toxaphene, Tetrachloroethylene or Perchloroethylene (Perc), Toluene, 1,1,1-Trichloroethane or Methyl Chloroform, Trichloroethylene (TCE), Vinyl Chloride, Xylene (Mixed isomers)</td>
</tr>
<tr>
<td>Food processing</td>
<td>Arsenic, Benzene, Cadmium, Copper, Carbon Tetrachloride, Dichloromethane or Methylene Chloride, Lead, Mercury, Picloram, Tetrachloroethylene or Perchloroethylene (Perc), Toluene, 1,1,1-Trichloroethane or Methyl Chloroform, Trichloroethylene (TCE), Xylene (Mixed isomers)</td>
</tr>
<tr>
<td>Funeral services / mortuary</td>
<td>Glyphosate, Dichloromethane or Methylene Chloride, Nitrate, Nitrile, Total Coliforms, Viruses</td>
</tr>
<tr>
<td>Furniture repair / manufacturing</td>
<td>Barium, 1,2-Dichloroethane or Ethylene Dichloride, Dichloromethane or Methylene Chloride, Ethylbenzene, Lead, Mercury, Selenium, Trichloroethylene (TCE)</td>
</tr>
<tr>
<td>Gas stations (see also above)</td>
<td>cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Tetrachloroethylene or Perchloroethylene (Perc), Trichloroethylene (TCE)</td>
</tr>
<tr>
<td><strong>Groundwater and ground vehicle drainage wells</strong></td>
<td></td>
</tr>
<tr>
<td>Graveyards / cemeteries</td>
<td>Dalapon, Lindane, Nitrate, Nitrile, Total Coliforms, Viruses</td>
</tr>
<tr>
<td>Hardware / lumber / parts stores</td>
<td>Aluminum (Fume or Dust), Barium, Benzene, Cadmium, Chlorobenzene, Copper, Dichloromethane or Methylene Chloride, Di(2-ethylhexyl) adipate, Di(2-ethylhexyl) phthalate, 1,4-Dichlorobenzene or P-Dichlorobenzene, Ethylbenzene, Lead, Mercury, Tetrachloroethylene or Perchloroethylene (Perc), 1,1,1-Trichloroethane or Methyl Chloroform, Trichloroethylene (TCE), Toluene, Xylene (Mixed isomers)</td>
</tr>
<tr>
<td>Historic waste dumps / landfills</td>
<td>Atrazine, Alachlor, Carbofuran, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloroacetonitrile, Dalapon, Glyphosate, Dichloromethane or Methylene Chloride, Nitrate, Nitrile, Oxamyl (Vydate), Sulfate, Simazine, Tetrachloroethylene or Perchloroethylene (Perc), Trichloroethylene (TCE)</td>
</tr>
<tr>
<td>Home manufacturing</td>
<td>Arsenic, Barium, Benzene, Cadmium, Chlorobenzene, Copper, Carbon Tetrachloride, 1,2-Dichlorobenzene or O-Dichlorobenzene, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Di(2-ethylhexyl) phthalate, Ethylbenzene, Lead, Mercury, Selenium, Styrene, Tetrachloroethylene or Perchloroethylene (Perc), 1,1,1-Trichloroethane or Methyl Chloroform, Trichloroethylene (TCE), Toluene, Turbidity, Xylene (Mixed isomers)</td>
</tr>
<tr>
<td>Industrial waste disposal wells (see UIC for more information on concerns, and locations)</td>
<td>Acrylamide, Arsenic, Atrazine, Alachlor, Aluminum (Fume or Dust), Ammonia, Barium, Benzene, Cadmium, Carbofuran, Carbon Tetrachloride, Chlorobenzene, Copper, Cyanide, 2,4-D, 1,2-Dibromoethane or Ethylene Dibromide (EDB), 1,2-Dichlorobenzene or O-Dichlorobenzene, 1,4-Dichlorobenzene or P-Dichlorobenzene, 1,1-Dichloroethylene or Vinylidene Chloride, cis 1,2 Dichloroethylene, Dichloromethane or Methylene Chloride, Di(2-ethylhexyl) adipate, Di(2-ethylhexyl) phthalate, 1,2-Dichloroethylene or Ethylene Dichloride, Dioxin, Endrin, Epichlorohydrin, Hexachlorobenzene, Hexachlorocyclopentadiene, Lead, Mercury, Methoxychlor, Oxamyl (Vydate), Polychlorinated Biphenyls, Selenium, Styrene, Sulfate, Tetrachloroethylene or Perchloroethylene (Perc), Toluene, 1,2,4-Trichlorobenzene, 1,1,1-Trichloroethane or Methyl Chloroform, Trichloroethylene (TCE), Vinyl Chloride, Xylene (Mixed isomers), Zinc (Fume or Dust)</td>
</tr>
<tr>
<td>Junk / scrap / salvage yards</td>
<td>Barium, Benzene, Copper, Dalapon, cis 1,2-Dichloroethylene, Dichloroacetonitrile, Lead, Polychlorinated Biphenyls, Sulfate, Simazine, Tetrachloroethylene (TCE), Tetrachloroethylene or Perchloroethylene (Perc)</td>
</tr>
<tr>
<td>POTENTIAL SOURCE</td>
<td>CONTAMINANT</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Commercial / Industrial continued</td>
<td></td>
</tr>
<tr>
<td>Machine shops</td>
<td>Arsenic, Aluminum (Fume or Dust), Barium, Benzene, Boric Acid, Cadmium,</td>
</tr>
<tr>
<td></td>
<td>Chlorobenzene, Copper, Cyanide, Carbon Tetrachloride 2,4-D, 1,4-Dichlorobenzene or P-Dichlorobenzene, 1,2-Dichloroethane or Ethylene Dichloride, 1,1-Dichloroethylene or Vinylidene Chloride, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Di(2-ethylhexyl) phthalate, Ethylbenzene, Fluoride, Hexachlorobenzene, Lead, Mercury, Polychlorinated Biphenyls, Pentachlorophenol, Selenium, Styrene, Tetrachloroethylene or Perchloroethylene (Perc), Toluene, 1,1,1-Trichloroethane or Methyl Chloroform, 1,1,2-Trichloroethane, Trichloroethylene (TCE), Xylenes (Mixed Isomers), Zinc (Fume or Dust)</td>
</tr>
<tr>
<td>Medical/vet offices</td>
<td>Arsenic, Acrylamide, Barium, Benzene, Cadmium, Copper, Cyanide, Carbon</td>
</tr>
<tr>
<td></td>
<td>Tetrachloride, Dichloromethane or Methylene Chloride, 1,2-Dichloroethane or</td>
</tr>
<tr>
<td></td>
<td>Ethylene Dichloride, Lead, Mercury, Methoxychlor, 1,1,1-Trichloroethane or Methyl Chloroform, Radionuclides, Selenium, Silver, Tetrachloroethylene or Perchloroethylene (Perc), 2,4,5-TP (Silvex), Thallium, Xylenes (Mixed Isomers)</td>
</tr>
<tr>
<td>Metal plating/finishing/fabricating</td>
<td>Antimony, Aluminum (Fume or Dust), Arsenic, Barium, Benzene, Cadmium, Carbon Tetrachloride, Chlorobenzene, Chromium, Copper, Cyanide, 1,4-Dichlorobenzene or P-Dichlorobenzene, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Di(2-ethylhexyl) adipate, Ethylbenzene, Lead, Mercury, Polychlorinated Biphenyls, Pentachlorophenol, Selenium, Styrene, Sulfate, Tetrachloroethylene or Perchloroethylene (Perc), Thallium, Toluene, 1,1,1-Trichloroethane or Methyl Chloroform, 1,1,2-Trichloroethane, Trichloroethylene (TCE), Vinyl Chloride, Xylenes (Mixed Isomers), Zinc (Fume or Dust)</td>
</tr>
<tr>
<td>Military Installations</td>
<td>Arsenic, Barium, Benzene, Cadmium, Chlorobenzene, 1,2-Dichlorobenzene or O-</td>
</tr>
<tr>
<td></td>
<td>Dichlorobenzene, 1,2-Dichloroethane or Ethylene Dichloride, cis 1,2-</td>
</tr>
<tr>
<td></td>
<td>Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene</td>
</tr>
<tr>
<td></td>
<td>Chloride, Hexachlorobenzene, Lead, Mercury, Methoxychlor, 1,1,1-Trichloroethane or Methyl Chloroform, Radionuclides, Selenium, Tetrachloroethylene or Perchloroethylene (Perc), Toluene, Trichloroethylene (TCE)</td>
</tr>
<tr>
<td>Mines/gravel pits</td>
<td>Lead, Selenium, Sulfate, Tetrachloroethylene or Perchloroethylene (Perc), 1,1,1-Trichloroethane or Methyl Chloroform, Turbidity</td>
</tr>
<tr>
<td>Motor pools</td>
<td>cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride,</td>
</tr>
<tr>
<td>Motor vehicle waste disposal wells</td>
<td>Arsenic, Barium, Benzene, Cadmium, Chlorobenzene, Copper, cis 1,2-</td>
</tr>
<tr>
<td>(gas stations, repair shops) See UIC</td>
<td>Dichloroethylene, trans 1,2-Dichloroethylene, 1,4-Dichlorobenzene or P-</td>
</tr>
<tr>
<td>for more on concerns for these sources</td>
<td>Dichlorobenzene, Lead, Fluoride, 1,1,1-Trichloroethane or Methyl Chloroform,</td>
</tr>
<tr>
<td><a href="http://www.epa.gov/safewater/uic-cy">http://www.epa.gov/safewater/uic-cy</a>.</td>
<td>Dichloromethane or Methylene Chloride, Tetrachloroethylene or Perchloroethylene (Perc), Trichloroethylene (TCE), Xylenes (Mixed Isomers)</td>
</tr>
<tr>
<td>ts.html</td>
<td></td>
</tr>
<tr>
<td>Office building/complex</td>
<td>Barium, Benzene, Cadmium, Copper, 2,4-D, Diazinom, 1,2-Dichlorobenzene or O-</td>
</tr>
<tr>
<td></td>
<td>Dichlorobenzene, Dichloromethane or Methylene Chloride, Ditquat, 1,2-</td>
</tr>
<tr>
<td></td>
<td>Dichloroethane or Ethylene Dichloride, Ethylbenzene, Glyoxalate, Lead, Mercury, Selenium, Simazine, Tetrachloroethylene or Perchloroethylene (Perc), 1,1,1-Trichloroethane or Methyl Chloroform, Trichloroethylene (TCE), Vinyl Chloride, Xylenes (Mixed Isomers)</td>
</tr>
<tr>
<td>Photo processing/printing</td>
<td>Acrylamide, Aluminum (Fume or Dust), Arsenic, Barium, Benzene, Cadmium,</td>
</tr>
<tr>
<td></td>
<td>Carbon Tetrachloride, Chlorobenzene, Copper, Cyanide, 1,1-Dichloroethylene or Vinylidene Chloride, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Di(2-ethylhexyl) phthalate, 1,2-Dichlorobenzene or O-Dichlorobenzene, 1,2-Dichloroethylene or Ethylene Dichloride, 1,2-Dibromoethylene or Ethylene Dibromide (EDB), Heptachlor epoxide, Hexachlorobenzene, Lead, Lindane, Mercury, Methoxychlor, Propylene Dichloride or 1,2-Dichloropropane, Selenium, Styrene, Tetrachloroethylene or Perchloroethylene (Perc), 1,1,1-Trichloroethane or Methyl Chloroform, Toluene, 1,1,2-Trichloroethane, Trichloroethylene (TCE), Vinyl Chloride, Xylenes (Mixed Isomers), Zinc (Fume or Dust)</td>
</tr>
<tr>
<td>POTENTIAL SOURCE</td>
<td>CONTAMINANT</td>
</tr>
<tr>
<td>------------------</td>
<td>------------</td>
</tr>
<tr>
<td><strong>Commercial / Industrial continued</strong></td>
<td></td>
</tr>
<tr>
<td>Synthetic / plastics production</td>
<td>Antimony, Arsenic, Barium, Benzenes, Cadmium, Carbon Tetrachloride, Chlorobenzene, Copper, Cyanide, 1,2-Dichlorobenzene or O-Dichlorobenzene, 1,4-Dichlorobenzene or P-Dichlorobenzene, 1,2-Dichloroethane or Ethylene Dichloride, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Di(2-ethylhexyl) adipate, Di(2-ethylhexyl) phthalate, Ethylbenzene, Hexachlorobenzene, Lead, Mercury, Methyl Chloroform or 1,1,1-Trichloroethane, Pentachlorophenol, Selenium, Styrene, Tetrachloroethylene or Perchloroethylene (Perk), Toluene, Trichloroethylene (TCE), Vinyl Chloride, Xylene (Mixed Isomers), Zinc (Fume or Dust)</td>
</tr>
<tr>
<td>RV/mini storage</td>
<td>Arsenic, Barium, Cyanide, 2,4-D, Endrin, Lead, Methoxychlor</td>
</tr>
<tr>
<td>Railroad yards/maintenance/fueling areas</td>
<td>Atrazine, Barium, Benzenes, Cadmium, Dalapon, 1,4-Dichlorobenzene or P-Dichlorobenzene, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Lead, Mercury, Tetrachloroethylene or Perchloroethylene (Perc), Trichloroethylene (TCE),</td>
</tr>
<tr>
<td>Research laboratories</td>
<td>Arsenic, Barium, Benzenes, Beryllium Powder, Cadmium, Carbon Tetrachloride, Chlorobenzene, Cyanide, 1,2-Dichloroethene or Ethylene Dichloride, 1,1-Dichloroethylene or Vinylidene Chloride, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Lead, Mercury, Polychlorinated Biphenyls, Selenium, Tetrachloroethylene or Perchloroethylene (Perc), Thallium, Thiosulfates, Toluene, 1,1,1-Trichloroethylene or Methyl Chloroform, Trichloroethylene (TCE), Vinyl Chloride, Xylene (Mixed Isomers)</td>
</tr>
<tr>
<td>Retail operations</td>
<td>Arsenic, Barium, Benzenes, Cadmium, 2,4-D, 1,2-Dichloroethene or Ethylene Dichloride, Lead, Mercury, Phenylenes, Tetrachloroethylene or Perchloroethylene (Perc), Toluene, 1,1,1-Trichloroethene, Vinyl Chloride</td>
</tr>
<tr>
<td>Underground storage tanks</td>
<td>Arsenic, Barium, Benzenes, Cadmium, 1,4-Dichlorobenzene or P-Dichlorobenzene, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Lead, Tetrachloroethylene or Perchloroethylene (Perc), Trichloroethylene (TCE)</td>
</tr>
<tr>
<td>Wood preserving/treating</td>
<td>cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Lead, Sulfate</td>
</tr>
<tr>
<td>Wood/pulp/paper processing</td>
<td>Atrazine, Barium, Benzenes, Carbon Tetrachloride, Copper, Dichloromethane or Methylene Chloride, Dioxin, 1,2-Dichloroethene or Ethylene Dichloride, Ethylbenzene, Lead, Mercury, Polychlorinated Biphenyls, Selenium, Styrene, Tetrachloroethylene or Perchloroethylene (Perc), Trichloroethylene (TCE), Toluene, 1,1,1-Trichloroethene or Methyl Chloroform, Xylene (Mixed Isomers)</td>
</tr>
<tr>
<td><strong>Residential / Municipal</strong></td>
<td></td>
</tr>
<tr>
<td>Airports (maintenance/fueling areas)</td>
<td>Arsenic, Barium, Benzenes, Cadmium, Carbon Tetrachloride, cis 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Ethylbenzene, Lead, Mercury, Selenium, Tetrachloroethylene or Perchloroethylene (Perc), 1,1,1-Trichloroethene or Methyl Chloroform, Trichloroethylene (TCE), Xylene (Mixed Isomers)</td>
</tr>
<tr>
<td>Apartments and condominiums</td>
<td>Atrazine, Alachlor, Colifom, Cryptosporidium, Dalapon, Dichl, Giardia Lamblia, Glyphosate, Nitrate, Nitrite, Picrocan, Sulfate, Simazine, Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>Camp grounds/RV parks</td>
<td>Benormyl, Coliform, Cryptosporidium, Dichl, Dalapon, Giardia Lamblia, Glyphosate, Isospropanol, Nitrate, Nitrite, Picrocan, Sulfate, Simazine, Turbidity, Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>Cesspools - large capacity (see UIC for more information)</td>
<td>Atrazine, Alachlor, Carbofuran, Coliform, Cryptosporidium, Dichl, Dalapon, Giardia Lamblia, Glyphosate, Nitrate, Nitrite, Oxauryl (Vydast), Picrocan, Sulfate, Simazine, Turbidity, Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>Drinking water treatment facilities</td>
<td>Atrazine, Benzene, Cadmium, Cyanide, Fluoride, Lead, Polychlorinated Biphenyls, Toluene, Total Trihalomethanes, 1,1,1-Trichloroethene or Methyl Chloroform</td>
</tr>
<tr>
<td>Gas pipelines</td>
<td>cis 1,2-Dichloroethene, trans 1,2-Dichloroethene, Dichloromethane or Methylene Chloride, Tetrachloroethylene or Perchloroethylene (Perc), Trichloroethylene or TCE</td>
</tr>
<tr>
<td>Golf courses and urban parks</td>
<td>Arsenic, Atrazine, Benzene, Chlorobenzene, Carbofuran, 2,4-D, Dichl, Dalapon, Glyphosate, Lead, Methoxychlor, Nitrate, Nitrite, Picrocan, Simazine, Turbidity</td>
</tr>
<tr>
<td>POTENTIAL SOURCE</td>
<td>CONTAMINANT</td>
</tr>
<tr>
<td>------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Residential / Municipal continued</td>
<td></td>
</tr>
<tr>
<td>Housing developments</td>
<td>Atrazine, Alachlor, Coliiform, Cryptosporidium, Carbofuran, Diquat, Dalapon, Giardia Lamblia, Glyphosate, Dichloromethane or Methylene Chloride, Nitrate, Nitrite, Picloram, Simazine, Trichloroethylene (TCE), Turbidity, Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>Landfills/dumps</td>
<td>Arsenic, Atrazine, Alachlor, Barium, Benzene, Cadmium, Carbofuran, cis 1,2-Dichloroethylene, Diquat, Glyphosate, Lead, Lindane, Mercury, 1,1,1-Trichloroethane or Methyl Chloroform, Dichloromethane or Methylene Chloride, Nitrate, Nitrite, Picloram, Selenium, Simazine, Trichloroethylene (TCE)</td>
</tr>
<tr>
<td>Public buildings (e.g., schools, town halls, fire stations, police stations) and Civic Organizations</td>
<td>Arsenic, Acrylamide, Barium, Benzene, Beryllium Powder, Cadmium, Carbon Tetrachloride, Chlorobenzene, Cyanide, 2,4-D, 1,2-Dichlorobenzene or O-Dichlorobenzene, 1,4-Dichlorobenzene or P-Dichlorobenzene, Dichloromethane or Methylene Chloride, Di(2-ethylhexyl) phthalate, 1,2-Dichloroethylene or Ethylene Dichloride, Endothall, Endrin, 1,2-Dibromomethane or Ethylene Dibromide (EDB), Lead, Lindane, Mercury, Methoxychlor, Selenium, Toluene, 1,1,1-Trichloroethane or Methyl Chloroform, Trichloroethylene (TCE), Vinyl Chloride, Xylene (Mixed Isomers)</td>
</tr>
<tr>
<td>Septic systems</td>
<td>Atrazine, Alachlor, Carbofuran, Coliiform, Cryptosporidium, Diquat, Dalapon, Giardia Lamblia, Glyphosate, Nitrate, Nitrite, Oxamyl (Vydate), Picloram, Sulfate, Simazine, Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>Sewer lines</td>
<td>Coliiform, Cryptosporidium, Diquat, Dalapon, Giardia Lamblia, Glyphosate, Nitrate, Nitrite, Oxamyl (Vydate), Picloram, Sulfate, Simazine, Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>Stormwater infiltration basins/injection wells (UIC Class V), runoff zones</td>
<td>Atrazine, Alachlor, Coliiform, Cryptosporidium, Carbofuran, Chlorine, Diquat, Dalapon, Giardia Lamblia, Glyphosate, Dichloromethane or Methylene Chloride, Nitrate, Nitrite, Nitrosamine, Oxamyl (Vydate), Phosphates, Picloram, Simazine, Trichloroethylene (TCE), Turbidity, Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>Transportation corridors (e.g., roads, railroads)</td>
<td>Dalapon, Picloram, Simazine, Sodium, Sodium Chloride, Turbidity</td>
</tr>
<tr>
<td>Utility stations</td>
<td>Arsenic, Barium, Benzene, Cadmium, Chlorobenzene, Cyanide, 2,4-D, 1,2-Dichlorobenzene or O-Dichlorobenzene, 1,2-Dichloroethane or Ethylene Dichloride, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Lead, Mercury, Picloram, Toluene, 1,1,2,2-Tetrachloroethane, Tetrachloroethylene or Perchloroethylene (Perc), Trichloroethylene (TCE), Xylene (Mixed Isomers)</td>
</tr>
<tr>
<td>Waste transfer/recycling</td>
<td>Coliiform, Cryptosporidium, Giardia Lamblia, Nitrate, Nitrite, Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>Wastewater treatment facilities/discharge locations (incl. land disposal and underground injection of sludge)</td>
<td>Cadmium, Coliiform, Cryptosporidium, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Dichloromethane or Methylene Chloride, Fluoride, Giardia Lamblia, Lead, Mercury, Nitrate, Nitrite, Tetrachloroethylene or Perchloroethylene (Perc), Selenium, sulfate, Trichloroethylene (TCE), Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>Agricultural / Rural</td>
<td></td>
</tr>
<tr>
<td>Auction lots/boarding stables</td>
<td>Coliiform, Cryptosporidium, Giardia Lamblia, Nitrate, Nitrite, Sulfate, Viruses</td>
</tr>
<tr>
<td>Animal feeding operations/ confined animal feeding operations</td>
<td>Coliiform, Cryptosporidium, Giardia Lamblia, Nitrate, Sulfate, Turbidity, Viruses</td>
</tr>
<tr>
<td>Bird rookeries/wildlife feeding/migration zones</td>
<td>Coliiform, Cryptosporidium, Giardia Lamblia, Nitrate, Nitrite, Sulfate, Turbidity, Viruses</td>
</tr>
<tr>
<td>Crops - irrigated + non-irrigated</td>
<td>Benzene, 2,4-D, Dalapon, Dinoseb, Diquat, Glyphosate, Lindane, Lead, Nitrate, Nitrite, Picloram, Simazine, Turbidity</td>
</tr>
<tr>
<td>Dairy operations</td>
<td>Coliiform, Cryptosporidium, Giardia Lamblia, Nitrate, Nitrite, Sulfate, Turbidity, Viruses</td>
</tr>
<tr>
<td>Drainage wells, lagoons and Liquid waste disposal - agricultural</td>
<td>Atrazine, Alachlor, Coliform, Cryptosporidium, Carbofuran, Diquat, Dalapon, Giardia Lamblia, Glyphosate, Nitrate, Nitrite, Oxamyl (Vydate), Picloram, Sulfate, Simazine, Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>Managed forests/grass lands</td>
<td>Atrazine, Diquat, Glyphosate, Picloram, Simazine, Turbidity</td>
</tr>
<tr>
<td>Pesticide/ fertilizer storage facilities</td>
<td>Atrazine, Alachlor, Carbofuran, Chlorodane, 2,4-D, Diquat, Dalapon, 1,2-Dibromo-3-Chloropropane or DBCP, Glyphosate, Nitrate, Nitrite, Oxamyl (Vydate), Picloram, Simazine, 2,4,5-T (Silvex)</td>
</tr>
<tr>
<td>POTENTIAL SOURCE</td>
<td>CONTAMINANT</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Agricultural / Rural continued</td>
<td></td>
</tr>
<tr>
<td>Rangeland/grazing lands</td>
<td>Coliform, Cryptosporidium, Giardia Lamblia, Nitrate, Nitrite, Sulfate, Turbidity, Viruses</td>
</tr>
<tr>
<td>Residential wastewater lagoons</td>
<td>Atrazine, Alachlor, Carbofuran, Coliform, Cryptosporidium, Diquat, Dalapon, Giardia Lamblia, Glyphosate, Nitrate, Nitrite, Oxamyl (Vynate), Picloram, Sulfate, Simazine, Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>Rural homesteads</td>
<td>Atrazine, Alachlor, Carbofuran, Coliform, Cryptosporidium, cis 1,2-Dichloroethylene, trans 1,2-Dichloroethylene, Diquat, Dalapon, Giardia Lamblia, Glyphosate, Nitrate, Nitrite, Oxamyl (Vynate), Picloram, Sulfate, Simazine, Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>MISCELLANEOUS SOURCES</td>
<td></td>
</tr>
<tr>
<td>Abandoned drinking water wells</td>
<td>Atrazine, Alachlor, Coliform, Cryptosporidium, Carbofuran, Diquat, Dalapon, Giardia Lamblia, Glyphosate, Dichloromethane or Methylene Chloride, Nitrate, Nitrite, Oxamyl (Vynate), Picloram, Simazine, Trichloroethylene (TCE), Turbidity, Vinyl Chloride, Viruses</td>
</tr>
<tr>
<td>(conduits for contamination)</td>
<td></td>
</tr>
<tr>
<td>Naturally occurring</td>
<td>Arsenic, Asbestos, Barium, Cadmium, Chromium, Coliform, Copper, Cryptosporidium, Fluoride, Giardia Lamblia, Iron, Lead, Manganese, Mercury, Nitrate, Nitrite, Radionuclides, Selenium, Silver, Sulfate, Viruses, Zinc (Fume or Dust)</td>
</tr>
<tr>
<td>Underground injection control (UIC) wells CLASS I - deep injection of hazardous and non-hazardous wastes into aquifers separated from underground sources of drinking water</td>
<td>see UIC at <a href="http://www.epa.gov/safewater/uic/index.html">http://www.epa.gov/safewater/uic/index.html</a></td>
</tr>
<tr>
<td>UIC wells CLASS II deep injection wells of fluids associated with oilgas production (for more detailed list of sites click here)</td>
<td>see UIC at <a href="http://www.epa.gov/safewater/uic/index.html">http://www.epa.gov/safewater/uic/index.html</a></td>
</tr>
<tr>
<td>UIC wells CLASS III re-injection of water/steam into mineral formations for mineral extraction</td>
<td>see UIC at <a href="http://www.epa.gov/safewater/uic/index.html">http://www.epa.gov/safewater/uic/index.html</a></td>
</tr>
<tr>
<td>UIC wells CLASS IV - officially banned. Inject hazardous or radioactive waste into or above underground sources of drinking water</td>
<td>see UIC at <a href="http://www.epa.gov/safewater/uic/index.html">http://www.epa.gov/safewater/uic/index.html</a></td>
</tr>
<tr>
<td>UIC wells Class V (SHALLOW INJECTION WELLS). Click here for more information on sources of UIC Class V wells</td>
<td><a href="http://www.epa.gov/safewater/uic/cv-fs.html">http://www.epa.gov/safewater/uic/cv-fs.html</a></td>
</tr>
</tbody>
</table>
Appendix B
Where to Get More Information

GMA Hearings Boards

GMA Hearings Boards were created by the legislature to hear cases related to the Growth Management Act. The three GMA Hearings boards are for Eastern Washington, Western Washington, and Central Puget Sound. Critical Areas planning and ordinance decisions are subject to review by the board. The board hears cases when a "Petition for Review" is filed.

The GMA Hearings Board website, http://www.gmhb.wa.gov/, contains a wealth of information about the boards and how they work. Each of the boards has a decision digest where decisions that effect critical aquifer recharge areas (CARAs) and other growth management issues can be viewed on-line.

You may also contact the Hearings Board as follows:

<table>
<thead>
<tr>
<th>Central Puget Sound</th>
<th>Western Washington</th>
<th>Eastern Washington</th>
</tr>
</thead>
<tbody>
<tr>
<td>King, Snohomish, Pierce and Kitsap counties</td>
<td>Western Washington counties not in the central Puget Sound</td>
<td>Counties and cities east of the crest of the Cascade Mountains</td>
</tr>
<tr>
<td>900 4th Avenue Suite 2470 Seattle, WA. 98164</td>
<td>905 24th Way SW, Suite B-2 Olympia, WA 98502</td>
<td>15 West Yakima, Suite 102 Yakima, WA 98902</td>
</tr>
<tr>
<td>Tel: (206) 389-2625 Fax: (206) 389-2588 E-mail to: <a href="mailto:central@cps.gmhb.wa.gov">central@cps.gmhb.wa.gov</a></td>
<td>P.O. Box 40953 (MS: 40953) Olympia, WA. 98504-0953 Tel: (360) 664-8966 Fax: (360) 664-8975 E-mail to: <a href="mailto:western@ww.gmhb.wa.gov">western@ww.gmhb.wa.gov</a></td>
<td>Tel: (509) 574-8980 Fax: (509) 574-6964 E-mail to: <a href="mailto:AAndreas476@EW.GMHB.WA.GOV">AAndreas476@EW.GMHB.WA.GOV</a></td>
</tr>
</tbody>
</table>

Ten counties and their included cities in Washington are not required to plan fully under the GMA. These jurisdictions must still plan for Critical Areas and Natural Resources Lands. These ten counties are Adams, Asotin, Cowlitz, Grays Harbor, Klickitat, Lincoln, Okanogan, Skamania, Whitman, and Wahkiakum.

GMA Laws and Regulations


The Revised Code of Washington and the Washington Administrative Code are also available at public libraries.
Planning Resources

Washington State Department of Community, Trade, and Economic Development is responsible for administering the Growth Management Act and has many helpful documents on-line: http://www.cted.wa.gov/portal/alias_CTED/lang_en/tabID_375/Default.aspx

The Municipal Research & Services Center of Washington has vast amounts of helpful on-line information for counties and cities, including Growth Management Act information: http://www.mrsc.org/

Recognition and technical assistance programs

Envirostars: http://www.envirostars.org/

Best available science for critical aquifer recharge areas


Local Pollution Prevention Information

King County Hazardous Waste: http://www.govlink.org/hazwaste/

Thurston County Hazardous Waste: http://www.co.thurston.wa.us/health/ehrphwaste.html

Spokane Joint Aquifer Board: http://www.spokanaquifer.org/Factsheet3_aquifer.pdf

Geologist/Hydrogeologist Licensing Laws and Regulations

Department of Licensing Geology License Website: http://www.wa.gov/dol/bpd/geofront.htm

Washington State Code Reviser Website, with links to RCWs and WACs: http://slc.leg.wa.gov/

Both of the above websites have links to the Geologist Licensing laws and regulations: RCW 18.220 and WAC 308-15.
State Ground Water Protection Programs

Washington State Department of Health Drinking Water Program

The Department of Health is responsible for administering the Safe Drinking Water Act program for the state. This includes regulating public water supplies and administering the Source Water Protection program.

The federal Safe Drinking Water Act includes provisions for preventing contamination of drinking water for Group A public water supply systems (15 or more connections).

Drinking Water Program website: http://www.doh.wa.gov/ehp/dw/default.htm

Source Water Protection website:

Here is a quote from this website:

_The SWAP program will result in an evaluation of the source water that provides drinking water to Group A public water systems in Washington state. This evaluation will estimate the degree to which a given public water source is at risk from contamination. Once completed, the assessment results will be used to assist local communities in targeting and implementing protection measures such as Best Management Practices (BMPs), zoning overlays, critical area ordinances and public education. The information can also be used to help focus technical assistance outreach efforts and compliance inspections._

Chapter 173-200 WAC, Ground Water Quality Standards,

Implementation Guidance for the Ground Water Quality Standards,


Chapter 173-303 WAC, Dangerous Waste Regulations,
http://www.ecy.wa.gov/biblio/wac173303.html

Washington State Department of Ecology Sand and Gravel General Permit:

Critical Aquifer Recharge Area Guidance
Washington State Underground Injection Control Program

- Website: http://www.ecy.wa.gov/programs/wq/grmdwtr/uic/index.html

Federal Ground Water Programs

U.S. Environmental Protection Agency - Drinking Water Program

EPA Safe Drinking Water Act Maximum Contaminant Levels (MCLs),
http://www.epa.gov/safewater/mcl.html

Delineation and Vulnerability Methods


http://www.epa.gov/safewater/uic/tad_sensitive_gw.pdf


FUGRO Airborne Surveys Groundwater Recharge Mapping

This is a private company who uses airborne geophysics for ground water and geology mapping. The method is used for TDS mapping, saltwater intrusion, water depth, and recharge area mapping. This is included here as an example of an airborne geophysical method for mapping recharge areas, and does not imply an endorsement.
Eastern Washington Studies


On-line USGS publication index, Columbia Basin NAWQA:
http://wa.water.usgs.gov/ccpt/pubs/

On-line USGS publication index, Yakima Basin NAWQA:
http://oregon.usgs.gov/projs_dir/yakima/pubs.html

Western Washington Studies


On-line USGS publication index, Puget Sound NAWQA:
http://wa.water.usgs.gov/ps.publication.index.html

Ground Water Quality Studies


Saltwater Intrusion

Impervious Surfaces


Database Sources

Washington State Department of Ecology on-line:

- Facility/Site:  [http://www.ecy.wa.gov/services/as/iss/fsweb/fshome.html](http://www.ecy.wa.gov/services/as/iss/fsweb/fshome.html)
  Facilities and sites that are regulated by the Department of Ecology.
  Environmental Information Management System; sampling, measurements, and monitoring results. The link to query EIM on-line and download data is on this page.

Washington State Department of Health on-line:

The Department of Health is launching a new website for water system data. The website is not yet available as of publication of this document. For currently available information on-line, and to check for new developments, see the Drinking Water Program website at [http://www.doh.wa.gov/ehp/dw/default.htm](http://www.doh.wa.gov/ehp/dw/default.htm).

U.S. Geological Survey on-line:

  This bibliography contains references to published reports, maps, journal articles, and proceedings related to the water resources of Washington State as published by the U.S. Geological Survey, or in cooperation with other Federal or State agencies.
  NWIS, or National Water Information System, is the USGS on-line database for water resources data. This site contains data collected in the state of Washington, including groundwater and surface water data and the associated water-quality data, meteorological data, and site information.
U.S. Department of Agriculture Natural Resource Conservation Service on-line:

- NRCS soils website: http://soils.usda.gov/
- NRCS snow survey and water resources information: http://www.wa.nrcs.usda.gov/snow/index.html

The NRCS Snow Survey Program provides mountain snowpack data and stream flow forecasts for the western United States.

Common applications of snow survey products include water supply management, flood control, climate modeling, recreation, and conservation planning.

Appendix C
Selected GMA Hearings Board Decisions

Examples of GMA Hearings Board Decisions from the Digests

Each GMA Hearings Board publishes a digest of decisions that makes it easier to find decisions related to various topics. Here are some examples of decisions from the digests from the Central Puget Sound, Western Washington, and Eastern Washington Growth Management Hearings Boards:

**Mapping and performance standards:**

Central Puget Sound:

"The use of performance standards is recommended in the Minimum Guidelines for ... circumstances where critical areas (e.g., aquifer recharge areas, wetlands, significant wildlife habitat, etc.) cannot be specifically identified." WAC 365-190-040(1). However, where critical areas are known, cities and counties cannot rely solely upon performance standards to designate these areas. [Pilchuck II, 5347c, FDO, at 41-42.]

**Local government discretion and the GMA framework:**

Western Washington:

The GMA provides that ultimate planning decisions rest with the local government. Such decisions are not unfettered but must be within the range of discretion allowed by the GMA. A GMHB does not substitute its judgment as to the best alternative available, but reviews the local government action to determine if it complies with the goals and requirements of the GMA. CCNRC v. Clark County 92-2-0001 (FDO 11-10-92)

Eastern Washington:

The Act requires protection of critical areas, and the county is given the opportunity to select the manner of that protection. Their choice is given great deference. Easy, et al. v. Spokane County, EWGMHB 96-1-0016, Order on Compliance (Sep. 23, 1997).

**What protecting Critical Areas (CA) means:**

Central Puget Sound:

The Act's directive that local governments are to "protect" critical areas means that they are to preserve the structure, value and functions of wetlands, aquifer recharge areas used for potable water, fish, and wildlife habitat conservation
areas, frequently flooded areas and geologically hazardous areas. [derived from WAC 365-195-825(2)(b)] [Pilchuck II, 5347c, FDO, at 20.]

Western Washington:

The GMA requirement to protect CAs directs a local government to adopt appropriate and specific criteria and/or standards. Willapa v. Pacific County 99-2-0019 (FDO 10-28-99)

Compliance monitoring and enforcement:

Western Washington:

If BMPs are relied upon for protection of CAs some form of monitoring and enforcement must be included to ensure that the plans are actually implemented and followed. ARD v. Shelton 98-2-0005 (FDO 8-10-98)

Eastern Washington:

Further, laws can be so vague that they simply are unenforceable. That is the case here. Such an ordinance cannot satisfy GMA’s duty to adopt enforceable “development regulations” to “protect” critical areas. A person should be able to determine what the law is by reading the published code. Ordinance no. 109-2003 (ICAO) relies on language too vague to create an enforceable standard and therefore cannot not operate to “control” land use activities and does not satisfy the county’s GMA obligation to adopt “development regulations” to protect critical areas. The enforcement measures adopted by the county provide only for ad hoc enforcement. This does not constitute a reasoned adaptive management program, particularly where, as here, there is no provision for the monitoring of compliance. Larson Beach Neighbors and Jeanie Wagenman v. Stevens County, EWGMHB 00-1-0016, EWGMHB, Order on Compliance, November 13, 2003.

Critical Aquifer Recharge Areas:

Eastern Washington:

The GMA directs counties to designate, classify and protect areas with a “critical recharging effect on aquifers used for potable water.” It is necessary to determine the location of recharge areas as a first step in designating and protecting them. The county must provide criteria necessary to indicate when an area needs specific scientific analysis to determine whether it is a critical aquifer recharge area. Save Our Butte Save Our Basin Society, et al. v. Chelan County, EWGMHB 94-1-0015, Compliance Hearing Order (Apr. 8, 1999).
Critical Areas and pre-existing land uses:

Western Washington:

A local government must regulate preexisting uses in order to fulfill its duty to protect critical areas. GMA requires any exemption for preexisting use to be limited and carefully crafted. PPF v. Clallam County 00-2-0008 (FO 12-19-00).

Critical Areas and best available science:

Central Puget Sound:

When any local government in the Central Puget Sound region adopts amendments to policies and regulations that purport to protect critical areas pursuant to RCW 36.70A.060(2), those enactments will be subject to meeting the best available science requirements of RCW 36.70A.172 and the potential of appeal to this Board pursuant to RCW 36.70A.280. [Tulalip II, 9313, 1/28/00 Order, at 4.]
Appendix D
Example Costs and Consequences of Ground Water Contamination

Former Pacific wood treating site (at the Port of Ridgefield)

A former Port tenant using wood treating chemicals contaminated ground water beneath the Port of Ridgefield and the National Wildlife Refuge. Sediments of Lake River have also been contaminated. The cost of this clean up has been estimated at $40 to $50 million dollars.


City of Tumwater, Palermo Well Field

In 1993, the city of Tumwater, Palermo well field was contaminated with trichloroethylene (TCE) and was threatened by perchloroethylene (PCE). Three of the city’s six wells were removed from service and replaced with two new wells at another location. The total cost for dealing with solvent contamination was $3.9 million dollars, the cleanup took more than six years, and three of six city wells were closed for five years.

Sources:
http://www.crcwater.org/issues5/0#0, from an article by Joel Coffidis in the Daily Olympian, July 1, 1998: WATER SAFETY: EPA tests pinpoint the origin of the toxic chemicals that have closed three wells.


Alexander Farms

In 1998, yellow water was found coming out of two domestic wells in the vicinity of the Alexander Farms site. The source was from a spill of dinoseb, a pesticide. More than 12,000 tons of Dinoseb-contaminated soils were excavated. The costs to owners for cleanup were said to top $1 million (Tri-city Herald, 4/26/2002).
**Boomsnub electroplating facility**

The Boomsnub Corporation is an electroplating facility located in Vancouver, Washington. Repeatedly and illegally, it has disposed of spent hexavalent chrome into the environment. As a result, the entire water supply for the city of Vancouver and the Clark County area was threatened.

The groundwater remediation project cost more than $3 million to the Washington State taxpayers. At least six thousand tons of highly contaminated soils have been removed. In 1995, it was estimated that $10 million would be spent in an attempt to save the city of Vancouver water supply.

**Sources:**

**Walkerton, Ontario, Canada**

The town of Walkerton, Ontario, Canada, endured a horrible contamination event that killed seven people and sickened hundreds in May 2000. The cause was the application of manure to fertilize a field near a town well. The town well was supposed to be monitored, but the person who was supposed to sample and make sure the well was chlorinated had fallen into improper practices.


> [Improper practices] included mislabeling sample bottles for microbiological testing, failing to adequately chlorinate the water, failing to measure chlorine residuals daily, making false entries on daily operating sheets, submitting false annual reports to the Ministry of the Environment (MOE), and operating wells without chlorination.

A groundwater protection program would have identified the well location and its importance, and would have identified the risk inherent in allowing manure to be spread on adjacent land. Only compliance monitoring would have caught the inadequate practices of the water system operator. Compliance monitoring is important for operators of facilities that are contamination risks for the same reasons.

A cost estimate of the remediation alone was set at $9,222,215. A fuller explanation of the costs is available at [http://www.newswire.ca/releases/November2001/26/c0319.html](http://www.newswire.ca/releases/November2001/26/c0319.html).

Appendix E
Example County Fact Sheets for Pollution Prevention

Photos courtesy of U.S. EPA Region 10

The following fact sheet is copied from the Thurston County Business Pollution Prevention Program Fact Sheet - Floor Drains (February 2004),
http://www.co.thurston.wa.us/health/ehtrp/hwaste.html

Floor Drains

“Convenient disposal to floor drains today can lead to expensive clean-up costs later when you try to sell or refinance your property.”

The Problem

Many types of businesses use floor drains as an easy way to dispose of floor cleaning or other wastes. What many business owners do not realize is that putting wastes down floor drains may violate the Thurston County Nonpoint Source Pollution Ordinance and several other federal and state laws.

Many floor drains send untreated wastes directly to storm drains, septic systems, dry wells, pits, or ditches. When wastes enter these types of drains, they pass through soil and may enter groundwater, or may enter streams or lakes directly -- they do not necessarily go to a sewage treatment plant. Moreover, even a treatment plant cannot effectively handle many types of wastes.
When commercial property is sold or refinanced, finance companies often require an environmental site assessment that evaluates whether property is contaminated. Convenient disposal to floor drains today can lead to expensive clean up costs later when you try to sell or refinance your property. Irresponsible disposal can also lead to contaminated drinking water that may affect your community and your family.

What You Can Do

Find out where your floor drains go. Consider each drain separately. Call your city or county public works department or local sewer utility and ask for help in identifying where your drains lead. If your business was built before 1970, or is located in a rural area, your floor drains most likely do not lead to a sanitary sewer.

If your floor drain is already connected to a sanitary sewer. You still need to meet local sewer discharge limits. All discharges to a sewer system are authorized by the LOTT Wastewater Alliance. LOTT may be reached at 753-8428.

If your floor drain is not connected to a sanitary sewer. Contact the Thurston County Business Pollution Prevention Program for help in determining if you have a pollution problem from this floor drain. Two options to consider for non-connected floor drains.

1. Connect the floor drain to a sanitary sewer and meet sewer discharge limits; or
2. Seal the floor drain and change your current disposal practices.

Practical tips to eliminate the use of and, therefore, the need for a floor drain:

3. Sweep and spot-mop floors frequently to minimize the need for complete floor cleaning. Improve general housekeeping practices.

4. Build a "dead-end" sump where occasional wastewater can collect and either evaporate or be pumped into a dedicated and labeled container. Sludge that may build up in the sump will need to be removed and possibly managed as hazardous waste.

5. If you must keep a floor drain, consider installing a recirculating floor scrubber or "closed-loop" wastewater recycling system, or investigate treatment-by-generator options.

Keep a Record of Your Actions

If you seal off a floor drain, create a record of past uses for the drain, the date when the drain was sealed, and describe the physical location of the drain before it was sealed. Keep records of flow into any drains that are connected to a sanitary sewer to demonstrate that you have met discharge permit requirements. This information may be important if the property is offered for sale, and it could be useful in reducing liability in the case of an investigation of contaminated soil or ground water.

Additional Information

Staff from the Thurston County Business Pollution Prevention Program is available to answer questions about floor drains, best management practices for wastewater, and other hazardous
waste related issues. We also offer free, non-regulatory on-site technical assistance. Please contact the Business Pollution Prevention Program at (360) 786-5457, Monday through Friday, from 8 a.m. to 5 p.m., or at TDD (360) 754-2933 or see our website at http://www.co.thurston.wa.us/health/ehr/hwaste.html.

Other Hazardous Waste Management and Disposal Fact Sheets

- Antifreeze, Used Oil, and Oil Filters
- Compliance with Nonpoint Source Pollution Ordinance
- Disposal of Petroleum-Contaminated Absorbent Materials
- Doing Business in a Wellhead Protection Area
- Hazardous Waste Management in Printing and Photography
- Secondary Containment
- Solvents and Parts Cleaners
- Spill Plans
- Storing and Labeling Hazardous Waste
- Used Shop Towels

February 2004

The following fact sheet is copied from the Thurston County Business Pollution Prevention Program Fact Sheet - Secondary Containment (February 2004), http://www.co.thurston.wa.us/health/ehr/hwaste.html

Secondary Containment

"Liquid hazardous materials such as petroleum products, antifreeze, and solvents can present a threat to soil, ground water, and surface water."

The Problem

Liquid hazardous materials such as petroleum products, antifreeze, and solvents can present a threat to soil, ground water, and surface water if accidentally spilled or leaked. These substances must be stored so that if a spill or leak does occur, the material remains contained and does not contaminate the environment. A solution to the problem is to use secondary containment when storing hazardous liquids.

The Regulatory Requirements

The Thurston County Nonpoint Source Pollution Ordinance (Article VI of the Sanitary Code) requires that hazardous waste, including petroleum products, be stored so that if a container leaks or ruptures the contents will not contaminate ground or surface water. The best way to ensure this is to provide secondary containment for all containers of liquid hazardous products and wastes.
The Thurston County Critical Areas Ordinance Chapter 17.15.520 C(2) also requires businesses that are located in aquifer recharge areas to provide secondary containment for hazardous materials that are stored on-site.

The Thurston County Mineral Extraction Code requires that fuel and hazardous materials are stored according to the requirements of the Nonpoint Source Pollution Ordinance. The Department of Ecology requires coverage and containment of hazardous materials through the “National Pollutant Discharge Elimination System and State Waste Discharge General Permit for Process Water and Stormwater Associated with Sand and Gravel Operations and Asphalt Batch Operations” RCW Chapter 90.48.

**What is Secondary Containment?**

Secondary containment is a liquid-tight barrier that will adequately contain hazardous materials that are released from a storage container. A simple example of secondary containment is placement of a 5-gallon drum (primary containment) inside a 55-gallon drum (secondary containment). Another example is placement of 55-gallon drums or a large fuel tank (primary containment) inside a liquid-tight concrete bunker (secondary containment). The outer wall of a double-walled fuel storage tank is also an example of secondary containment.

The size and design of a secondary containment unit or device depends on the type and amount of material that it holds.

**The Options**

Four secondary containment method options will satisfy Thurston County regulatory requirements. Liquid hazardous materials, including petroleum products, can be:

1. Stored indoors on a liquid-tight concrete floor without secondary containment if the storage area is able to contain 100 percent of the largest container in the event of a spill and prevent it from flowing or leaking out of the building. Also, spilled or leaked materials must be prevented from entering floor drains that are not part of a liquid-tight containment system designed to capture and hold hazardous materials.

2. Stored in outdoor or indoor covered secondary containment that can hold 110 percent of the volume of the largest storage container or 10 percent of the total volume stored, whichever is greatest, plus the displacement volume of any items inside the containment.

3. Stored in outdoor uncovered secondary containment that can hold 120 percent of the volume of the largest storage container or 10 percent of the total volume stored, whichever is greatest, plus the displacement volume of any items inside the containment.

4. Stored in UL-certified double-walled storage tanks. The volume requirements that are listed in options 1, 2 and 3 do not apply to these storage tanks, because they do not require additional containment provisions.
Secondary Containment Criteria

Chemical Compatibility and Structural Integrity

- The structural materials used in secondary containment units, including expansion joints and seals (if applicable) must be chemically compatible with the substance(s) that will be contained.
- Secondary containment must be maintained liquid-tight at all times, except when draining storm water under direct supervision (see below).
- Secondary containment must be physically adequate to hold a release and remain liquid-tight.
- Discharge valves must be closed and locked when not in use. The key or combination for the lock must be kept on-site and available during all work shifts.

Stormwater Accumulation and Discharge

- Stormwater must be managed in accordance with the Thurston County Drainage Design and Erosion Control Manual.
- Outdoor uncovered containments must be maintained free of stormwater accumulation.
- An operator must be present during stormwater discharge from secondary containment.
- All discharge valves must be closed and locked after a supervised discharge is completed.
- Stormwater discharged from petroleum product secondary containment must be treated through an oil/water separator. (See the Thurston County fact sheet “Oil/Water Separators.”)

Spills

Keep secondary containment areas free of small spills and drips. Drip pans that can be conveniently cleaned are helpful in preventing contamination of the secondary containment area. Hazardous materials, liquid hazardous waste and petroleum product spills must be cleaned up immediately.

Remember that even a small spill, drip, or leak must be cleaned up and disposed of as a hazardous waste. Absorbents and other cleanup materials that are contaminated must also be managed as a hazardous waste. Up to 55 gallons of petroleum-contaminated absorbents may be deposited at the Thurston County Waste and Recovery Center annually, as explained in the Thurston County fact sheet “Disposal of Petroleum Contaminated Absorbent Materials.”

Additional Information

Please call the Thurston County Business Pollution Prevention Program at (360) 786-5457 or TDD 754-2933 or see our website at http://www.co.thurston.wa.us/health/ehrp/hwaste.html.

June 2004
Tapping unsustainable groundwater stores for agricultural production in the High Plains Aquifer of Kansas, projections to 2110

David R. Steward (search?author=1+DavidR.+Steward&sortspec=date&submit=Submit)
Paul J. Brusius (search?author=1+PaulJ+.Brusius&sortspec=date&submit=Submit)
Xiaoying Yang (search?author=1+XiaoyingY.+Yang&sortspec=date&submit=Submit)
Scott A. Staggenborg (search?author=1+ScottA.+Staggenborg&sortspec=date&submit=Submit)
Stephen M. Welch (search?author=1+StephenM.+Welch&sortspec=date&submit=Submit)
Michael D. Apley (search?author=1+MichaelD.+Apley&sortspec=date&submit=Submit)

Author Affiliations

Edited by Dieter Garten, Potsdam Institute for Climate Impact Research, Potsdam, Germany, and accepted by the Editorial Board July 16, 2013 (received for review November 20, 2012)

Significance

Society faces the multifaceted crossroads dilemma of sustainably balancing today’s livelihood with future resource needs. Currently, agriculture is tapping the High Plains Aquifer beyond natural replenishment rates to grow irrigated crops and livestock that augment global food stocks, and science-based information is needed to guide choices. We present new methods to project trends in groundwater pumping and irrigated corn and cattle production. Although production declines are inevitable, scenario analyses substantiate the impacts of increasing near-term water savings, which would extend the usable lifetime of the aquifer, increase net production, and generate a less dramatic production decline.

Abstract

Groundwater provides a reliable tap to sustain agricultural production, yet persistent aquifer depletion threatens future sustainability. The High Plains Aquifer supplies 30% of the nation’s irrigated water, and the Kansas portion supports the congressional district with the highest market value for agriculture in the nation. We project groundwater declines to assess when the study area might run out of water, and comprehensively forecast the impacts of reduced pumping on corn and cattle production. So far, 30% of the groundwater has been pumped and another 39% will be depleted over the next 50 y given existing trends. Recharge supplies 15% of current pumping and would take an average of 900–1,300 y to completely refill a depleted aquifer. Significant declines in the region’s pumping rates will occur over the next 15–20 y given current trends, yet irrigated agricultural production might increase through 2040 because of projected increases in water use efficiencies in corn production. Water use reductions of 20% today would cut agricultural production to the levels of 15–20 y ago, the time of peak agricultural production would extend to the 2070s, and production beyond 2070 would significantly exceed that projected without reduced pumping. Scenario analyses evaluate incremental reductions of current pumping by 20–40%, the latter rate approaching natural recharge. Findings substantiate that saving more water today would result in increased net production due to projected future increases in crop water use efficiencies. Society has an opportunity now to make changes with tremendous implications for future sustainability and livability.

http://www.pnas.org/content/110/37/E3477.full

7/23/2015
Groundwater provides a reliable water supply that has contributed to the intensification of agriculture and increased food production occurring over the past 50 y (1). Large increases in crop and livestock production commonly co-occur with associated aquifer depletion throughout the semiarid grasslands of the world (2, 3) Yet, the gains in agricultural productivity achieved through tapping groundwater beyond the rate of replenishment threaten its long-term prospects (4). Water is a precious, unique resource that is important for life and a commodity for which no substitute exists (5).

Humanity faces the challenge of balancing the water needs of the present with the long-term needs of the future (6, 7). The consequences of our actions and responses to dealing with the water demands of today and those associated with future changes in population and economic development will overshadow the impacts of changes in climate on future water supplies (8). Although consumption of freshwater supplies has not yet crossed a potentially dangerous planetary threshold (9), crop yields have begun to fall in many regions because of water scarcity, and global food security remains a worldwide concern (10). There is a clear need for society to become prepared for the consequences of reductions in groundwater use that shall occur in the foreseeable future.

The wise management of groundwater resources requires a more comprehensive understanding of the relationships between aquifer pumping and the capacity of the terrestrial environment to provide ecosystem goods and services upon which society depends (8, 11). Informed decision making in processes involving the control and feedbacks between society and ecosystems is founded on increased understanding of the relevant interdisciplinary linkages (3, 12). Human activity has a significant impact on the structure and function of the earth (13), and changes driven by economic development and population growth are occurring faster than our understanding (14). We developed integrated methods to forecast groundwater depletion into the future, to retain the impacts of pumping on the crop and livestock sectors, and to study the impacts of changes in water use on agricultural production We show that water limitations will begin to have a significant impact on food production over the next few decades; yet, the changes we might implement today could significantly alter future possibilities

An Integrated System with Groundwater Depletion Supplying Irrigated Corn and Cattle Production

The consequences of aquifer depletion are studied in a region of national and international importance for agricultural production. The High Plains Aquifer in western Kansas lies in the arid region of the central plains of the United States, which was formed by John Wesley Powell (15) to need irrigation for successful agriculture. The region experiencing the worldwide tragedy of the commons, with aquifer depletion from a common pool resource used to support irrigated agriculture (16), is one of four "critical areas" for "annual renewable water" in the western hemisphere and one of 22 worldwide (17). Irrigation began in the late 1800s and intensified through the 1900s (18, 19). As a result, the western Kansas congressional district has the highest total market value of agriculture products in the nation (20). Corn-based cattle revenues far overshadow those from other agricultural sectors (21), and the important region supports the second highest state inventory for cattle on feed (22).

The study region lies near the geographical center of the contiguous United States in the central plains of Kansas, and its data sources are illustrated in Fig. S1 (footsupp supplied 1.0.0.37/1.0.0.37/pnas 20122203615.0.0.37/1.0.0.37/1.0.0.37). Mean annual precipitation varies from less than 0.5 m y in western Kansas to over 1.5 m toward the southeast, and land elevation decreases from over 1,200 m above mean sea level (m.s.l.) in the west to 250-300 m in eastern Kansas. Groundwater is readily tapped in the drier west where the High Plains Aquifer and the natural pattern of irrigated corn production follows that of groundwater use. Cattle production is focused near irrigated corn and within the west's higher elevations, where cool nights and lower humidity help cattle dissipate heat and maintain high growth potential within the summer and warmer, drier conditions help maintain production in the winter. Although many data sources are reported annually for Kansas's 105 counties, results are aggregated in this study to the nine agricultural districts (shown in Fig. S1 with 2000 data).

Projections of aquifer depletion are illustrated in Fig. 1. Our methods are articulated in Appendix A along with a description of how they compare with previous studies. Briefly, measurements of groundwater level in observation wells are fit to a logistic curve for each well to approximate water level change over time; these projected values are triaged across all wells at fixed times to provide a set of groundwater level surfaces, and the volume of water between surfaces gives change in storage. The saturated thickness in Fig. 1A exhibits a persistent declining trend that began before 1980 and continues for the foreseeable future. Groundwater measurements are plotted in Fig. 1B using dimensionless variables that enable all data to be plotted on one graph; the logistic function very accurately reproduces the observations. (The average absolute difference between the observed and approximated levels is 1.52 m across all measurements in all wells, from Eq. 3 in Appendix A.) These methods also result in estimates of the computed volume of groundwater use in Fig. 1C that very accurately reproduce previous studies of aquifer depletion (in Table S1 (footsupp supplied 1.0.0.37/1.0.0.37/pnas 20122203615.0.0.37/1.0.0.37/1.0.0.37/1.0.0.37/1.0.0.37))...
only 70% remained in 2010, and the declining trend continues through 2110 and beyond. Three distinct stores exist: the west central district has experienced a larger fraction of depletion and subsequent decreases in well yields, whereas larger predevelopment stores in the southwest and northwest will begin to experience regional limitations in the capacity to pump over the next two decades given current trends. Future pumping approaches a long-term asymptotic limit equal to the rate of recharge (R), which is 0.01 x 10^6 m^3/y, and 15% of current pumping. If existing trends continue to total depletion, then, depending on the district, projected replenishment times would average between 500–1,300 y (obtained from the volume of predevelopment groundwater storage, S, divided by the annual volume of recharge, R). Although refilling generally is recognized as being a long-term proposition, this method provides holistic estimates as to how long it actually might take, although spatial and temporal heterogeneities would result in some areas recovering more quickly and others more slowly.

Groundwater-supported agriculture has led to vertically integrated regional industries, in which economic forces drive irrigated corn production to support a concentration of cattle feedlots that provide a continuous flow of supply for slaughterhouses (24). Recent increases in cattle production (Fig. S3 (footsupplid:10.1073/pnas.1220351110)) reflect a national redistribution to this region with proximity to slaughterhouses, inherent climate for cattle production, and abundant feed (Fig S1 (footsupplid:10.1073/pnas.1220351110)). Although North America supplies over 40% of the global supply of corn (25), variability in precipitation has a significant impact on dryland production (Fig. S2 (footsupplid:10.1073/pnas.1220351110)). This is observed in Fig. 2, in which irrigated and dryland corn production are plotted along with the corn consumed by cattle on feed. Cattle consumption far exceeds dryland production, and its volatility makes it an unreliable source for feedlots. The increases observed in irrigated corn production are the result of both increased crop water use efficiencies and farming practices in which a larger fraction of irrigated land is being used to grow corn (Fig. S4 (footsupplid:10.1073/pnas.1220351110)). We incorporate both drivers of change in our model relating groundwater pumping and irrigated corn production in Eq. 11 to quantify the impacts of groundwater limitations on the region’s agricultural production.
The historical trends in groundwater pumping and agricultural production are projected into the future in Fig. 3. The existing trends in groundwater use for each agricultural district are plotted using the same curves as those in Fig. 1C from 1980 to 2110. Future pumping rates reflect reductions in the capacity to extract large discharges of groundwater that have been in use in central Kansas and must occur throughout the region as the aquifer water levels decline and pumping eventually approaches, at most, the rate of recharge. A set of water reduction scenarios limit current water use by factors of 20%, 40%, 60%, and 80%. Reducing pumping rates in 2010, $Q_{2010}$, by these percentages leads to greater future groundwater availability, which is quantified using a coefficient $D = (Q_{2010} - R)/(Q_{2010} - R)$ for each scenario that equals the reduction in groundwater removed from storage. Note that an 80% reduction represents pumping at close to, but slightly more than, the recharge rate, $R$, in each district. For each scenario, the pumping rate in each 5-y interval is reduced to the recharge rate plus $D$ times the groundwater removed from storage ($Q_{2010} - R$) and the time period is extended by 10. These factors conserve mass balance because the same volume of aquifer is just dewatered over a longer time, and each curve reduces future well pumping to eventually approach recharge. The dewatered volume is computed for each scenario through 2110, and the remaining groundwater in storage is reported in Fig. 3 for each scenario.

A: Dewatered volume is less than or not reached in 2010
B: Pumping rate is less than or not reached in 2010
C: Dewatered volume is less than or not reached in 2010

Fig. 2.

In a new window (E3477/F2 expansion.html) | Download PPT (e.powerpoint/110/37/E3477/F2)

Cattle production in western Kansas increased over the past few decades. Overall, cattle consumed more corn than was produced in the region throughout the 1980s, and more recently, the hard red has leveled off and regional corn production (irrigated plus dryland) is approximately equal to the net corn consumption by cattle.

Fig. 2.

In a new window (E3477/F2 expansion.html) | Download PPT (e.powerpoint/110/37/E3477/F2)

An integrated system with cattle consuming irrigated corn grown with groundwater (A). A set of hypothetical yet realistic scenarios are developed to illustrate how changes in water use would affect agricultural production (B and C). The groundwater pumping follows current trends from Fig. 1, and optional scenarios are developed that scale the current annual water use by a factor and then extend the time of aquifer depletion. Thus, the same volume of water eventually would be extracted from the aquifer for each scenario. The water use efficiencies in Fig. 54 also enable prediction of the additional corn produced from irrigation, as well as the number of cattle the value-added corn production would support. The two upper right graphs (C) show only the southwestern district for clarity, and the same graphs are repeated in Fig. 55 (lookupPubPollDOC:10.1073/pnas.1220351110/DCSupplemental/pnas.201220351S1.pdf?targetId=namedDest=SFS) along with the other two districts. The integral of corn and cattle production...
The corn produced from irrigation is computed by multiplying the groundwater use Q in Eq. 3 by the water use efficiency \( W \) from Fig. S4C (footnote/supp/1104.1073/pnas.1223551110-04CSupplemental/pnas.201223551S1.pdf)
target=\_blank\_target=S4C) and gives confidence of the functional form in Eq. 11. The good fit of these curves to data also supports the recharge rates in Fig. 1C, as changes in \( R \) would result in shifting the confidence interval higher or lower along the y-axis. The net median corn produced from irrigation from 2010 to 2110 is tabulated in Fig. 3 for each scenario, along with the corn that could be grown in 2110 using the remaining groundwater storage.

The cattle production supported by groundwater is computed by multiplying the irrigated corn production by the corn fed per head of cattle, \( F \) in Eq. 7. Results are aggregated to the High Plains region of western Kansas, and the median and confidence intervals are computed from the sum of corn production across the three irrigation districts. Data are plotted for the number of cattle obtained by multiplying the tabulated head of cattle in January by the factor in Eq. 6. Note that the number of cattle is larger than those supported by irrigation, because the irrigation calculations do not include dryland corn production nor the component of irrigated corn resulting from precipitation. Data are also plotted for the number of cattle minus these dryland corn components times \( F \). Results reflect the trends on which cattle consumed more corn than was produced in Kansas from 1990 to 1995, and more recent cattle consumption is approximately the total corn crop. The net cattle production from irrigation is tabulated in Fig. 3 for each scenario for corn grown from 2010 to 2110 and also for the corn production remaining in 2110 (e.g., current trends gave projected median production of 414 million head (Mhead) of cattle through 2110 along with the net production capacity from groundwater stores remaining at the end for an additional 138 Mhead). Results show a net increase in cattle production with increasing water reductions today.

The Challenge of Stemming Groundwater Declines Today to Sustain Agriculture’s Future

Adoption of groundwater and agricultural management actions that move toward balancing current and future benefits requires a better understanding of the impacts of groundwater depletion and increased interdisciplinary understanding of the consequences of change (27). Irrigation practices in the region are adapting to groundwater depletion and reduced pumping capacity by transitioning from full irrigation to limited irrigation rates on the same land area, by decreasing irrigated acreage, and by applying presoak irrigation to increase the duration of pumping (2). Such adaptive strategies reduce the risk of crop failure and are observed in the west central district, where increased water limitations have promoted higher water use efficiencies than in the other districts (Table S2). The water laws that affect groundwater pumping practices and data are recorded by Kansas Statutes Annotated (K.S.A.), the Kansas Water Appropriation Act (K.S.A. 82a-701) defines a water right as a real property right to lawfully divert and use water, and annual water use reports have been required for every water right in Kansas since 1981 (K.S.A. 82a-733). These data are available publicly via the Water Rights Information System (WRIS) database at the Kansas Division of Water Resources, and the annual groundwater pumping data points in Fig. 1C were obtained by summing this reported water use for the wells in each agricultural district. A steep increase in reported pumping is observed through 1981 (Illustrated in Fig. 1C); these recopetered water use reports do not reflect the consistent levels of corn grain production and acres harvested for several years before and after 1981 observed in US Department of Agriculture (USDA) National Agricultural Statistics Service (NASS) data. Groundwater use declined slightly after 1981, and this may be the result of wells taken out of production because of depleted groundwater (as has occurred in portions of west central Kansas). However, it also may be a consequence of more accurate water use reports as a result of the increasing use of flowmeters, as irrigators have been found to overreport water use before flowmeter installation (28). The Groundwater Monitoring District Resulting from K.S.A. 82(1020) created local governance with the jurisdiction to require flowmeters on wells starting in 1987, and these have been in place.
in much of Kansas for the past 5–30 y (ref. 29, p. 53). All data used in this study are limited to the period of mandatory water use reporting beginning in 1981 (i.e., data shown before 1981 in Fig. 1C were not used for model development).

Trends in irrigated and dryland corn production (Fig. S2 (footnote/supplement10.1073/pnas.1220351110/JDCSupplemental/nnas.201220351JSl.pdf?targetid=namediest=ST1)) illustrate the importance of irrigation to the study region. Across the United States, annual increases in yield averaged 1–2% per year over the period 1960–2000 (ref. 30, figure 2). In western Kansas, both dryland yields and annual precipitation exhibit stationary trends over the past 30 y. Although recent no-till dryland farming practices have higher yield potential than conventional cropping systems as the result of more available water and increases in soil organic matter, the actual yields may be lower because of diseases (31). Note that dryland yields do not account for unharvested fields due to failed crops during dry years or for fields that went unplanted because of low subsoil moisture during planting time. Irrigated yields have increased steadily by 1.5% per year in the study area, illustrating the emergence of higher yields with lower irrigation rates. These increases are a result of the adoption of farming practices that increase infiltration and reduce runoff by improvements in soil and residue management (such as no-till), conversion from less efficient flood irrigation to center-pivot low-pressure drop irrigation and some subsurface drop irrigation, which enable better irrigation use efficiency, and the introduction of hybrids with better genetics (25). The assumption of a linearly increasing trend in yields is observed in the agricultural data (32) and may be expected into the future, as evidenced by Monsanto's goal of doubling 2000 yields in the United States by 2030 (33).

Realizing the potential of improved future prospects requires collective action to design solutions that reduce aquifer depletion today while rewarding participation (34, 35). The scenarios in Fig. 3 illustrate the impact of regional reduction in groundwater use on agricultural production. Current pumping rates have peaked (as constrained by both hydrogeology and water law), and withdrawal rates will begin to decrease over the next 15–20 y throughout western Kansas given existing trends in aquifer depletion. Corn and cattle production is projected to increase through the next 30–40 y because of increasing water use efficiencies. Although the western central district, with its larger fraction of aquifer depletion, faces more limited prospects for improvements through water savings, there is still time in the southwest and northwest districts to make changes today with significant implications for the future. The water reduction scenario that reduces pumping by 20% would cut current agricultural production back to the levels of 15–20 y ago, yet, the time of peak agricultural production would extend to the 2070s, and agricultural production significantly improves beyond 2070. Increasing water savings from 20%, 40%, and 60–80% extends the time to peak production further into the future, and, ultimately, results in more corn and cattle because of more available water when increased water use efficiencies are realized. The production levels at 80%, which approach the limitations imposed by regional recharge, can support only 12% of today's cattle population—0.5 Mhead of cattle, rising to 1.4 Mhead in 2110 as the result of increased water use efficiencies (as quantified using Eq. 13 in Appendix B).

Essentially, we show that value is added by water conservation today through increased future and net agricultural production. The reality of the situation is causing stakeholders to consider conservation options such as the 2012 K.S.A. 62-1041 that established Local Enhanced Management Areas (LEMAs), such as High Priority Area 6 in Sheridan County, where 29% reductions are planned (36).

Our model accurately reproduces historical aquifer declines in Table S1 (footnote/supplement10.1073/pnas.1220351110/JDCSupplemental/nnas.201220351JSl.pdf?targetid=namediest=ST1) giving credence to our projections of future water stores. Note that our results are presented for the Ogallala Aquifer portion of the High Plains Aquifer in the three western agricultural districts of Kansas and that the previous studies are presented for the entire High Plains Aquifer, which also includes the eastern Great Bend Prairie and Equus Beds aquifers in southern central Kansas. The predevelopment storage was 2392 × 10^6 m^3 and that for the High Plains is 430 × 10^6 m^3 (Table S1 (footnote/supplement10.1073/pnas.1220351110/JDCSupplemental/nnas.201220351JSl.pdf?targetid=namediest=ST1)). The results comparing retrospective studies in Table S1 (footnote/supplement10.1073/pnas.1220351110/JDCSupplemental/nnas.201220351JSl.pdf?targetid=namediest=ST1) are presented in terms of the change in storage that has occurred since predevelopment. It is a reasonable comparison across the Ogallala and High Plains aquifers because the Great Bend Prairie and Equus Beds aquifers have smaller volumes of groundwater that are managed for safe yield and have not experienced groundwater level declines as large as that of the Ogallala (37). The changes in groundwater storage in Table S1 (footnote/supplement10.1073/pnas.1220351110/JDCSupplemental/nnas.201220351JSl.pdf?targetid=namediest=ST1) compare favorably from predevelopment to 1980 and to 1992, and although differences exist between our results and those published for 2000, 2007, and 2009, a brief interpretation of methods illustrates that our results are consistent with all previous studies. A major difference between previous studies in 1980 and 1992 and those in the 2000s is that a new surface of predevelopment water level was adopted (ref. 38, p.13) that changed the predevelopment volume of groundwater from 430 × 10^6 m^3 (ref. 15, Table S1) to 246 × 10^6 m^3 (ref. 26, Table 1). Consequently, although 63 × 10^6 m^3 had been pumped by 1992 (39) using the original surface, the change in storage from predevelopment to 2000 removes only 58 × 10^6 m^3 using the new surface (38). Yet, groundwater levels continued to go down between 1992 and 2000, as measured by the spatially averaged decline in saturated thickness of 5.27 m (17.3 ft) in 1992 (ref. 39, p. 34) and 5.55 m (18.2 ft) in 2000 (ref. 39, p. 32). This follows the steady declining trend in cumulative change in storage occurring from 1980 to 2009 (ref. 40, p. 6). When groundwater storage is subtracted from the earlier estimates of predevelopment storage, the results show a depleted volume of 95 × 10^6 m^3 in 2000 compared
with our results of $60 \times 10^8$ m$^3$, and $112 \times 10^8$ m$^3$ in 2007 compared with $111 \times 10^9$ m$^3$. Therefore, our results very closely match those from previous studies when they use the predevelopment water level from ref. 18 that we also use.

Our regional estimates of recharge integrate across the spatially and temporally varying local recharge processes. The deeper "fossil water" from recharge over the past 13,000 yr (41, 42) is overtopped by more recently recharged water (43) that historically supplied stream flow to perennial rivers and streams (44). Many streams no longer flow because of groundwater depletions that diverted this base flow component to wells, creating dry channels and ephemeral streams that recharge groundwater during runoff events (45). As groundwater stores decline, the groundwater budget eventually will transition to a new equilibrium in which extractions equal recharge (46). We computed recent recharge rates to preserve conservation of mass, at which the annual pumped volume is equal to the change in storage plus the recharge captured by wells. This gave the recharge volume in Fig. 1, which was used to compute the average recharge rate over each agricultural district by dividing the surface area in Table S3 [(footnote suppl.10.1073/pnas.1220351110-DCCSupplemental/pnas 201220351S/pdf)]
targetd=nameddest=S3). Note that the lines of recharge plus storage in Fig. 1 C very closely approximate the recent data points of measured groundwater pumping rates. The recharge rates for our study compare well with those for other studies summarized in Table S3 [(footnote suppl.10.1073/pnas.1220351110-DCCSupplemental/pnas 201220351S/pdf)]
targetd=nameddest=S3), although they are smaller than the estimates used by the Kansas Division of Water Resources in Table S3 [(footnote suppl.10.1073/pnas.1220351110-DCCSupplemental/pnas 201220351S/pdf)]
targetd=nameddest=S3). Obtained by spatially integrating recharge (39, 47) over the extent of the High Plains Aquifer and dividing by surface area. This suggests that the water captured by wells using our mass balance approach may reflect the entire recharge from the terrestrial ecosystem, some of which may be destined for base flow to the streams and rivers that still flow in the region. Note that our methods do not capture the recent additions to recharge that may occur from excess irrigation that returns to groundwater through the vadose zone (45) or hysteresis effects. Although trend time estimates from the surface to the groundwater table in Kansas are on the order of 50–2,000 yr (ref. 46, p. 42), recharge beneath topographical depressions where surficial water concentrates may reach groundwater over periods of months to decades (49). Although artificial recharge projects such as those in central Kansas (37) are being considered to provide more groundwater, it would take time for such systems to infiltrate water and affect groundwater levels.

Our methods of forecasting changes in groundwater stores and agricultural production are applicable to other areas where regional groundwater depletion supports crop and livestock production, although the simplicity of the groundwater system (an aquifer that responds to pumping as an unconfined aquifer (41)) and the socioeconomic system (population with vertical integration of regional industries supported by cost-efficient water extraction technology (50)) may limit application. Likewise, our assumption of linearly increasing crop yields already may have tapped out the maximal impacts available through advances in irrigation technology (conversion from flood to center-pivot to LEPA irrigation), and crop function may evolve over time in response to a more dynamic, changing climate. Changes in water use by other industries (dairy, hog, cattle, etc.) would influence our projections of irrigated corn and cattle production.

**Conclusions**

Eventually, the southwest and northwest districts in Kansas will realize the fate emerging in the west central district, where shallower groundwater stores have resulted in decreased well yields, well abandonment, and conversion back to dryland, although a reduced number of ideally situated and constructed wells may continue to capture new recharge indefinitely. The capacity to pump water will be affected in the 2020s; yet, aggregate corn and cattle production will increase through the 2040s, reflecting current trends of linearly increasing water use efficiency over time and the ability to grow more corn with less water. The future is bright in the near term but bleak beyond, and increased agricultural production may be realized before limnient reductions occur. Our scenario analysis in Fig. 3 substantiates the impacts of water savings on today's production levels and on future prospects.

Although agricultural practices and technologies have led to advances in crop and cattle production (Fig. 3), water policies have not yet realized substantial reductions in the rate of groundwater use (Fig. 1). Instead, pumping decreases as wells go dry. Short-term crop production leads to long-term sustainability challenges due to groundwater depletion, and tradeoffs exist. The excess short-term capacity might be used to support projected increases in the demand for the region's nationally and internationally important livestock sector (51). Alternatively, current increases could supply the biofuel industry for a while, although water limitations raise concerns for long-term biofuel production (52), or saving water now could provide a future store that builds resilience and stability for agricultural ecosystems to weather the future impacts of climate variability and change (53).

Our scenario analysis provides a foundation toward understanding the impacts of changes in groundwater tapping on agricultural production today and into the future. Society has an opportunity now to make changes with tremendous implications for future sustainability and livability. The time to act will soon be past.

**Appendix A. Groundwater Methods**

http://www.pnas.org/content/110/37/E3477.full

7/23/2015

015582
Groundwater is studied using a variety of data sources. A network of observation wells exists where the groundwater level is measured over time and made available through the Kansas WIZARD database (54). Contour maps of the elevation of the base of the aquifer and the predevelopment groundwater level (before large-scale pumping) were developed by the US Geological Survey (USGS) (18) for the High Plains Aquifer as part of the Regional Aquifer-System Analysis project. This source has been used extensively to study groundwater in the Kansas region, and digital forms exist (55, 56). Within Kansas, more recent borehole data led to construction of an enhanced contour map of bedrock elevation (57). The values of bedrock elevation and predevelopment water level at the observation wells were obtained by applying the ArcGIS Topo to Raster tool to the contour maps to produce grids and by assigning the values at each well using the raster cells. Likewise, the ground elevation at each well was estimated using the USGS Digital Elevation Model (DEM) data. Together, these data provide the base elevation, B, the land elevation, L, the predevelopment groundwater level, h0, and a set of M measurements of groundwater levels h_m at times t_m at each observation well.

These data were used in previous studies of groundwater level and storage. Surfaces of groundwater level have been developed by spatially extrapolating measurements at observation wells at specified times, and changes in storage have been obtained from the groundwater volume between surfaces (40). Such surfaces also have been used to linearly extrapolate the rate of change in groundwater level across 10-y periods (58). One issue in such studies involves the temporal changes in water level during pumping (levels commonly drop in wells by tens of meters during seasonal pumping schedules). This problem is dealt with by screening data to use water level measurements only when wells have recovered from the drawdown associated with irrigation; in this study, we use only measurements taken in December and January because irrigation typically ends in late summer and early spring preirrigation of fields has not yet begun (16). Another issue is related to an ever-changing set of observation wells over time (some wells were measured decades ago whereas others just in recent years). One consequence is that considerable changes in the surfaces of groundwater level emerge as hills and valleys from where observations wells are added and removed across periods of study. One method of dealing with this problem is to develop surfaces over a range of years in which the same observation wells exist at the start and end; this gives 566 wells between predevelopment to 2006 for all of Kansas (40) and fewer than 10 wells per county over most of the High Plains Aquifer (ref. 36, p. 22). Changes in storage also have been computed by adding the results during recent periods using a larger set of wells (36, 40) to regional contour maps of observed changes in groundwater level from predevelopment to 1980 (59), obtained by subtracting maps of groundwater observations from predevelopment (16) and connecting points with equal change.

We developed a model that correctly reproduces the trend in groundwater level from predevelopment to depleted conditions for all 3,025 observation wells in the High Plains Aquifer region of Kansas. This is accomplished using a dimensionless saturated thickness (60)

\[
\eta = \frac{h - B}{h_0 - B} \quad [1A]
\]

that varies between 1 (predevelopment with \( h = h_0 \)) and 0 (depleted with \( h = B \)). A mathematical function that reproduces these asymptotic limits is given by the logistic function

\[
\eta = \frac{1}{1 + e^{-T}} \quad [1B]
\]

where the dimensionless time \( T \) is approximated as a linear function of time:

\[
T = \theta_0 + \sigma t. \quad [1C]
\]

This gives the well functional used to approximate groundwater level over time:

\[
h(t) = B + \frac{h_0 - B}{1 + e^{\theta_0 + \sigma t}}. \quad [2]
\]

The coefficients \( \theta_0 \) and \( \sigma \) are obtained using regression over the \( M \) measurements of groundwater level \( h_m \) at time \( t_m \) for each well (60).

A set of criteria was developed to correct discrepancies between data sources.

1. The measured groundwater levels in some observation wells were not between the base elevation and the predevelopment water level. This was rectified by lowering the base elevation and raising the predevelopment level so that all observations fit within this range. This method was chosen because observed water level (surveyed land elevation minus measured depth to water) is more accurate than the layered surfaces for base and predevelopment levels.

2. Extra measurement data were added to reproduce the long-term declining trends. A point was added for wells not measured recently from a kriged surface of observation wells at 2005, and measurement points were added at 1930 and 2000 from a linear extrapolation of observations while keeping these points within the saturated aquifer. The year 1930 starts the period when technological capabilities began to develop for significant groundwater extraction (19, 45), and 2000 represents the end of the "estimated usable lifetime" for significant portions of the High Plains Aquifer from a previous study that used linear trends (58).

Wells with inconsistent or incomplete data were excluded because of

1. Predevelopment groundwater level greater than land elevation

2. Predicted well water level at 1930 more than 5 m below predevelopment water level

http://www.pnas.org/content/110/37/E3477.full

7/23/2015

015583
3. Drop in predicted water level by more than 10 m between 1930 and 1980 (excluding wells with too-large predevelopment water level)

4. Fewer than \( M = 10 \) measurements

The data used includes 1,601 observation wells with 45,038 measurements. The average of the absolute difference between the approximate function and observations across all measurements is

\[
\frac{\sum_{\text{all}} \sum_{\text{all}} |z_{\text{approx}} - z_{\text{obs}}|}{\sum_{\text{all}} |z_{\text{approx}}|} = 1 \text{ ft} \text{ m} \tag{3}
\]

Although the coefficients for each well provide the capacity for changes in storage to occur over different periods of time at different points in the aquifer (as is actually happening across the region), the approximated function and all measurements may be plotted on the same graph using the dimensionless coefficients \( T \) vs \( T' \) in Fig. 1-a.

Our well function, Eq. 2, provides the saturated thickness and changes in storage in Fig. 1. The groundwater level was calculated at each observation well by evaluating the function at times 1980, 2010, 2000, and 2110. Surfaces of saturated thickness were obtained by applying the universal kriging algorithm with a second-order trend to these water levels and subtracting from the surface of bedrock elevation (57). The volumes of water in storage at these times and at predevelopment were computed by multiplying this saturated thickness by the specific yield (18, 61) to get the water content, clipping to the extent of the High Plains Aquifer (47) in each agricultural district, and summing using zonal statistics in ArcGIS. The changes in storage over 5-y periods were computed by evaluating the well function at the start and end of each period, kriging the differences to derive a surface across wells, multiplying this by the specific yield, clipping, and summing. The results are reported as the change in storage per year by dividing these results by the 5-y period.

---

Appendix B. Agricultural Production Methods

The production and consumption of corn are quantified using recent agricultural data. The irrigated and dryland corn production for western Kansas is Fig. 2 is obtained by aggregating the USDA NASS's annual reports (62). Although these data are reported as volume (bushels), the findings are multiplied by density (56 lb/bushel) and converted to units of metric tons. The USDA data also report the number of cattle in feedlots on January 1 of each year. The following conversion factors document the practices of cattle operations in Kansas and were used to compute feed requirements. Calves gain 0.3 t/weight gain and consume 0.9 t/dry corn. The overall dry matter conversion is 5.5 t/weight gain for 1 t of weight gain, 90% of feed is corn, and corn contains 86% dry matter. Yearling cattle gain 0.2 t/weight gain and consume 0.8 t/dry corn. These performance parameters were selected in an attempt to provide median estimates across differences encountered depending on breed, sex, entry weight within age category, and market conditions.

These parameters form a basis for our estimates of corn requirements to feed calves and yearlings to finished weight:

\[
\begin{align*}
0.3 \text{ t-weight gain } & \times \frac{5.5 \text{ t-feed}}{\text{calf}} \\
0.9 \text{ t-dry corn} & \times \frac{1.75 \text{ t-corn}}{\text{calf}} \\
0.9 \text{ t-dry corn} & \times \frac{0.85 \text{ t-dry corn}}{\text{calf}}
\end{align*}
\]

and

\[
\begin{align*}
0.2 \text{ t-weight gain } & \times \frac{6 \text{ t-feed}}{\text{yearling}} \\
0.9 \text{ t-dry corn} & \times \frac{1.25 \text{ t-corn}}{\text{yearling}} \\
0.9 \text{ t-dry corn} & \times \frac{0.85 \text{ t-dry corn}}{\text{yearling}}
\end{align*}
\]

Calves typically are fed for 220–240 d, and the potential exists to feed 1.5 calves per year for every calf on feed in January; yearlings are fed for 120–140 d, and there is a potential to feed 2.5 per year for every yearling on feed in January. Of the cattle fed in a given year, we used the estimate that =30% are calves and 70% are yearlings at the time of feedlot entry. This gives a relation between the total annual cattle per year vs. the cattle counted during the annual survey in January:

\[
\begin{align*}
1.5 \text{ cattle} & \times 0.3 \text{ calf in Jan.} \\
\text{calf in Jan.} & \times \text{cattle in Jan.} \\
2.5 \text{ cattle} & \times 0.7 \text{ yearling in Jan.} \\
\text{yearling in Jan.} & \times \text{cattle in Jan.} \\
2.2 \text{ cattle} & \times \text{cattle in Jan.}
\end{align*}
\]

We validated our estimates by comparing USDA NASS cattle data to our estimate, in which the fed cattle sold in Kansas (ref. 63, p. 434) divided by the cattle on feed on January 1 (63, p. 420) was 2.08 and 2.21 in 2002 and 2003, respectively. The ratios in the last equations combine to give the corn requirement per cattle counted in January.

http://www.pnas.org/content/110/37/E3477.full

7/23/2015

015584
The corn consumption by cattle in Fig. 2 was obtained by multiplying this ratio by the aggregated cattle-on-feed data from the USDA (62). Together, the last two ratios give the corn feed needed per head of cattle for feedlot practices in Kansas:

$$\frac{1.73 \text{ t-corn}}{\text{ calf in Jan.}} \times \frac{1.5 \text{ calf}}{\text{ calf in Jan.}} \times \frac{0.7 \text{ calf in Jan.}}{\text{ 0.7 yearling in Jan.}} \times \frac{2.5 \text{ yearling}}{\text{ 2.5 yearling in Jan.}} = \frac{3.0 \text{ t-corn}}{\text{ cattle in Jan.}}$$

Data related to crop production are aggregated to the level of the nine agricultural districts in Kansas. The yields of irrigated corn, \( Y_i \), and dryland corn, \( Y_0 \), are reported by the USDA (62), and precipitation data were gathered from the Kansas Weather Data Library (64). These time series are shown in Fig. S2 (https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1220351110/-/DCSupplemental/pnas.2012203511S.pdf?targetId=nameddest=SF4) from 1961 to 2009 along with the annual irrigation, \( I \), which was obtained by dividing the pumping rate of all irrigation wells in each district by the total irrigated area reported on the annual WRIS water use reports (65). These data illustrate that the west’s higher irrigation leads to higher irrigated yields, and the east’s higher precipitation leads to higher dryland yields. Data for the eastern and central districts are not reported on other figures to aid in visual interpretation. The median level and 95% confidence intervals are also shown in this figure for the western districts, their construction is detailed next.

We used the bootstrap method (66) to extrapolate and project data trends over time as follows. The data sets for each variable in each district in Fig. S2 (https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1220351110/-/DCSupplemental/pnas.2012203511S.pdf?targetId=nameddest=SF4) contain \( n = 29 \) data points. The median value and 95% confidence intervals are constructed for each dataset using sampling with replacement for data with linear trends as follows:

1. Develop a set of \( n = 10,000 \) randomly generated samples that select \( n \) data points with replacement.

2. For each sample, calculate the slope and intercept using linear regression with least squares, and calculate the residual difference between each data point and the linear estimate.

3. For every time for which statistics are to be evaluated, project each of the \( N \) regression lines to that year, and detrend the data by adding the residuals of the sample set to the value of the regression line at its intercepts.

4. Sort the \( n \times N = 290,000 \) points for each year, take the median 50% value, and drop the lower and upper 2.5% to get the 95% confidence interval.

5. Repeat the last two steps for each year for which statistics are evaluated, and connect points on the lines for the median and confidence intervals across years.

The probability of an independent trial exceeding a \( 1 - \alpha/2 \) confidence limit has a binomial distribution (here, \( \alpha = 0.95 \)). When the number, \( N \), of independent trials is large, the binomial distribution closely approximates a normal distribution with mean \( \bar{N}p \) and variance \( Np(1-p) \), where \( p = \alpha/2 \). When \( n = 10,000 \), one has a 99% confidence that the realized confidence limits are between 94.84% and 95.36%.

The increase in corn production from recent water use efficiencies is illustrated in Fig. S4A (https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1220351110/-/DCSupplemental/pnas.2012203511S.pdf?targetId=nameddest=SF4) by plotting the difference between irrigated and dryland yields divided by the rate of irrigation, \( (Y_i - Y_0)/I \). The bootstrapping procedures are applied to construct median lines and confidence limits for each district and demonstrate the linear trend in data. Corn production also has increased because of changes in land use practices over the past 30 y, where a larger fraction of irrigated fields, \( f \), now is used for corn production. This is illustrated in Fig. S4B (https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1220351110/-/DCSupplemental/pnas.2012203511S.pdf?targetId=nameddest=SF4), in which the USDA-reported (62) harvested area of irrigated corn in each agricultural district is divided by the total irrigated area in the WRIS annual water use reports (65). These data follow a functional form that transitions from a lower limit, \( f_{\text{min}} \), to an upper limit, \( f_{\text{max}} \), and it is approximated here using the same logistic equation as for the observation wells:

$$f(x) = f_{\text{max}} \frac{f_{\text{max}} - f_{\text{min}}}{1 + e^{-a(x - b)}}$$

This trend reflects USDA reports (62) in which irrigated corn has become the primary irrigated crop, but that water also is used for irrigated alfalfa, soybeans, wheat, and sorghum. Only a small fraction of groundwater in western Kansas is used by municipalities, industry, and feed yards. It is likely that current land use practices will continue as long as markets continue to make corn profitable, and other competing uses may limit \( f \) from becoming larger in the future.

We used nonlinear regression to determine the coefficients in Eq. 8 that minimize the least-square objective function containing the difference between the \( n = 29 \) estimate \( f \) at time \( x \) and the data points \( f_n \)

$$\mathcal{F} = \sum_{n=1}^{N} \left[ f_n - f(x_n) \right]^2$$

http://www.pnas.org/content/110/37/E3477.full

7/23/2015

015585
This nonlinear function was minimized using the Levenberg–Marquardt method (67, 68) to obtain the coefficients $b_0$ and $b_1$. This may be written as follows:

\[
\left( \frac{f(\hat{y}_b + \lambda \hat{y}_i)}{b_0 + b_1} \right) \left( \hat{y}_b + \lambda \hat{y}_i \right) = f(b_0) - b_1 
\]

using the Jacobian matrix $J$ with

\[
J = \begin{bmatrix}
\frac{\delta f(\hat{y})}{\delta b_0} & \frac{\delta f(\hat{y})}{\delta b_1} \\
\frac{\delta g(\hat{y})}{\delta b_0} & \frac{\delta g(\hat{y})}{\delta b_1} \\
\vdots & \vdots \\
\frac{\delta h(\hat{y})}{\delta b_0} & \frac{\delta h(\hat{y})}{\delta b_1}
\end{bmatrix}, \quad \hat{J} = \begin{bmatrix}
\frac{\delta f(\hat{y})}{\delta b_0} \\
\frac{\delta f(\hat{y})}{\delta b_1} \\
\vdots \\
\frac{\delta f(\hat{y})}{\delta b_1}
\end{bmatrix}.
\]

where $J$ is the identity matrix, and $\lambda$ is adjusted using the values of $b_0$ and $b_1$ for the $q^\text{th}$ iterate as per ref. 68.

The bootstrap procedures were applied using this functional form and optimization technique to get the median lines and confidence intervals in Fig. 5A8 (lookup/suppl/doi:10.1073/pnas.1202035110/ICDSupplemental/pnas.2012020351S1pdf.pdf targetID=namedest=S4F4). The coefficients $f_{2\text{na}}$ and $f_{2\text{na}}$ were found ad hoc by repeating the bootstrap method with different values and determining those that minimized the objective function. The results for $f_{2\text{na}}$ are reported in Table S2 (lookup/suppl/doi:10.1073/pnas.1202035110/ICDSupplemental/pnas.2012020351S1pdf.pdf targetID=namedest=S2) for the three western agricultural districts, along with values of $b_0$ and $b_1$ for $t/4$ 2010 that closely approximate the median lines of $f$ (within 0.7% for all values in Fig. 5A8 (lookup/suppl/doi:10.1073/pnas.1202035110/ICDSupplemental/pnas.2012020351S1pdf.pdf targetID=namedest=S4F4)).

The recent increases in the fraction of irrigated land used for corn production reflect increases in demand for corn in cattle production. The number of head of cattle on feed in January is shown in Fig S3 (lookup/suppl/doi:10.1073/pnas.1202035110/ICDSupplemental/pnas.2012020351S1pdf.pdf targetID=namedest=S3F3F4). The median lines and confidence intervals were constructed for each agricultural district using bootstrap with the logistic equation in Eq. 8. These trends reflect a redistribution of cattle feed locations over the last 30 y as operations in the Midwest focused on the large feedlots of western Kansas with ideal weather for cattle production, local irrigated corn, and proximity to major slaughterhouses. Thus, the use of the logistic function to redistribute irrigated corn production in Eq. 8 follows the trends observed in the cattle production that consumes corn.

We developed a function to relate groundwater pumping to corn production. Data measurements for the fraction of irrigated area in corn times the increase in yield divided by the irrigation rate, $(Y_t - Y_0)/I$, are plotted in Fig. S4C (lookup/suppl/doi:10.1073/pnas.1202035110/ICDSupplemental/pnas.2012020351S1pdf.pdf targetID=namedest=S4F4) and Table S2 (lookup/suppl/doi:10.1073/pnas.1202035110/ICDSupplemental/pnas.2012020351S1pdf.pdf targetID=namedest=S2). The median lines and confidence intervals for the corn water requirements in Fig. S4C (lookup/suppl/doi:10.1073/pnas.1202035110/ICDSupplemental/pnas.2012020351S1pdf.pdf targetID=namedest=S4F4) were obtained using the bootstrap procedures with coefficients $c_0$ and $c_1$ obtained using least-squares regression. The values of $c_0$ and $c_1$ that closely approximate the median lines (within 0.11 the bootstrap $t$-value) in the lines in Fig. S4C (lookup/suppl/doi:10.1073/pnas.1202035110/ICDSupplemental/pnas.2012020351S1pdf.pdf targetID=namedest=S4F4) are reported in Table S2 (lookup/suppl/doi:10.1073/pnas.1202035110/ICDSupplemental/pnas.2012020351S1pdf.pdf targetID=namedest=S2). The variable $c_0$ represents the corn production per irrigation, and the median values of 22.8–25.6 t/ha-m compare well with the Kansas State Research and Extension (71) estimates of 25 t/ha-m (3,000 gallons of water per bushel of corn yield). The ratio $c_1/c_0$ represents the median increase in water use efficiency, and the values of 1.7–2.1% per year reflect recent national increases in yield of 1–2% per year (30). Eq. 11 takes on a simpler form at future times when $f = f_{2\text{na}}$ and the expressions and coefficients for $\tilde{y}_t$ for these asymptotic forms are found in Table S2 (lookup/suppl/doi:10.1073/pnas.1202035110/ICDSupplemental/pnas.2012020351S1pdf.pdf targetID=namedest=S2).

Our estimates of the corn water requirements $W$ in Eq. 11 represent the fraction of irrigation used for corn times the increase in yield per irrigation. If we write $f$ as the area under the rainfall, $A_T$, divided by the area of groundwater pumping, $A_P$,

\[ W = \frac{Y_t - Y_0}{I} = A_T (Y_t - Y_0) = \frac{c_1}{AQ}, \]
then the irrigated corn production is \( C_I = A_I Y_c \), and the annual pumped volume of groundwater is \( Q = A_Q \).

Thus, the corn produced by irrigation may be obtained by multiplying \( W \) in Fig. 5AC (assuming supplemental water is 100% pumped groundwater). Note that this gives the corn resulting from irrigation; the corn production resulting from precipitation alone, \( A_I Y_c \), must be added to this value to get the total irrigated corn production.

A simplified projection may be computed for the number of cattle produced from recharge alone. The sum across agricultural districts of the annual volume of recharge, \( R \), in Fig. 1, times the long-term estimates of corn production from irrigation, \( \mu_c \) in Eq. 11, with coefficients from Table S2 (supplemental file), gives the feed requirements for cattle, \( F \) in Eq. 7, as

\[
B = \sum_i \left( \frac{0.07 \times 10^6}{y^{0.39}} \right) \left( \frac{15.570 - 0.2838(y - a_i)}{10^3} \right) \times 0.73 \text{ cattle} y^{-1} \text{ ha}^{-1}
\]

Thus, the annual corn production from recharge can support 0.5 head of cattle today and could support 1.4 head in 2110 if existing production trends continue.

Acknowledgments

The authors give special thanks to the editor and two anonymous reviewers for their very constructive comments that helped clarify contributions. The authors gratefully acknowledge financial support from the National Science Foundation (Grant GEO 0909515), the USDA Agricultural Research Service (Opalita Aquifer Initiative), and the US Department of Transportation (Kansas State University Transportation Center).

Footnotes

1To whom correspondence should be addressed. E-mail: stewart@ksu.edu (maisto stewart@ksu.edu)

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. D.G. is a guest editor invited by the Editorial Board.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1220351110/-/DCSupplemental (lookup/suppl/doi:10.1073/pnas.1220351110/-/DCSupplemental)

Freely available online through the PNAS open access option.

References


http://www.pnas.org/content/110/37/3477.full

7/23/2015

015587


http://www.pnas.org/content/110/37/E3477.full


http://www.pnas.org/content/110/37/E3477.full

7/23/2015

015590


HighWire Press-hosted articles citing this article

Empowering people to change occupational behaviours to address critical global issues: Habiller le es a changer leurs comportements occupationnels en vue d’aborder les grands enjeux mondiaux

Canadian Journal of Occupational Therapy (Canadian Journal of Occupational Therapy) 2015 82 (3)
194-204

Abstract (http://journals.sagepub.com/doi/content/abstract/0321016414567919) Full Text (HTML)
(http://journals.sagepub.com/doi/full/0321016414567919) Full Text (PDF) (http://journals.sagepub.com/doi/pdf/0321016414567919)

Development of a new long-term drought resilient soil water retention technology

Journal of Soil and Water Conservation (Journal of Soil and Water Conservation) 2014 69 (5):131A-140A

Full Text (HTML) (http://www.njabstract.org/jstor/content/abstract/10166335) Full Text (PDF) (http://www.njabstract.org/jstor/pdf/10166335)

Importance of a sound hydrologic foundation for assessing the future of the High Plains Aquifer in Kansas


Reply to Butler et al.: A sound hydrologic foundation for interdisciplinary studies of the High Plains Aquifer

http://www.pnas.org/content/110/37/E3477.full 7/23/2015
Tapping unsustainable groundwater stores for agricultural production in the High Plain...
Meeting Hydrologic and Water Quality Goals through Targeted Bioretention Design

William F. Hunt, M.ASCE1; Allen P. Davis, F.ASCE2; and Robert G. Traver, M.ASCE3

Abstract: Bioretention is one of the most commonly used stormwater control measures (SCMs) in North America and Australasia. However, current design is not targeted to regulatory need, often reflecting an outdated understanding of how and why bioretention works. The purpose of this manuscript is to synthesize research to recommend a suite of design standards focused on the purpose of bioretention SCM. Both hydrologic (peak flow mitigation, infiltration, annual hydrology, and stream stability) and water quality [total suspended solids (TSS) and particulates, pathogen-indicator species, metals, hydrocarbons, phosphorus, nitrogen, and temperature] regulatory and stream ecology needs are addressed. Bioretention cells designed to meet a prioritized subset of these measures would be substantially different than cells that are designed for a different subset of needs. Designers have the ability to adjust bowl volume, media composition, media depth, underdrainage configuration, and vegetation type. This study examines how each of those design parameters can be adjusted such that a "one size fits all" approach is no longer the norm. DOI: 10.1061/(ASCE)EE.1943-7870.0000504. © 2012 American Society of Civil Engineers.

CE Database subject headings: Stormwater management; Infiltration; Biological processes; Water quality; Pathogens; Temperature effects; Nutrients.

Author keywords: Stormwater; Infiltration; Bioretention; Hydrology; Water quality; Design; Pathogens; Temperature; Nutrients; Bioinfiltration.

Introduction

Bioretention, and related bioinfiltration, are among the most commonly used stormwater control measures (SCMs) in North America and Australasia. Most stormwater regulating authorities have a wide range of bioretention design guidelines that primarily rely on a "one size fits all" approach. Whereas there is some commonality among design standards, substantial variability exists among each jurisdiction. Bioretention review articles have recently been published to summarize performance capabilities (Dietz 2007; Davis et al. 2009; Roy-Poirier et al. 2010); but as yet, a science-based design guidelines document does not exist. The purpose of this manuscript is to synthesize research findings to create design standards that meet a variety of hydrologic and water quality needs for use by the regulatory and design communities. The limitations of bioretention are discussed, water quality improvements are featured by focusing on treatment mechanisms, and novel modifications are highlighted to assist with specific water quality challenges.

Terminology

Before design recommendations are established, some terminology of bioretention components is presented (Table 1), as this terminology is not universal. A cross section of bioretention illustrates the different components of the bioretention system (Fig. 1). A biofiltration cell is similar in all aspects, except that no underdrain is used.

Design for Hydrologic Goals

Designers are asked to meet a variety of hydrologic design goals. Among the most common are mitigating peak flow or volume to mimic predevelopment surface water hydrology, infiltrating a specified fraction of runoff, and releasing outflow at rates sensitive to stream geomorphology (EISA 2007; NCSU 2009; PaDEP 2006). Design requirements vary by jurisdiction.

Mitigate Peak Flow/Storm Volume

Perhaps the longest-standing design goal for SCMs is to mitigate flow from infrequent storm events [1.1-yr, 2-yr, or 10-yr Average Return Interval (ARI)]. Of all design goals, peak flow mitigation may have been the most difficult for bioretention to realize. This is because of many design requirements limiting the depth of the bowl to 150–300 mm (6–12 in.) (Davis et al. 2009). The reasons for shallower depth include (1) ensuring vegetation health, (2) concerns over the compaction of sediments, and (3) health and safety considerations, including vector control. A shallow pool of water in a parking lot does not need to be fenced, but deeper volumes may require fencing or other risk-reducing infrastructure. A bioinfiltration cell examined for nearly a decade at Villanova University has an average bowl depth of 430 mm (17 in.) (Heasom et al. 2006). Vegetation die-off has not been an issue because of the use of plants able to withstand inundation and dry periods. The site was first
Table 1. Components of a Bioretention Design; see Fig. 1

<table>
<thead>
<tr>
<th>Bioretention Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowl</td>
<td>The surface-ponding zone. Depth and volume must be specified.</td>
</tr>
<tr>
<td>Media</td>
<td>An engineered fill media with moderately high permeability. Depth, composition, and infiltration rate must be specified.</td>
</tr>
<tr>
<td>Root zone</td>
<td>Upper layer of the media available to the plant roots. Water stored in this region is available for both evapotranspiration and exfiltration.</td>
</tr>
<tr>
<td>Lower media zone</td>
<td>Lower media layer not readily available to roots. Water stored in this region is released through exfiltration.</td>
</tr>
<tr>
<td>Underdrain (optional)</td>
<td>Typically small-diameter (100-150 mm), plastic pipes. These drainage lines are located in the gravel layer below the fill media to collect water and convey it to the storm sewer network or receiving stream. Underdrains are most often used when bioretention cells are located in slowly draining soils and are required when impermeable liners are used. Can be constructed with gate valves when soil conditions are marginally permeable. Underdrains should be below the root zone to prevent clogging.</td>
</tr>
<tr>
<td>Internal water storage (IWS) (optional)</td>
<td>A subsurface portion of the media that provides additional storage volume in the bioretention cell. In permeable soils, water stored in this layer is principally released through exfiltration. The IWS layer is created by elevating the exit of the underdrain. Examples of IWS (also termed the saturation zone) are further described by Dietz and Claussen (2006); Blecken et al. (2009); Davis et al. (2009); and Brown and Haat (2011).</td>
</tr>
</tbody>
</table>

Fig. 1. Cross section of bioretention cell (image by Shawn Kennedy, NC State Univ.)

designed with a maximum depth of 300 mm (12 in.), however it was immediately altered to take advantage of available storage. Other anecdotally observed bioretention cells near Atlanta have excess storage depths of 600 mm, and an operational site at Villanova is roughly 900 mm in depth. The vegetative health of the Georgia bioretention cells was reasonably good, but recruitment of less desirable weed species was apparent over time.

Whereas peak flow mitigation requirements still permeate the regulatory environs, this concept has little scientific support as an environmental or ecological performance metric. Understanding complete-flow durations will provide better protection against urban-stream syndrome (Walsh et al. 2005a). When designing for peak outflows, the volume-removal capacity of the bowl and media storage must be considered, as discussed later in this article. Modeling of bioretention SCMs in the mid-Atlantic region shows that the peak flow of somewhat infrequent events (such as 1.1-year or 2-year ARIs) can be mitigated through additional storage, either with increasing bowl depth (between 300-600 mm), or by additional volume capture (difference in runoff volume). Field research supports this finding to some extent (Davis et al. 2012), but the required volume may not be available as a result of restrictions on depth or available space. Also important is that the media and drainage configuration do not allow relatively high flow rates through the cell. Rates associated with gravel and sand media mixes could approach 250 mm/h infiltration rates, minimizing bioretention’s peak-flow mitigation.

In many instances, it may be prudent to pair bioretention with either an underground or above-ground detention facility in an overflow treatment-train configuration. An in-series SCM approach allows the bioretention detention volume to be reduced. Models are becoming available for designers to take credit for the bioretention peak-flow mitigation capabilities (Heasom et al. 2006). A summary of peak-flow mitigation design guidance is presented in Table 2.

Annual Water Budget

Some communities are promoting one of two related design goals: meeting predevelopment surface water hydrology (EISA 2007; NCSU 2009) and infiltrating or evapotranspiring a set volume of runoff (PaDEP 2006). In short, jurisdictions are asking that surface runoff be “converted” to infiltration and evapotranspiration (ET) to more closely mimic these pathways within a vegetated landscape.

Bioretention designers have many options by which to achieve these goals, including designing cells with (1) proportionally larger media-to-runoff volume ratios, (2) deeper media depths, (3) internal water storage (IWS) layers, and (4) vegetation with deeper roots to promote ET, while (5) ensuring an adequate bowl volume is maintained. Davis et al. (2012) define and describe the bioretention abstraction volume (BAV), which accounts for many of the factors above. BAV is the available storage volume in the bioretention cell and is calculated as the sum of the storage in the surface bowl and that within available media porosity in the root depth. The larger the BAV relative to the contributing watershed, the more infiltration, ET, and lower flow release a bioretention cell will have.

Table 2. Designing Bioretention for Peak-Flow Mitigation

<table>
<thead>
<tr>
<th>Design component</th>
<th>Guidance</th>
<th>Supporting studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowl depth</td>
<td>Increased bowl/media void space volume or depth above what is required for water quality. Verify that flow-through rate of media and underdrain system is sufficiently low. May be best designed as a treatment train.</td>
<td>Mostly anecdotal evidence, but Davis et al. (2012) provides some support.</td>
</tr>
</tbody>
</table>
If regulation requires that predevelopment surface-water hydrol-
logic conditions are to be met, the BAV could be selected such that it,
along with other landscape depression storage, would equal the
initial abstraction of a target, or a volumetric-predevelopment,
condition.

To achieve a required BAV, the designer selects the bioretention
surface area, the fill-media depth, and the plant-root depth. Other
choices include the options to use an undersoil or create an IWS
zone. Davis et al. (2012) provide tools to calculate the BAV, as
described in Eqs. (1)-(3). Eq. (1) is specifically for a bioinfiltration
cell, which has no undersoil, but would also be applicable to an
IWS with underlying soils that limit exfiltration. Eq. (2) is for a
conventionally undersaturated (no IWS) cell. Note that as soon as
field capacity is reached, water starts exiting the undersoil,
thereby increasing the value of the lower media storage and negati-
ating the bowl volume for average conditions. An IWS cell over
permeable underlying soils would add bowl volume to Eq. (2), as
expressed in Eq. (3)

$$BAV = Bowl\ Vol. + RZMS \cdot (SAT - WP)$$ \hspace{1cm} (1)

$$BAV = RZMS \cdot (SAT - WP) + LMS \cdot (SAT - FC)$$ \hspace{1cm} (2)

$$BAV = Bowl\ Vol. + RZMS \cdot (SAT - WP) + LMS \cdot (SAT - FC)$$ \hspace{1cm} (3)

where BAV = bioretention abstraction volume; RZMS = root-zone
media-storage volume; SAT = saturation point; WP = wilting point;
LMS = lower media-storage volume (deeper than plant roots); and
FC = field capacity.

While the research of Li et al. (2009) predates that of Davis et al.
(2012), in which the BAV concept is introduced, the theory behind
the BAV is evident in Li et al’s work. Proportionally larger cells,
and cells with deeper media depths, reduced outflow volumes more
than smaller surface area and shallow media-depth cells. Jones and
Hunt (2009) compared the frequency of outflow for four bioretention
cells, and the cells that had the proportionally largest surface
areas (and media volumes) had the fewest occurrences of outflow,
previously a result of having more opportunity for intra and inter-
event exfiltration and ET. Brown and Hunt (2011) demonstrate how
IWS depth impacts the frequency and volume of outflow in high-
exfiltration soils. By increasing the size of an IWS, the designer can
limit the amount and occurrence of undersoil discharge. Meeting
required infiltration goals is a subset of meeting predevelopment, or

**Table 3. Design Guidance for Matching Target Hydrology and Required Infiltration**

<table>
<thead>
<tr>
<th>Design component</th>
<th>Guidance</th>
<th>Supporting studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowl depth/volume</td>
<td>Bowl depth must be adequate to store water-quality volume, allowing intra-event percolation through the media. Bowl volume is part of BAV.</td>
<td>Davis et al. (2012)</td>
</tr>
<tr>
<td>BAV</td>
<td>Combines bowl volume with available media storage to calculate the amount of water captured by a cell during a storm. Adjust the BAV to match abstraction of the target hydrologic condition.</td>
<td>Davis et al. (2012)</td>
</tr>
<tr>
<td>Media depth</td>
<td>Part of BAV. Deeper media depths allow for more storage of runoff. Exact depth required is determined by capture demand.</td>
<td>Li et al. (2009)</td>
</tr>
<tr>
<td>Root zone</td>
<td>Part of BAV and media depth. Use of more deeply rooted plants increases the overall volume reduction, and that of the volume evaporated.</td>
<td>Davis et al. (2012)</td>
</tr>
<tr>
<td>Surface area</td>
<td>Part of BAV. Increasing the surface area allows more storage of runoff. Exact surface area determined by storage demand.</td>
<td>Jones and Hunt (2009)</td>
</tr>
<tr>
<td>IWS</td>
<td>In cells employing underdrainage, IWS creates an exfiltration zone. Thicker IWS zones allow for greater amounts of runoff to infiltrate in non-restrictive soils.</td>
<td>Brown and Hunt (2011)</td>
</tr>
<tr>
<td>Construction technique</td>
<td>The final 0.3 m of excavation should be &quot;raked&quot; rather than smeared.</td>
<td>Brown and Hunt (2010)</td>
</tr>
</tbody>
</table>

**Outflow Rates to Protect Stream Geomorphology**

As stream geomorphology becomes an important component of
urban water management, SCMs must be designed to release water
at, or near, rates that preserve stream stability. Little research has
focused on relating bioretention outflow rates to stream flow rates,
but DeBusk et al. (2011) determined that normalized bioretention
outflow from several cells related very closely to normalized stream
flow rates associated with postevent shallow groundwater interflow
over multiple year-long periods of record. Similarly, Oliszewski
(2010) note that cumulative flow discharge from a bioretention cell
is greatly reduced and spread over a much longer time than that of
the input runoff. It approaches, but does not match, that of a for-
ested watershed.

The BAV design concept can be used such that the depression
storage of a developed watershed, coupled with a bioretention BAV,
is similar to that of watersheds in geomorphically stable stream
reaches. If bioretention overflow or bypass occurs only as fre-
quently as it would under natural or target conditions, both erosive
and benthic-macroinvertebrate stream health (Walsh et al. 2005a, b)
are likely to be maintained. If concern exists of increased exfiltration
as baseflow for arid regions, the root zone can be expanded.
to accelerate ET and conventional underdrains (no IWS) can be included to reduce infiltration.

**Designing for Water Quality**

Currently in most SCM applications, many pollutants are targeted for removal by bioretention, but rarely does every pollutant require treatment at a given location. Watershed needs, such as compliance with total maximum daily loads (TMDLs), often dictate which pollutant(s) should be removed or sequestered by bioretention. Bioretention design guidance for most common pollutants is reviewed here: total suspended solids (TSS) and particulate matter, pathogens (as represented by surrogate bacterial indicator species), oil and grease, metals, total phosphorus (TP), total nitrogen (TN), and temperature/thermal pollution.

Water-quality treatment should not be viewed in isolation from hydrologic improvement, as reducing outflow volume from bioretention is an important part of reducing total pollutant loads, as the product of concentration and annual volume (Hunt et al. 2006; Li and Davis 2009). In many cases, the only reason an outflow pollutant load is less than the inflow load is the result of modifications in hydrology and the water balance.

Pollutant removal of nearly any kind is predicated on the hydraulic retention time ($T_{H}$) of an SCM. Separation of a pollutant from the flowing runoff requires time for the various removal mechanisms to be effective. $T_{H}$ should be considered as an important design component for all SCMs (Bäckström 2002). Any design that increases $T_{H}$ should be expected to enhance water-quality improvement. Higher $T_{H}$ will increase the probability of the bowl not being empty for the next storm, negatively impacting bioretention hydrologic performance. Fortunately this risk is small, as it would affect only back-to-back larger events.

**TSS and Particulate Matter**

The mechanisms for removing particulate matter are sedimentation and filtration. All water that passes through the media is filtered, capturing fine particles. Larger, higher-density particles are effectively trapped by sedimentation. The use of relatively fine media and the resulting relatively low flow rates, compared to the rapid sand filtration common in drinking-water systems, can provide excellent removal of particulate matter. Large particles should be strained out at the media surface. Smaller particles are captured by the media through sedimentation, interception, and diffusion-transport mechanisms. Consequently, the large bulk of the captured-solids load is at the surface of the bioretention cell, and TSS removal by bioretention is highly effective (Davis 2007; Li and Davis 2008; Hatt et al. 2009a). Fixed by the BAV, the majority of the yearly runoff volume will ultimately enter the bioretention media, ensuring high levels of particulate-matter treatment.

Because of efficient straining and surface filtration, the depth of bioretention media is minimally important for particulate removal. Other design issues, such as infiltration rate or the presence of an IWS zone, impact TSS and particulate sequestration only through their role in ensuring that the flow passes through the media. Design goals (hydrologic or water-quality) other than TSS removal will normally dictate the required depth, infiltration rate, or presence of an IWS layer.

The presence of vegetation has modest benefit, as it slows water flow, allowing more time for sedimentation to occur (increased $T_{H}$). Also, the roots of the vegetation would be expected to maintain adequate infiltration rates, should particulate matter begin to restrict flow through the fill media (Hatt et al. 2009a). The long-term study conducted at Villanova for a well-vegetated bio-infiltration cell has demonstrated no reduction in surface infiltration (Emerson and Traver 2008). Nonetheless, at this time, no specific guidance is available for the type and density of vegetation needed to promote TSS removal. Table 4 summarizes design needs for TSS and particulate removal.

Many cases of failure have been related to massive sediment inflow during construction and periods when surrounding ground surfaces were yet stabilized. In addition to standard erosion-control techniques, bioretention construction should be scheduled late in the site-construction sequence, and completed cells can be surrounded by sod to minimize erosion and sediment impacts (Hunt and Lord 2006).

**Bacteria/Pathogen-Indicator Species**

Because of monitoring costs, rarely have actual pathogens been studied in stormwater—rather indicator species of bacteria are used. Bioretention has numerous treatment mechanisms for indicator bacteria. Filtration is the primary sequestration mechanism, as microbes can strongly sorb to organic media components and soils. In general, field studies of bioretention have shown promise with respect to indicator species capture (Hathaway et al. 2009; Passeport et al. 2009). A long-term laboratory study has shown that *E. coli* removal efficiency will increase with age (Zhang et al. 2011). A minimum media depth/maximum flow rate combination may exist, however, where indicator species are no longer removed. In fact, bacteria could be stripped away from the collector particles to which they had been bound if the hydraulic loading is too high. Hathaway et al. (2011) suggest a minimum of 0.6 m (2 ft) for a fill-media depth, on the basis of field research in North Carolina. Media with relatively low infiltration rates have been demonstrated in laboratory studies to have higher rates of sequestration (Rusciano and Obropta 2007; Bright et al. 2010; Zhang et al. 2010), suggesting that infiltration rates in the lower range of accepted values should be specified (25–50 mm/h or 1–2 in./h). When including pathogen collection, designers should not grossly undersize bioretention cells, thereby moderating the hydraulic loading rate.

The benefits or costs of including an IWS have not been fully explored with respect to pathogens; however, Passeport et al. (2009) did show sequestration of fecal coliforms in cells with an

| Table 4. Bioretention Design Needs for TSS sequestration |
|---------------------------------------------|---------------------------------------------|
| Design component | Guidance | Supporting studies |
| Media depth | TSS and particulate matter require only shallow media depth. However, a minimum of 0.3 m is suggested for plant survival and growth. | Li et al. (2008); DiBlasi et al. (2009) |
| Infiltration rate | Up to 0.04 mm/s (6 in./h) | Emerson and Traver (2008); Hatt et al. (2009a) |
| Vegetation | Presence helpful to slow flows and facilitate long-term infiltration as a result of particulate accumulation | Hunt and Lord (2006) |
| Construction technique | Protection of site from sediment mounding during construction and site stabilization. Use of sod around perimeter. | |
IWS layer present. If an IWS is used in the design to comply with other design goals (such as increasing infiltration), care must be taken to ensure that water is not stored near the surface of the bioretention cell, as that would aid in the persistence of bacteria. Considering the lack of information available and Hathaway’s (2010) minimum soil-depth recommendation, the IWS submerged zone should be no closer than 0.6 m (2 ft) from the surface. Therefore, the media depth of bioretention cells employing an IWS will necessarily be deeper than 0.6 m.

Separate from capture is the die-off of captured microbes, which is controlled by other environmental factors. Exposure to sunlight [ultraviolet (UV) radiation], desiccation, predation, temperature, and nutrient availability can all influence microbial survival in SCMs. Indicator bacteria have been shown to persist in natural systems (Struck et al. 2008). Studies by Jeng et al. (2005) suggest that indicator bacteria can persist in sediments given the right environmental conditions. Laboratory studies by Zhang et al. (2010, 2011) demonstrated rapid die-off of accumulated E. coli organisms in bioretention media, within a few days. Evidence suggests that protozoan predation is the primary die-off mechanism in this case.

The role of vegetation in bioretention pathogen sequestration has not been examined. Vegetation does attract some animals to the bioretention cell, resulting in direct defection, and exposure to UV light is a removal mechanism that is hindered by high vegetation densities. All of the field bioretention cells examined (Hathaway et al. 2009, 2011; Hathaway 2010; Passer et al. 2009) were vegetated. Passer et al. (2009) specifically examined turf grass, with the hypothesis that this vegetation would enable some UV light to reach captured bacteria. Further research is needed to provide a definitive assessment. Table 5 summarizes bioretention design guidance for pathogen (indicator species) removal.

**Metals, Hydrocarbons, and Oil**

The bioretention fill media and overlaying mulch layer provide numerous opportunities for the adsorption of metals and petroleum-based pollutants. Hydrophobic organic compounds such as polycyclic aromatic hydrocarbons (PAH) and other fuel-based hydrocarbons will partition into organic matter at either the surface (mulch) or in the media. A bioretention media may not normally be high in supplemental organic matter (for reasons described in later sections), but the opportunity for adsorption is high in organic-rich media (Schwarzenbach et al. 2003). Heavy metals also tend to bind strongly to components of soil media. Both the organic and inorganic fractions, particularly hydroxides (iron and aluminum oxides) provide complexation sites for the binding of metals. Metal adsorption is generally a strong function of pH, with increasing metal adsorption at higher pH. Because of the low metal concentrations typical of urban runoff (10^(-10)^2 (µg/L)), effective adsorption tends to occur within the pH range of the bioretention media (6–7).

Hong et al. (2006) demonstrated that most (> 80%) hydrocarbons (toluene, naphthalene) in a runoff application are captured in a thin mulch layer, where they are subsequently broken down as a result of microbial activity. PAHs were found to primarily be associated with particulates and were consequently captured in the top few centimeters of media in a field study (DeBlasi et al. 2009). Likewise, the preponderance of metals (Pb, Cu, Zn) were found to be trapped in the top 20 cm (8 in.) of bioretention media (Li and Davis 2008). High metal-removal rates have been found for bioretention cells in the field (Davis et al. 2003; Hunt et al. 2008; Hatt et al. 2009b). Because of the strong affiliation between the media and metals and hydrocarbons, sequestration occurs at the surface of the cell, as summarized in Table 6.

At some point, provision must be made for the removal of metals that have accumulated in the media. If the facility is regularly maintained, often to preserve an adequate infiltration rate or through routine landscaping, removal of a few centimeters of surface material may extend the metals-removal capacity indefinitely. Leaving the media in place for many years may necessitate removal and disposal of metal-laden media. Toxicity characteristic leaching procedure (TCLP) testing of the metal-laden media may be necessary. Nonetheless, metal-laden media should be only about 10-cm deep (Li and Davis 2008) and will be concentrated in the direct vicinity of the flow inlets to the bioretention cell (Jones 2009).

Phytoextraction of metals and hydrocarbons has been the subject of minimal study, as vegetation can take up some of these pollutants for later harvest. Studies by Sun and Davis (2007) suggest that only a small fraction of the total metals load will be taken up by bioretention grasses. A very thick growth of grasses would be necessary to have a measurable impact on the metals balance. As of now, an exact specification for plant selection cannot be made, but the presence of vegetation in a bioretention cell is recommended.

| Table 5. Designing Bioretention for Pathogen-Indicator Species Sequestration |
|-------------------------------|-----------------|-------------------|
| Design component              | Guidance         | Supporting studies |
| Fill media depth              | 0.6 m (2 ft) minumum | Hathaway et al. (2011) |
| Infiltration rate             | Longer T<sub>50</sub> preferable. 0.007 to 0.014 mm/s (1–2 in/h) | Ruscianno and Obrapta (2007); Bright et al. (2010); Zhang et al. (2010) |
| IWS                           | Acceptable to include, but keep top of submerged zone at least 0.6 m from surface of media | Hathaway (2010) |

| Table 6. Bioretention Design Needs for Metals and Hydrocarbon Sequestration |
|-------------------------------|-----------------|-------------------|
| Design component              | Guidance         | Supporting studies |
| Mulch layer                   | Important for trapping hydrocarbons. A minimum of 75–100 mm (3–4 in.) is recommended for weed prevention. | Hong et al. (2006); Hunt and Lord (2006) |
| Media depth                   | PAH sequestration occurs within a few centimeters of surface. Metal removal occurs in the top 200 mm (8 in.) of tested media. A minimum of 0.3 m (1 ft) is suggested. | Li and Davis (2008); DeBlasi et al. (2009); Hatt et al. (2009b) |
| Infiltration rate             | Up to 0.04 mm/s (6 in./h) | |
| Vegetation                    | Provide phytoremediation benefit that is as yet unquantified. Vegetation is recommended | |
Phosphorus

The principal mechanisms for phosphorus (P) removal in bioRetention are the filtration of particulate-bound P and chemical sorption of dissolved P. As discussed in the TSS section, filtration by bioRetention is highly effective. Particulate-bound phosphorus is trapped with TSS; therefore, to remove particulate P, only a shallow media layer is required (Hsieh et al. 2007). The chemical sorption of P, however, must be directly addressed by the designer and manifests itself in media selection.

Media selection is critical. Phosphorus-laden fill media is too often utilized, which can have extremely detrimental impacts on system performance (Hunt et al. 2006; Hatt et al. 2009b). Care must be taken to limit the fraction of organic matter (OM) as well; organic matter will break down and lead to leaching of phosphorus from the media (Clark and Pitt 2009), although different forms of OM will leach P to various degrees. Too frequently, high OM contents are specified for bioRetention fill mixes, intended to aid plant growth rather than improve water quality. High OM-content fill media will generally export high contents of phosphorus and should be avoided (Clark and Pitt 2009). Carefully selected fill media, low in phosphorus content, has reliably produced good phosphorus sequestration (Hsieh and Davis 2005; Hsieh et al. 2007; Hunt et al. 2008; Hatt et al. 2009a; Passeport et al. 2009; Lucas and Greenway 2011).

The next generation of media selection for phosphorus sequestration will incorporate tailored media for enhanced P uptake. P sorption in acidic soils is controlled by the amorphous iron and aluminum content of the soils, and successful bioRetention-media mixes will target iron and aluminum-rich substances. Native iron-rich soils, such as those in the Piedmont of the Mid and Southern Atlantic USA, or Krasnozem soil in Australia, are possible ingredients for a bioRetention mixture (Lucas and Greenway 2011). Iron or aluminum-based water-treatment residuals, a byproduct of drinking-water treatment, can be amended to media to greatly enhance P capacity (Lucas and Greenway 2011; O’Neill and Davis 2012a, b). Quantifying amorphous iron and aluminum contents in bioRetention media will allow the estimation of annual P loading rates, unit-volume media-storage capacity of phosphorus, and fill-volume media to determine how long a medium will be able to capture P before it needs to be replaced (Lucas and Greenway 2011).

Removal of dissolved phosphorus requires a comparatively high $T_{wp}$, and therefore a deeper medium (Hsieh et al. 2007). If dissolved P is of concern, a minimum media depth would be 0.6 m, with 0.9 m recommended. Field studies of bioRetention cells with 0.6-0.9 m media depths (Davis 2007; Hatt et al. 2009b; Passeport et al. 2009) have found good TP sequestration (provided that an appropriate media is used). Phosphorus removal is expected for a rather wide range of infiltration rates, with a suggested range of 0.007-0.028 mm/s (1-4 in. /h). Often soils that have the required soil composition adapted for effective phosphorus sequestration also have lower media-infiltration rates.

An IWS layer can complicate phosphorus removal if the layer stores water too close to the top of the media. Saturated conditions within the media, at locations where phosphorus would be stored, are likely to leach P (Hunt et al. 2006; Hatt et al. 2009b). If an IWS is utilized for other goals, it must be located below the P-sequestering portion of the media. As such, a 0.45-0.6 m (1.5-2 ft) separation is recommended between the top of the IWS layer and the media surface.

While chemical and physical processes associated with the fill media are the primary controlling factors for P sequestration, the presence of vegetation (provided that it is not fertilized) appears to be important as well. Batch experiments that investigated bioRetention, with and without vegetation (Lucas and Greenway 2008), clearly showed that the presence of vegetation improved P removal. Table 7 summarizes design recommendations for P removal.

Nitrogen

Nitrogen (N) is primarily removed from stormwater through biological nitrification-denitrification reactions. Under aerobic conditions, nitrification occurs such that nitrifying bacteria convert ammonia to nitrate, NO₃. Denitrification is an anoxic process, in which nitrate acts as the electron acceptor and the electron donor is typically an organic material. Nitrogen gas, $N_2$, is the product of denitrification—a benign nitrogen form that can be released to the atmosphere. Therefore, anoxic conditions play a critical role in bioRetention for nitrogen management. Plant uptake is a second mechanism of N removal. Lucas and Greenway (2008) demonstrated that a substantial portion of nitrogen could be removed from water and soil by vegetation. The vegetation-soil-water complex also creates an environment conducive to nitrification and denitrification.

The nitrification-denitrification process is temperature-dependent and denitrification rates are particularly low in colder conditions. Therefore N removal is generally limited by contact time under anoxic conditions. As such, a deeper media layer and lower infiltration rate are needed. A minimum of 0.75 m (2.5 ft) of fill media is required for nitrogen treatment, but at least 0.9 m (3 ft) is recommended.

### Table 7. Designing BioRetention for Phosphorus Removal

<table>
<thead>
<tr>
<th>Design component</th>
<th>Guidance</th>
<th>Supporting studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media composition</td>
<td>Critical component for TP removal. Select media with high P-sorption potential. Likely fill-media components for enhanced P-removal include iron and aluminum-rich soils and water-treatment residuals that can be used as amendments. Modest amounts of mulch appear satisfactory. Most composts will leach excessive P.</td>
<td>Hunt et al. (2006); Hsieh et al. (2007); Clark and Pitt (2009); Hatt et al. (2009b); Lucas and Greenway (2011); O’Neill and Davis (2012a, b)</td>
</tr>
<tr>
<td>Media infiltration rate</td>
<td>Between 0.007-0.028 mm/s (1-4 in./h)</td>
<td>Hsieh et al. (2007); Hatt et al. (2009b); Passeport et al. (2009)</td>
</tr>
<tr>
<td>Media depth</td>
<td>Minimum 0.6 m (2 ft), but 0.9 m recommended as a conservative value.</td>
<td>Kim et al. (2003); Passeport et al. (2009)</td>
</tr>
<tr>
<td>Underdrainage</td>
<td>IWS must not saturate portions of media where P is expected to be sequestered; 0.45-0.6 m (1.5-2 ft) separation between top of IWS and top of media.</td>
<td></td>
</tr>
<tr>
<td>Vegetation selection</td>
<td>Vegetation appears to improve P removal, but P uptake is expected to be somewhat limited.</td>
<td>Brattieres et al. (2008); Lucas and Greenway (2008)</td>
</tr>
</tbody>
</table>
Like P removal, media composition is important for N removal. Many initial bioretention design specifications have required the media to contain large fractions of organic matter. Unfortunately, organic matter in bioretention cells will break down and leach N (Hunt et al. 2006; Clark and Pitt 2009). However, a small amount of organic matter (no more than 5% of total weight or 10% of total volume) is needed in the media to provide a carbon source for the nitrifying and denitrifying bacteria. Postconstruction carbon can be supplied from plant roots, leaf litter, and as mulch breaks down. Because urban stormwater TN concentrations are generally < 5 mg/L, available carbon is not expected to limit the denitrification process.

IWS layers were initially designed specifically to foster denitrification (Kim et al. 2003; Dietz and Claussen 2006; Hunt et al. 2006). The original purpose of an IWS was to force an anoxic zone in the bottom media layer of bioretention. This may or may not occur depending upon underlying soil infiltration rates and the depth of an IWS. A deeper IWS with low-infiltration in-situ soils is more apt to produce anoxic conditions. If a bioretention cell has an IWS zone, the media depth will most likely be 0.9 m or greater.

Vegetation is an important component in determining the ability of a bioretention cell to remove N. Lucas and Greenway (2008) showed that the presence of vegetation enhances TN removal, suggesting that bioretention cells that are planted at a low density may not provide as much nitrogen treatment as those more densely planted. Brattieres et al. (2008) found that plant type is important, and the authors were able to recommend specific species for use in Victoria, Australia. Vegetation that produces a sizeable root mass in the media is preferred, but must not be so aggressive as to pose a clogging threat to underdrains. A pair of turfgrass bioretention cells in North Carolina did show positive N reduction (Passeport et al. 2009), but more deeply rooting grasses are expected to provide even better performance. Table 8 is a summary of nitrogen-specific design guidelines.

**Temperature/Thermal Pollution**

Heat transfer and infiltration are the two mechanisms employed by bioretention that reduce thermal impacts to surface waters. SCMs that store water underground partially mitigate thermal pollution (Natarajan and Davis 2010). Maximizing the $T_w$ in cooler sub-layers of the bioretention cell, or in the underlying soil, is the key design component. Jones and Hunt (2009) demonstrated that bioretention cells with deeper soil depths were more likely to cool runoff than cells with shallower depths. This is because soil near the surface is influenced by ambient air temperatures and direct sunlight to a greater degree than those in lower media profiles—i.e., further away from the bioretention bowl.

The best way to mitigate thermal pollution is to infiltrate runoff. The most effective bioretention cells for thermal abatement were those that were proportionally larger vis-à-vis the contributing drainage area (Jones and Hunt 2009)—in essence those with larger BAVs. As discussed in the hydrology design section of this study, Li et al. (2009) demonstrated that cells with high media volume-to-watershed size ratios were more successful in reducing outflow. Clear limits exist with respect to how large to make a bioretention cell, but deeper depths and larger cells will produce the coolest, and least quantity, of effluent. Without infiltration, it is highly unlikely that bioretention cells (or any SCMs) would be able to meet stringent cold-water discharge standards (Jones and Hunt 2009, 2010; Natarajan and Davis 2010).

While not studied for thermal control purposes, IWS systems (1) store runoff in cooler layers of the fill media for long periods (increasing $T_w$ internally), and (2) exfiltrate more water to the underlying soil (increasing $T_w$ externally). As such, the use of IWS is considered beneficial for thermal abatement (Brown and Hunt 2009). The IWS should be located such that water is not stored within the top 0.6 m (2 ft) of the media, as Jones and Hunt (2009) observed substantially higher soil temperatures in the top 0.6 m of the media.

Bioretention cells with vegetative cover provide shading to the media, preventing direct sunlight from warming the media surface. Vegetation that produces a canopy that shades the surface of the bioretention cell is recommended. A summary of thermal pollution abatement guidance is found in Table 9.

---

**Table 8. Designing Bioretention for Nitrogen Removal**

<table>
<thead>
<tr>
<th>Design component</th>
<th>Guidance</th>
<th>Supporting studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media composition</td>
<td>Modest OM (high OM leaches N); some carbon necessary for denitrification</td>
<td>Clark and Pitt (2009); Hunt et al. (2006)</td>
</tr>
<tr>
<td>Media infiltration rate</td>
<td>Between 0.007 and 0.014 mm/s (1-2 in./h)</td>
<td></td>
</tr>
<tr>
<td>Media depth</td>
<td>$T_w$ is important, so deeper media is preferred (0.9-m, or 3-ft, minimum)</td>
<td></td>
</tr>
<tr>
<td>Underdrainage</td>
<td>IWS provides longer $T_w$; allows denitrification to occur under anoxic conditions</td>
<td>Kim et al. (2003); Passeport et al. (2009)</td>
</tr>
<tr>
<td>Vegetation selection</td>
<td>Large root mass vegetation appears to be best</td>
<td>Brattieres et al. (2008); Lucas and Greenway (2008)</td>
</tr>
</tbody>
</table>

**Table 9. Designing Bioretention for Thermal Pollution Abatement**

<table>
<thead>
<tr>
<th>Design component</th>
<th>Guidance</th>
<th>Supporting studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media depth</td>
<td>At least 0.9 m, with 1.2 m preferred</td>
<td>Jones and Hunt (2009)</td>
</tr>
<tr>
<td>Media volume/surface area</td>
<td>Increasing the media volume relative to the drainage catchment is recommended. A threshold of 7% may be considered to promote infiltration, though more research is needed</td>
<td>Jones and Hunt (2009); Li et al. (2009)</td>
</tr>
<tr>
<td>Infiltration rate</td>
<td>Longer $T_w$ is preferable, 0.007-0.014 mm/s (1-2 in./h) is recommended</td>
<td></td>
</tr>
<tr>
<td>IWS</td>
<td>Recommended for the bottom 0.3–0.45 m of a 1.2-m deep bioretention cell</td>
<td>Brown and Hunt (2009)</td>
</tr>
<tr>
<td>Vegetation</td>
<td>Shading of soil surface cools surface of fill media. Vegetation that produces a near 100% canopy cover is recommended.</td>
<td>Jones and Hunt (2010)</td>
</tr>
</tbody>
</table>
Table 10. Designing a Bioretention Cell to meet Comprehensive Design Goals

<table>
<thead>
<tr>
<th>Design component</th>
<th>Guidance</th>
<th>Reason(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowl volume/depth</td>
<td>BAV to meet design requirements</td>
<td>Needed for volume/peak reduction; consider treatment train for extreme storm-event peak control</td>
</tr>
<tr>
<td>Mulch layer</td>
<td>75–100 mm (3–4 in.)</td>
<td>Needed for hydrocarbon sequestration</td>
</tr>
<tr>
<td>Media depth</td>
<td>1.2 m (4 ft)</td>
<td>Temperature mitigation. Other pollutants addressed at shallower depths.</td>
</tr>
<tr>
<td>Infiltration rate</td>
<td>0.007–0.014 mm/s (1/2 in./h)</td>
<td>Maximize root zone for BAV.</td>
</tr>
<tr>
<td>Media composition</td>
<td>Phosphorus-sorptive material; fines fraction between 8 and 12%; limited OM</td>
<td>Organic matter will break down and leach N and P, so must be limited. Fines fraction relates to above infiltration rate. Amendments for enhanced P removal are needed, such as iron or aluminum-based soils or water-treatment residuals</td>
</tr>
<tr>
<td>Internal water storage</td>
<td>Yes, with thickness to saturate bottom 0.6 m of fill media and gravel envelope</td>
<td>Increases infiltration. Cools temperatures. Reduces TN. Kept from top 0.6 m of media to prevent leaching of pollutants trapped at the top of the soil column. The surface area can be made larger if the BAV required for the cell is not met. However, 1.2 m of media (with 0.75 m of IWS accounting for 0.15 m of gravel envelope) is likely sufficient to meet most hydrologic design goals.</td>
</tr>
<tr>
<td>Surface area</td>
<td>Select what is needed to capture water-quality volume in a 300-mm bowl</td>
<td>The one part of the design that is in conflict. In theory, more shading provides better temperature reduction and less shading improves pathogen die-off. If either temperature or pathogen mitigation needs are dropped, the decision as to vegetation type and density becomes clearer. Clearly some vegetation is needed to maintain adequate infiltration and facilitate removal of certain pollutants, especially N.</td>
</tr>
<tr>
<td>Vegetation</td>
<td>Needed at moderate density</td>
<td>Increases exfiltration of the bioretention cell. Protects media surface from TSS inundation during construction and in contributing area stabilization.</td>
</tr>
<tr>
<td>Excavation technique</td>
<td>Rake method</td>
<td></td>
</tr>
<tr>
<td>Surface protection</td>
<td>Multiple; sod around bioretention cell perimeter may be good option.</td>
<td></td>
</tr>
<tr>
<td>and stabilization</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Cross section of bioretention cell designed to meet requirements in Table 10 (image by Brad Wardynski, NC State Univ.)

Putting It All Together

It is possible to design a bioretention cell to accomplish nearly all of the aforementioned goals, addressing many hydrologic and water-quality targets. A bioretention cell with 1.2 m of media to mitigate temperature is excessive, and more costly to construct, to address only regulations of trapping TSS and particulates. Fundamentally, however, one cell can accomplish both needs. For water quality, the pollutant with the most restrictive $T_{15}$ will dictate the media and underdrainage design. Requirements to mitigate peak flow lead to surface-area and bowl-depth design. An example of a bioretention cell to meet multiple needs is presented in this section.

The example bioretention cell is expected to provide the following services: mitigate the 2-year ARJ event, replicate the long-term (surface) hydrology of the predevelopment condition, have flows that do not contribute to excessive stream erosion, trap TSS and pathogen-indicator species, sequester metals and hydrocarbons, remove nitrogen and phosphorus, and discharge at cooler temperatures. A suggested design is provided in Table 10 and Fig. 2. The only conflicting guidance is for vegetation selection. While the need for vegetation is nearly universal among design needs, the type of vegetation does vary, namely for temperature control versus pathogen-indicator species treatment.

Summary

This research overview and summation provides design guidelines that target specific hydrologic and water-quality goals. It is possible for bioretention to accomplish many, if not most, regulatory needs associated with urban stormwater treatment. Bioretention facilities are versatile practices, but do need to be carefully constructed; regular inspection and maintenance provides greater assurance that the intended goals of the design are met. The eventual design of a bioretention cell can, and should, be different, depending upon regulatory needs and local conditions. Bioretention research continues to provide fundamental understanding of the mechanisms of performance and the days of “one size fits all” bioretention design specification, without appealing to research findings, are limited.

Acknowledgments

This work was supported by the Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET), NOAA Grant No. NA06NOS4190167. The authors are grateful to Shawn Kennedy and Brad Wardynski for their graphic production.
References


PADEP, Division of Watershed Management, Harrisburg, PA.
Abstract

In the Pacific Northwest of the United States, adult coho salmon (Oncorhynchus kisutch) returning from the ocean to spawn in urban basins of the Puget Sound region have been prematurely dying at high rates (up to 90% of the total runs) for more than a decade. The current weight of evidence indicates that coho deaths are caused by toxic chemical contaminants in land-based runoff to urban streams during the fall spawning season. Non-point source pollution in urban landscapes typically originates from discrete urban and residential land use activities. In the present study we conducted a series of spatial analyses to identify correlations between land use and land cover (roadways, impervious surfaces, forests, etc.) and the magnitude of coho mortality in six streams with different drainage basin characteristics. We found that spawning mortality was closely and positively correlated with the relative proportion of local roads, impervious surfaces, and commercial property within a basin. These and other correlated variables were used to identify unmonitored basins in the greater Seattle metropolitan area where recurrent coho spawning die-offs may be likely. This predictive map indicates a substantial geographic area of vulnerability for the Puget Sound coho population segment, a species of concern under the U.S. Endangered Species Act. Our spatial risk representation has numerous implications for urban growth management, coho conservation, and basin restoration (e.g., avoiding the unintentional creation of ecological traps). Moreover, the approach and tools are transferable to areas supporting coho throughout western North America.

Introduction

In recent decades, human population growth and development have continued to increase along the coastal margins of North America [1]. The associated changes in land cover and human land use have elevated land-based sources of pollution, and toxic stormwater runoff in particular, to become one of the most important threats to the biological integrity of basins, lakes, estuaries, and nearshore marine environments [2]. In the United States, concerns related to non-point source pollution have gained momentum over the past decade (e.g., [3,4]). This has culminated most recently in the designation of “water quality and sustainable practices on land” as one of nine National Priority Objectives for the newly established National Ocean Council, together with ecosystem-based management, marine spatial planning, climate change and ocean acidification, and changing conditions in the Arctic [2]. For toxic runoff, however, the connections between unsustainable practices on land and the decline of ecological resilience in aquatic habitats remain poorly understood.

In western North America, semelparous anadromous salmonids (Oncorhynchus spp.) typically migrate thousands of kilometers in their lifetimes. They hatch and rear in freshwater, migrate seaward to capitalize on the productivity of the oceans to grow rapidly and reach sexual maturity, and then return to their natal streams to spawn and die. Certain salmonids, including pink (O. gorbuscha) and chum (O. keta) migrate to the ocean relatively soon after hatching. Others, however, such as Chinook (O. tshawytscha), steelhead (O. mykiss), sockeye (O. nerka), and coho (O. kisutch) may spend one or more years in freshwater lakes, rivers and streams. Because of this extended freshwater residency, juveniles of these species are potentially more vulnerable to anthropogenic modifications of freshwater habitat quality [5].

In contrast to the high mortality experienced by juvenile salmonids, mortality at the adult spawner life stage is relatively low. Familiar natural causes of mortality include predation, disease [6,7,8,9], strandings (following high flows), elevated stream temperatures, and competition—e.g., in habitats with abundant salmon returns and limited spawning substrate. Various human activities such as recreational and commercial fishing, stream dewatering, and the placement of migration barriers can also increase salmon spawner mortality. In general, however, salmon spawner mortality has not been attributed to toxic chemical contaminants in stormwater runoff—a data gap that may be due, in part, to 1) the relative rarity of salmon spawners in urban basins with poor water quality, and 2) the logistical difficulty of implementing toxicity studies on migratory, seawater-to-freshwater transitional adults.

The exception is a recently documented phenomenon of returning adult coho salmon dying at high rates in urban and urbanizing streams in lowland Puget Sound region, which includes
the greater Seattle metropolitan area [10]. Coho return to small coastal stream networks to spawn each fall. Entry into freshwater is triggered by early autumn rainfall and rising stream flows. Since there had been extensive habitat degradation and loss in these lowlands, many basins were targeted for stream restoration projects in the 1990s. Subsequent surveys to evaluate project effectiveness discovered that many coho salmon were dying in newly-accessible stream reaches before they were able to spawn – i.e., female carcasses were found in good condition (ocean bright colors) with skeins (membrane or sac that contains the eggs within the fish) filled with unspawned eggs [10]. In addition, affected coho from several different urban basins showed a similar progression of symptoms leading up to death, including disorientation, lethargy, loss of equilibrium, mouth gaping, and fin spaying. Systematic daily spawner surveys in recent years (2002–2009) have shown that adult mortality rates in urban streams are consistently high (relative to spawning coho salmon in more pristine areas), ranging from ~25–90% of the total fall runs [10]. Mortality rates of this magnitude likely have important negative consequences for maintaining viable coho populations [11]. Consistent with this, most coho mortalities observed over the past decade were spawners that strayed (did not home to their natal stream reaches) into these restored urban freshwater habitats.

The precise underlying cause of recurrent coho die-offs remains under investigation. An initial weight-of-evidence forensic study has systematically ruled out stream temperature, dissolved oxygen, poor overall spawner condition, tissue pathology (e.g., gill), pathogen prevalence or disease, and other factors commonly associated with fish kills in freshwater habitats (Scholz et al., unpublished data). These findings, together with the rapid onset of the syndrome, the nature of the symptoms (e.g., gapping and disequilibrium), and the consistent occurrence within and between urban basins over many years together point to toxic stormwater runoff from urban landscapes as the likely cause of coho spawner mortality. Urban runoff and stormwater-influenced combined sewer overflows (CSOs) can contain an exceptionally complex mixture of chemical contaminants. Specifically, urban streams are receiving waters for runoff and discharges containing pesticides [12], metals [13], petroleum hydrocarbons [14], plasticizers, flame retardants, pharmaceuticals, and many other potentially toxic chemicals. The list of possible causal agents is therefore long.

The above chemical complexity notwithstanding, there are several reasons to suspect motor vehicle sources as toxicants that are killing returning coho. Vehicles deposit many compounds on road surfaces via exhaust emissions, leaking fluids, and the wearing of tires, brake pads and other friction materials [15]. Emissions contain nitrogen and sulfur dioxide, benzene, formaldehyde, and a large number of polycyclic aromatic hydrocarbons (PAHs). Fluids, including antifreeze and motor oil, contain ethylene and propylene glycol and PAHs. Tire wear releases zinc, lead, and PAHs onto road surfaces [16], and brake pad wear is a major source of copper, zinc, nickel, and chromium [16,17]. Collectively, these contaminants accumulate on streets and other impervious surfaces until they are mobilized by rainfall and transported to aquatic habitats via runoff. Polycyclic aromatic hydrocarbons and metals such as copper are known to be toxic to fish, although acute lethality usually occurs at exposure concentrations that are higher (by orders of magnitude) than those typically detected in urban streams. It is likely that fall stormwater pulses contain higher concentrations than winter and spring due to the potential buildup of contaminants during the relatively dry summer months.

Although the adult die-off phenomenon has been observed in all Seattle-area urban streams where coho salmon occur, the overall rate of mortality has varied among basins. In qualitative terms, a higher proportion of returning animals have survived to spawn in basins that have more open space (e.g., parks and woodlands). Conversely, mortality rates have been consistently higher in basins with proportionately greater “urban” land cover and land uses. This raises the possibility of a quantitative relationship between discrete basin characteristics and coho survival and spawning success. Such a relationship would be important for several reasons. First, if coho mortality is significantly correlated with one or more land cover or land use variables, the latter could be used to identify unmonitored lowland basins where coho populations are at greatest risk. Second, it could provide a means to evaluate how future human population growth and development might impact wild coho populations in Puget Sound (and elsewhere) that are currently healthy. Finally, it could narrow the list of potentially causative pollution sources in urban basins, thereby focusing future toxicological studies to identify the specific contaminants involved.

In this study we performed a spatial analysis to identify landscape variables that correlate most closely with surveyed rates of coho spawner mortality across six different basins in Puget Sound. The variables included land use and land cover, tax parcel types, roadways, and impervious surfaces. We then used the information from these correlations to generate spatially explicit predictions of recurrent spawner losses in unmonitored basins throughout the four most densely populated counties in the greater Seattle metropolitan area.

Materials and Methods

Study Sites

We characterized habitat conditions within the drainage basins from streams at six sites in the Puget Sound lowlands (Figure 1). We chose these sites because coho spawner mortality has been monitored at these locations for several years (2000–2009; [10]). The sites represent a wide range of anthropogenic alteration, from highly urbanized (e.g., Longfellow Creek) to relatively undisturbed (e.g., Fortson Creek). Fortson Creek is considered a non-urban site, whereas the other five sites are urban streams and have varying degrees of development. The urban streams have all been a focus of varying restoration project efforts aimed at enhancing habitat quality for anadromous Pacific salmon. With the exception of the relatively unaltered Fortson Creek site, all site basins had impervious surface proportions well above the levels (5–10%) commonly associated with the decline of biological integrity in streams [18,19].

Confirmed observation of the coho spawner mortality syndrome (see below) within a stream system was a key factor in study site selection. Importantly, natural production of coho in Seattle-area urban streams is very low. Not unexpectedly, recent modeling that shown that local coho population abundance declines precipitously at rates of spawner mortality documented for these drainages [11]. The adult returns to these streams are thus likely to be animals straying into sink or attractive nuisance habitats. Conversely, the syndrome has not been documented in streams where coho are relatively abundant – i.e., non-urban basins, as confirmed by a full season of daily stream surveys on Fortson Creek. Therefore, to evaluate the phenomenon in relation to land cover, we were constrained to streams where coho are affected, even if adult returns to these basins were low in certain years. Lastly, there is no evidence that the mortality syndrome is related to the origin of the spawners (i.e., hatchery vs. wild fish). For example, artificially propagated coho that return as adults to regional hatchery facilities in non-urban basins are unaffected.
Figure 1. Six study sites where coho spawner mortality was monitored and landscape conditions were quantified. Main map depicts the Greater Seattle Metropolitan Area in Washington State, which is within the Puget Sound/Georgia Basin of the Pacific Northwest, United States of America (USA). Inset map illustrates location of the study sites within Washington State and the location of Washington State within the USA. For reference, red shading on main map represents the relative intensity of urbanization (light-medium and dense urban [23,24]). Drainage basins depicted in yellow shaded polygons represent the total basin flowing into a given stream reach site. Key for site numbers: 1 = Des Moines; 2 = Fauntleroy; 3 = Fortson; 4 = Longfellow; 5 = Piper's, and, 6 = Thornton Creek.

doi:10.1371/journal.pone.0023424.g001

Study Subjects

Coho salmon in this study were all within the Puget Sound/Suquamish of Georgia Evolutionarily Significant Unit (ESU). An ESU is defined as a group of populations that 1) are substantially reproductively isolated from conspecific populations and 2) collectively represent an important component in the evolutionary
legacy of the species [20]. Currently, Puget Sound/Straits of Georgia coho are designated a “species of concern” under the U.S. Endangered Species Act [21].

Coho typically spawn in small (lower order) streams in the Puget Sound lowlands in late fall and early winter and their fry emerge from stream substrates from March to May. Fry reside in riverine habitats for 14–18 months, smolt, migrate to marine environments where they grow rapidly and mature (16–20 months), and finally migrate to their natal basins where they spawn and die [22]. The adult spawners from the six study basins were both marked (adipose fin clipped) and unmarked, suggesting a mix of hatchery and wild origins.

Coho Spawner Mortality

We used existing monitoring data collected as part of daily and weekly spawner surveys in each of the six study locations (Table 1). Data were collected during the fall spawning season from 2000–2009 by Seattle Public Utilities (SPU), the Wild Fish Conservancy, and the Northwest Fisheries Science Center (NWFSC). Streams were checked every few days in the early fall (usually the first or second week in October, depending on rainfall) until the first adult coho was observed. The streams were then surveyed daily for the duration of the fall run, until the last carcass was documented, typically in the first or second week of December. For several years, biologists working for the City of Seattle (Wild Fish Conservancy) also surveyed many of the same urban streams for coho spawner mortality on a weekly basis. Side-by-side comparisons of daily and weekly survey data (e.g., for Longfellow Creek in 2005 and 2007) revealed practically no loss of carcasses to scavengers. Accordingly, we included the weekly survey data in our analyses.

The entirety of the available spawning habitat within a given urban drainage was surveyed for premature adult coho mortality. For some streams, including Longfellow Creek, mid-stream barriers to upstream migration confined adults to the lower portions of the drainage. This made it possible, in the course of a few hours as part of a daily survey, to inspect all sections of the stream that (1) had a gravel substrate suitable for redds (spawning “nests” built by females), and (2) were focal areas for repeated (year-to-year) redd building during successive spawner runs.

Monitoring data were not collected at all sites for all years (Table 1). Mortality among returning coho was quantified only for females on the basis of egg retention — i.e., the number of partially spawned or unspawned female carcasses observed in streams over an entire spawning season. Notably, the total number of returning adults was low for some years and some basins (Table 1). Nevertheless, the aggregate spawner survey data used in this analysis are the most comprehensive currently available.

Geospatial Datalayers

We used existing geospatial datalayers as our source of potential predictor variables and as a proxy for habitat type and condition. The datalayers were generated by a variety of organizations for planning and analytical purposes, making them suitable for running spatial analyses on habitat. They were also available over the entire spatial domain of our predictive model. We used four geospatial datalayers: Land-cover of the Greater Puget Sound Region [23,24]; impervious and impacted surfaces [25]; property type (compiled from King [26], Kitsap [27], Pierce [28] and Snohomish county [29] tax parcel databases), and roadways (Puget Sound Regional Council; PSRC [30]).

The Land-cover of Puget Sound datalayer is the highest quality and most accurate depiction of land use and land cover in the Puget Sound lowlands. The datalayer used 30 m gridded LANDSAT TM imagery from 2002, which was extensively analyzed and corrected to produce an accurate (83% overall accuracy, [24]) depiction of land use and land cover conditions. To reduce the total number of potential predictor variables, we only used the dense urban (>75%); light to medium urban (<75%); and grass, crops and/or shrubs categories. We also combined the mixed and deciduous forest with the coniferous forest category and named it forests.

The impervious and impacted surfaces datalayer was derived from a 2001 LANDSAT TM image with 30 m pixels and an accuracy of 83–91% [25]. This datalayer depicts high to completely impermeable surfaces such as building roofs; concrete or asphalt roads and parking lots; concrete, asphalt or brick sidewalks, pedestrian walkways, and mailboxes; etc.

One of the limitations of these two datalayers was that the pixel size of the source LANDSAT TM imagery is 30 m, so smaller

Table 1. Coho spawner mortality proportion and cumulative number of female carcasses enumerated (in parentheses) by site (columns) and year (rows).

<table>
<thead>
<tr>
<th>Year</th>
<th>Des Moines</th>
<th>Fauntleroy</th>
<th>Fortason</th>
<th>Longfellow</th>
<th>Piper's</th>
<th>Thornton</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>-</td>
<td>0.25 (12)</td>
<td>-</td>
<td>0.74 (135)</td>
<td>0.18 (17)</td>
<td>0.88 (33)</td>
</tr>
<tr>
<td>2001</td>
<td>-</td>
<td>0.22 (9)</td>
<td>-</td>
<td>0.61 (111)</td>
<td>0.70 (37)</td>
<td>0.62 (11)</td>
</tr>
<tr>
<td>2002</td>
<td>-</td>
<td>0.00 (1)</td>
<td>0.01 (114)*</td>
<td>0.86 (57)*</td>
<td>0.60 (10)</td>
<td>0.80 (5)</td>
</tr>
<tr>
<td>2003</td>
<td>-</td>
<td>0.00 (1)</td>
<td>-</td>
<td>0.67 (188)*</td>
<td>0.00 (1)</td>
<td>1.00 (2)</td>
</tr>
<tr>
<td>2004</td>
<td>0.03 (30)*</td>
<td>0.00 (0)</td>
<td>-</td>
<td>0.89 (9)*</td>
<td>0.33 (3)</td>
<td>1.00 (1)</td>
</tr>
<tr>
<td>2005</td>
<td>-</td>
<td>0.75 (4)</td>
<td>-</td>
<td>0.72 (75)*</td>
<td>0.74 (5)</td>
<td>0.50 (8)</td>
</tr>
<tr>
<td>2006</td>
<td>-</td>
<td>0.00 (0)</td>
<td>-</td>
<td>1.00 (4)*</td>
<td>1.00 (0)*</td>
<td>1.00 (4)</td>
</tr>
<tr>
<td>2007</td>
<td>-</td>
<td>0.75 (4)</td>
<td>-</td>
<td>0.73 (41)*</td>
<td>0.20 (3)</td>
<td>0.80 (5)</td>
</tr>
<tr>
<td>2008</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.67 (32)*</td>
<td>-</td>
<td>1.00 (2)</td>
</tr>
<tr>
<td>2009</td>
<td>-</td>
<td>-</td>
<td>0.00 (0)</td>
<td>0.78 (36)*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Overall</td>
<td>0.63 (30)</td>
<td>0.37 (30)</td>
<td>0.01 (114)</td>
<td>0.72 (498)</td>
<td>0.57 (86)</td>
<td>0.83 (71)</td>
</tr>
</tbody>
</table>

A dash (-) indicates survey was not conducted for that year/site.
*Northwest Fisheries Science Center (NWFSC) daily surveys, all others were weekly and collected by Seattle Public Utilities (SPU) or the Wild Fish Conservancy [51,52].
1Non-urban site.
1 doi:10.1371/journal.pone.0023424.t001
features, such as roads and precise land cover boundaries, were not adequately captured. In order to address this deficiency, we analyzed property types and roadways, as they are represented as precise polylines and polygon delineations of the corresponding land cover variables. The boundaries in these geospatial data layers were derived from precise survey data from major metropolitan areas, collected over many years by King, Kitsap, Pierce and Snohomish Counties.

The property types (parcels) data layer was based on ground surveyed delineations of property, which are used for taxation purposes, with positional accuracy of $+/-12$ m or less [26,27,28,29]. The original number of parcel types described by each county was between 103 and 292. Using the descriptions in the documentation that accompanied the data layers, we were able to place each of the original parcel types into one of the five following categories: apartments and condominiums; commercial; industrial; parks and open space; and, residential.

The roadways data layer was based on ground surveyed road and street centerlines. Each segment had a corresponding functional classification (FC#) code and width, as defined by the Federal Highway Administration [31] Highway Performance Monitoring System, and the Puget Sound Regional Council [30], respectively. We reduced the original nine functional classification types down to two categories: 1) heavily used roads (rural minor collector [FC08]; urban principal arterial - interstate [FC11]; urban principal arterial - other freeways and expressways [FC12]); urban principal arterial - other [FC14]; urban or rural minor arterial [FC16 or FC06]; urban collector [FC17]); and, 2) urban or rural local access roads (FC09 or FC19). We then calculated the total area (total length of green street centerline segment multiplied by its width) of each street functional classification for each corresponding site basin.

Spatial Analyses

We defined the area of influence of the surrounding landscape for each site as the total area draining into that site (basin). Drainage basins for each site were generated using the ‘flowaccumulation’ command in Environmental Systems Research Institute (ESRI) ArcGIS (v. 9.3). We used a United States Geological Survey (USGS) 10 m digital elevation model (DEM) as the underlying terrain for generating basins. We then interacted the corresponding basin boundary for each of the six sites with each of the geospatial data layers and their associated categories using ArcGIS. We quantified each geospatial data layer and its associated category in a given basin as the fraction or proportion of the total area of the basin occupied by that geospatial data layer or category. Longfellow Creek stood apart from the other sites in terms of the accuracy of the flow accumulation model because an unknown fraction of stormwater runoff in this drainage is diverted into the municipal sewer system. Therefore, the theoretical basin area, based on the terrain represented in the DEM, was not as representative of the true basin area compared with the other five sites.

Statistical Analyses

We used generalized linear mixed-effects models (GLMMs; [32,33]) to test the relationships between geospatial variables and coho spawner mortality. The response was binomial (observed number of female spawner mortalities each year, given the total number of female coho that returned to each site) and the models used a logit link function. All models included a random effect of site on the intercept, which accounts for nonindependence of the repeated samples taken at each site. We constructed a set of 139 candidate models by considering all combinations of the 12 predictors taken one, two, three or four at a time, with the restriction that a model could include at most one predictor from each of the four geospatial data layers (land cover, impervious surfaces, property types, and roadways). We also excluded combinations of predictors that had a pairwise Spearman rank correlation exceeding 0.9 in absolute value. The candidate set included an intercept-only model as a no-effect baseline against which we could assess the predictive power of the geospatial variables.

We fitted the models using the Laplace approximation to the marginal likelihood [32] in the lme4 package in R [34,35]. We then used Akaike weights [36], which represent the relative support for each model, normalized so the weights sum to unity across the candidate set. We used these weights to compute model-averaged estimates and unconditional standard errors (SEs) for the fixed regression coefficients, and we quantified the relative importance of each predictor using variable weights (i.e., the summed Akaike weights of all models that included that predictor; [36]). These model averaging calculations were based on the 95% confidence set of models (i.e., the top-ranked models whose cumulative Akaike weight is 0.95), after re-normalizing the weights.

Mapping coho spawner mortality

Using the fitted models, we built a map of predicted coho spawner mortality throughout the four counties (King, Kitsap, Pierce and Snohomish) representing much of the Puget Sound lowlands, by applying the GLMM equations to geospatial data from unmonitored basins. We used basins delineated in the National Hydrography Dataset Plus [37] as the underlying mapping unit (300 ha mean, 466 ha SD) and intersected the NHDPPlus data layer with each of the geospatial data layers used in the statistical analyses. Within the four-county region, we only made spawner mortality predictions in basins where coho salmon presence has been documented, based on current geospatial data layers generated by the Washington Department of Fish and Wildlife [38]. We then calculated the proportion of each basin that was covered by the selected landscape feature. We generated predicted values of the proportion of mortalities from each model in the 95% confidence set and then model-averaged these values using the normalized Akaike weights [36]. These predictions apply to the average basin in the Puget Sound coho ESU with some given set of habitat conditions, in the sense that the random effect of site was set to zero. To be conservative in representing the precision of the predicted values, we divided the calculated rates of likely coho spawner mortality into three bins: <10%, 10-50%, and >50%. These break points were chosen somewhat arbitrarily to represent low, medium and high spawner mortality rates.

Results

We found strong associations between land use and land cover attributes and rates of coho spawner mortality. Across the 95% confidence set of fitted models, three variables were particularly important for predicting mortality based on high variable weights: impervious surfaces, local roads, and commercial property type (Table 2 and Figure 2). There was substantial model selection...
Table 2. AIC weights, model averaged parameter estimates and unconditional confidence intervals for each variable, ranked by AICc weight.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AICc weight</th>
<th>AICc averaged coefficient</th>
<th>Unconditional SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impervious surfaces</td>
<td>0.7158</td>
<td>15.8425</td>
<td>14.5376</td>
</tr>
<tr>
<td>Local roads</td>
<td>0.5647</td>
<td>-15.6199</td>
<td>68.3331</td>
</tr>
<tr>
<td>Commercial</td>
<td>0.5107</td>
<td>7.9375</td>
<td>8.2616</td>
</tr>
<tr>
<td>Dense urban</td>
<td>0.3865</td>
<td>-7.7776</td>
<td>16.1614</td>
</tr>
<tr>
<td>Apartments &amp; condominiums</td>
<td>0.2409</td>
<td>-9.5330</td>
<td>31.1917</td>
</tr>
<tr>
<td>Heavy used roads</td>
<td>0.2019</td>
<td>5.3445</td>
<td>31.5073</td>
</tr>
<tr>
<td>Forest</td>
<td>0.1163</td>
<td>-0.7793</td>
<td>6.2249</td>
</tr>
<tr>
<td>Light to medium urban</td>
<td>0.1149</td>
<td>0.3250</td>
<td>2.9751</td>
</tr>
<tr>
<td>Grass, shrubs &amp; corps</td>
<td>0.0993</td>
<td>0.1664</td>
<td>5.4517</td>
</tr>
<tr>
<td>Residential</td>
<td>0.0975</td>
<td>0.0738</td>
<td>16.6820</td>
</tr>
<tr>
<td>Industrial</td>
<td>0.0547</td>
<td>-0.2475</td>
<td>4.7008</td>
</tr>
<tr>
<td>Parks &amp; open space</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

DOI:10.1371/journal.pone.0023424.t002

uncertainty, reflected in a large 95% confidence set and large number of models with ΔAICc<2.0 (57 and 8 of 139 candidate models, respectively; Table 3). In addition, although we excluded highly collinear combinations of variables (|r|>0.9), many variables were still strongly correlated, resulting in unstable parameter estimates and large unconditional SE estimates (Table 2). Nonetheless, predictive models that included land use and land cover attributes as predictors were clearly superior to the intercept-only model (ΔAICc = 20.4; Table 3), supporting the association of these variables with coho mortality.

While the multicollinearity among potential predictors made causal interpretation of the models difficult, it did not preclude predictions of where coho salmon are likely to be affected along an urbanization gradient. Not surprisingly, the highest predicted mortality rates were clustered around the major metropolitan areas of eastern Puget Sound, contained within Snohomish, King, Kitsap, and Pierce counties (Figure 3). In addition, there is a significantly sized area in Eastern Puget Sound that has considerable proportions of the variables (local roads, impervious surface and commercial parcel) most correlated with substantial mortality rates. It is important to note that these predicted values have substantial associated uncertainty and should therefore be interpreted cautiously; however, it is reasonable to use them for assigning the break points for the low, medium, and high mortality rate categories represented on the map.

Discussion

Overall, we have used conventional tools in landscape ecology to shed light on an unusually complex ecotoxicological challenge. Our analyses strongly support that specific characteristics of basins in the Puget Sound lowlands are linked to the die-offs of coho spawners that have been widely observed in recent years. Across basins, the strength of the association is greatest for impervious surfaces, local roads, and commercial property. We did not evaluate hydrologic or geomorphic basin characteristics as part of our analysis. Nevertheless, our findings support the hypothesis that coho are being killed by as-yet unidentified toxic chemical contaminants that originate from these types of surfaces and are transported to salmon spawning habitats via stormwater runoff.

Our results extend a large body of scientific information linking urbanization (broadly defined) and degraded water quality to a loss of biological integrity (sensu Karr [39]) and productivity in freshwater stream networks [18,40,41]. Previous studies have generally related land use and land cover variables to macroinvertebrate assemblages in streams [42], or to the relative abundance of salmon and other fish (e.g., [22,43,44]). The present analysis is novel because it relates basin characteristics directly to salmon health and survival, versus species presence or absence. Moreover, it offers new insights on the water quality aspects of urban runoff. The focus of most salmon restoration projects is physical characteristics of spawning and rearing habitat [45]. Most salmon specific restoration projects are deemed successful if they simply restore the physical habitat to a suitable state for a given species [46]. Our study suggests that suitable spawning and rearing habitats may not be supportive of coho salmon persistence when the surrounding landscape is urbanized. The linkages between increased impervious coverage within a basin, increased stormwater runoff, altered hydrologic processes, and ecological decline are well established (e.g., [18]). However, stormwater impacts encompass both physical and chemical drivers of decline, and it can be difficult to distinguish between these via in situ assessments because stream invertebrate communities integrate both stressor categories. Coho salmon spawners, by contrast, appear to be promising and specific sentinels for the degraded water quality aspect of urban runoff. Compared to macroinvertebrate sampling and taxa identification, the coho mortality syndrome is relatively easy and inexpensive for non-specialists to monitor in the form of digital video recordings of symptomatic fish, or the presence of unspawned female carcasses in streams.

Interestingly, the mortality syndrome appears to be specific to coho salmon. For example, there were temporally overlapping runs of coho and chinook salmon (O. keta) in Piper's Creek in the fall of 2006. Whereas all of the adult coho succumbed to the mortality syndrome, the chum were unaffected, with nearly all surviving to spawn (190 of 135 spawned out female carcasses; Scholz et al., unpublished data). Consistent with this, the survey
Figure 2. Female coho spawner mortality as a function of the proportion of each of the top three predictors in a given site basin, at the six study sites. Individual points correspond to specific years for each site. Mortality expressed as proportion of all returning females that died in a given year. Solid circle = Des Moines; hollow circle = Fauntleroy; solid square = Fortson; hollow square = Longfellow; solid triangle = Piper’s; hollow triangle = Thornton Creek.

doi:10.1371/journal.pone.0023424.g002

teams have not observed the characteristic symptoms (e.g., surface swimming, gaping) among other fish species that inhabit urban streams such as sticklebacks and cutthroat trout. Not only are coho unusual in this respect, the phenomenon appears to be restricted to the adult life stage. In the fall of 2003, surface flows from Longfellow Creek were diverted through streamside sheds housing aquaria that contained individual juvenile coho from the NWFSC hatchery. The juveniles (n=20) were maintained and observed daily throughout the fall spawner run. Overall juvenile survival was 100%, and the juveniles behaved normally, even on days when symptomatic adults were observed in the nearby stream (Scholz et al., unpublished data). The underlying reasons
<table>
<thead>
<tr>
<th>Model</th>
<th>Equation</th>
<th>∆MIC</th>
<th>wACC</th>
</tr>
</thead>
<tbody>
<tr>
<td>a+b</td>
<td>-4.5664+19.70(b)+44.41(b)</td>
<td>0.000</td>
<td>0.0953</td>
</tr>
<tr>
<td>c+d+b</td>
<td>-3.9215-109.56(b)+8.75(c)-29.98(c)</td>
<td>0.046</td>
<td>0.0912</td>
</tr>
<tr>
<td>c+ref</td>
<td>-3.6355+10.41(c)+20.21(c)</td>
<td>0.372</td>
<td>0.0775</td>
</tr>
<tr>
<td>cd+re</td>
<td>-4.4921+12.61(c)+14.31(c)-5.34(d)</td>
<td>0.579</td>
<td>0.0698</td>
</tr>
<tr>
<td>c+da</td>
<td>-4.4858+14.31(d)+5.21(d)-3.62(g)</td>
<td>0.666</td>
<td>0.0668</td>
</tr>
<tr>
<td>c+eab</td>
<td>-2.6065+15.89(c)+20.67(b)-2.38(b)</td>
<td>1.150</td>
<td>0.0525</td>
</tr>
<tr>
<td>c+eab</td>
<td>-4.6229+16.37(c)+35.26(b)+2.70(c)</td>
<td>1.357</td>
<td>0.0473</td>
</tr>
<tr>
<td>c+eab</td>
<td>-4.7001+17.52(c)+43.83(b)+1.62(b)</td>
<td>1.576</td>
<td>0.0424</td>
</tr>
<tr>
<td>c+eab</td>
<td>-4.5043+19.70(c)-53.28(c)</td>
<td>2.255</td>
<td>0.0277</td>
</tr>
<tr>
<td>cd+d+b</td>
<td>3.0628-3.484(b)-56.38(b)-46.28(b)-7.82(b)</td>
<td>2.485</td>
<td>0.0269</td>
</tr>
<tr>
<td>cd+eib</td>
<td>-7.555-13.72(b)+21.23(c)+11.65(c)</td>
<td>2.543</td>
<td>0.0262</td>
</tr>
<tr>
<td>c+d+eb</td>
<td>-3.9266-94.52(b)+43.32(b)+25.00(b)+16.05(k)</td>
<td>2.613</td>
<td>0.0253</td>
</tr>
<tr>
<td>cd+eb</td>
<td>-4.5174+20.39(c)+43.96(c)-0.52(b)</td>
<td>2.722</td>
<td>0.0236</td>
</tr>
<tr>
<td>c+d+eb</td>
<td>-4.0854+2.39(c)-76.45(b)+38.23(b)-22.27(b)</td>
<td>2.885</td>
<td>0.0221</td>
</tr>
<tr>
<td>c+d+eb</td>
<td>-4.7368+15.57(c)+6.88(c)-9.22(c)-22.10(c)</td>
<td>2.925</td>
<td>0.0216</td>
</tr>
<tr>
<td>cd+eib</td>
<td>-3.9607-100.49(b)+46.40(c)+27.43(b)-5.46(e)</td>
<td>2.954</td>
<td>0.0213</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-3.8347+12.37(c)+10.49(c)-40.59(c)-39.28(b)</td>
<td>3.280</td>
<td>0.0181</td>
</tr>
<tr>
<td>cd+eib</td>
<td>-3.8534+12.46(c)-40.45(c)+38.73(c)-0.18(b)</td>
<td>3.294</td>
<td>0.0180</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-3.9360+12.46(c)-40.28(c)+39.30(c)-0.31(b)</td>
<td>3.326</td>
<td>0.0177</td>
</tr>
<tr>
<td>cd+eib</td>
<td>-4.6134+16.52(c)+5.79(c)-13.40(c)+4.05(b)</td>
<td>3.378</td>
<td>0.0172</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-1.1996+46.26(c)-55.97(c)-24.83(c)</td>
<td>3.423</td>
<td>0.0168</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>3.9315+13.97(b)+17.40(b)+15.89(b)</td>
<td>3.858</td>
<td>0.0136</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>2.2747+22.99(c)+47.30(c)-7.31(b)</td>
<td>3.931</td>
<td>0.0313</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>1.2512+60.31(c)-0.13(b)</td>
<td>4.028</td>
<td>0.0205</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-5.8874+16.71(c)+32.45(b)+7.72(c)-0.75(b)</td>
<td>4.299</td>
<td>0.0190</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>5.8364-27.35(b)+11.35(b)+5.97(b)</td>
<td>4.837</td>
<td>0.0083</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-4.4535+18.76(c)-59.31(c)+13.3(c)</td>
<td>4.915</td>
<td>0.0080</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-2.4511-52.30(b)+20.45(c)-13.34(c)+7.50(c)</td>
<td>5.097</td>
<td>0.0079</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-4.7422+20.37(c)+45.05(c)+5.43(c)</td>
<td>5.141</td>
<td>0.0071</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-4.4680-1.36(b)-19.52(c)-52.48(c)</td>
<td>5.158</td>
<td>0.0071</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-4.5797+19.60(c)+53.24(c)-0.02(c)</td>
<td>5.188</td>
<td>0.0070</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>8.1283+20.52(b)-45.07(c)+14.67(c)</td>
<td>5.509</td>
<td>0.0059</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-4.2426+12.30(c)-5.31(b)</td>
<td>5.649</td>
<td>0.0055</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-5.6725+141.73(b)+22.77(c)+17.24(b)</td>
<td>5.821</td>
<td>0.0051</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-3.9208-12.84(b)+14.63(b)+6.46(b)</td>
<td>5.896</td>
<td>0.0049</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>0.4930+6.81+19.67(c)-5.22(b)</td>
<td>6.083</td>
<td>0.0045</td>
</tr>
<tr>
<td>c+d+eib</td>
<td>-1.0049+68.63(c)-59.91(c)-6.94(c)+26.58(b)</td>
<td>6.343</td>
<td>0.0039</td>
</tr>
</tbody>
</table>

Model weights shown here are re-normalized for the set of 37 top-ranked models shown: a = commercial; b = local roads; c = impervious; d = dense urban; e = apartments and condominiums; f = heavily used roads; g = light to medium urban; h = forest; i = residential; j = grass, crops and/or shrubs; and, k = industrial.

for the syndrome’s surprising uniqueness to adult coho are not yet known.

Daily or weekly stream surveys are labor intensive, and for this reason only a subset of urban drainages in Puget Sound have been monitored to date. The GIS-based mapping tool developed for this study can be used to identify future monitoring efforts on basins with a higher likelihood of coho die-offs based on land cover attributes. In addition to the basins we have identified within the range of the Puget Sound/Glacier Bay ESU, this approach could be extrapolated to other geographic areas where coho return to spawn along a gradient of urban growth and development. This includes, for example, coho from the Lower Columbia River ESU, a threatened population segment with a spawner range encompassing the greater metropolitan area of Portland, Oregon. Overall, future surveys will ground-truth initial model outputs and provide additional data that can be used to improve the predictive accuracy of the mapping tool.

Our findings likely "hotspots" for coho spawner mortality throughout central Puget Sound. Given that recurring adult losses at a rate greater than approximately 10% are likely to substantially reduce local population abundances, the high mortality basins in Figure 3 (10-50% and >50% predicted mortality categories) may represent sink habitats for the Puget Sound/Glacier Bay ESU. This is an important consideration for coho recovery planning at the local, county, and regional scales. Second, our results indicate areas where toxic exposure could potentially undermine stream restoration efforts - specifically, strategies that improve physical and biological habitat conditions (flow, connectivity, channel complexity, riparian function, etc.) as a means to boost coho population productivity.

The potential influence of rainfall, including timing, frequency, and individual storm intensity, remains an area of active investigation. Throughout the years of stream surveys, it has been qualitatively evident that rainfall influences the mortality syndrome. For example, salmon that arrive and enter a stream during an extended dry interval (a week or more) often survive and then become symptomatic and die when it next rains (Scholz et al., unpublished data). One of our aims in surveying Longfellow Creek (the stream with the most abundant overall returns) for more than a decade was to evaluate inter-annual variation in coho spawner mortality in relation to rainfall. However, a quantitative analysis has proven problematic due to highly variable rainfall patterns in combination with low adult returns in some years. It is clear, however, that the syndrome is not a simple first-flush phenomenon. In most years, both egg retaining and spawned out carcasses were observed across the 8-10 week fall run, irrespective of the number and size of rain events over that interval.

Over the longer term, an approach similar to the one developed here could be used to forecast the likely impacts of future human population growth and development on Puget Sound coho populations that are currently healthy. For example, the expansion of local road networks is a core focus for urban growth planning, and these projections could serve as a basis for evaluating how and where coho spawner mortality will increase under different growth management scenarios. This, in turn, would inform strategies to reduce or mitigate toxic runoff in highly productive basins, in advance of expanding transportation infrastructure - i.e., prevention vs. costly retrofits to the built environment. Also, our modeling approach could be expanded to include the timing and intensity of rainfall as potential drivers for coho spawner mortality. Rainfall patterns may be a key determinant of stormwater quality, although more work in this area is needed. Climate change is expected to shift regional rainfall patterns, and it should be possible to explore how this will interact with changing land cover (urbanization) to influence stormwater quality and toxic runoff to coho spawning habitats.
While not definitive, our results reinforce the parsimonious explanation that coho deaths are caused by one or more contaminants originating from motor vehicles. As noted earlier, this is important because it narrows the list of candidate toxics in complex urban landscapes. Future toxicological studies should focus on two ubiquitous urban runoff contaminant classes in particular. The first are metals in brake pads and other vehicle friction materials. Copper, zinc, and other metals are known to specifically target the fish gill, thereby disrupting respiration and osmoregulation [47]. The second, PAHs, [14,48,49] are taken up across the fish gill, and can impair cardiac function and respiration [50]. The symptoms displayed by affected coho (surface swimming, gaping, loss of equilibrium, etc.) are consistent with a disruption of respiration, osmoregulation, or circulation, or some combination of these.

Notably, PAHs and metals usually cause the above toxicological effects at concentrations well above those typically detected in urban streams. However, the majority of conventional toxicology studies using salmonids focus on freshwater species (e.g., rainbow trout) or the freshwater life stages of juvenile anadromous species. There are practically no toxicity data for coho salmon at the adult spawning stage. Many important osmoregulatory changes take place during the transition from seawater prior to spawning, and these may render adult coho more vulnerable to metals and PAHs than freshwater-resident salmonids. Adding to this complexity is the possibility of interactive toxicity (e.g., synergism) among contaminant mixtures. Studies that experimentally reproduce the familiar symptomology and mortality in adult coho, under controlled exposure conditions with environmentally realistic mixtures of metals and PAHs, will likely be necessary to definitively implicate motor vehicles.

Acknowledgments

We thank John Williams and an anonymous reviewer for significantly improving previous drafts of this manuscript. Disclaimer: the findings, conclusions and views expressed herein are those of the authors and do not necessarily represent those of the National Oceanic and Atmospheric Administration or the U.S. Fish and Wildlife Service.

Author Contributions

Conceived and designed the experiments: BEF JWD NLS Performed the experiments: BEF ERB FA. Analyzed the data: BEF ERB FA. Wrote the paper: BEF ERB NLS.

References

Recurrence Die-Offs of Adult Coho Salmon Returning to Spawn in Puget Sound Lowland Urban Streams

Nathaniel L. Scholz1*, Mark S. Myers1, Sarah G. McCarthy2, Jana S. Labenia1, Jenifer K. McIntyre1, Gina M. Yllitalo1, Linda D. Rhodes3, Cathy A. Laetz1, Carla M. Stehr1, Barbara L. French1, Bill McMillan4, Dean Wilson2, Laura Reed4, Katherine D. Lynch4, Steve Damm5, Jay W. Davis5, Tracy K. Collier1

1 Northwest Fisheries Science Center, NOAA Fisheries, Seattle, Washington, United States of America. 2 Department of Natural Resources and Parks, King County, Seattle, Washington, United States of America. 3 Wild Fish Conservancy, Duvall, Washington, United States of America. 4 Seattle Public Utilities, City of Seattle, Seattle, Washington, United States of America. 5 Washington Fish and Wildlife Office, U.S. Fish and Wildlife Service, Lacey, Washington, United States of America

Abstract

Several Seattle-area streams in Puget Sound were the focus of habitat restoration projects in the 1990s. Post-project effectiveness monitoring surveys revealed anomalous behaviors among adult coho salmon returning to spawn in restored reaches. These included erratic surface swimming, gaping, fin slapping, and loss of orientation and equilibrium. Affected fish died within hours, and female carcasses generally showed high rates (>90%) of egg retention. Beginning in the fall of 2002, systematic spawning surveys were conducted to 1) assess the severity of the adult die-offs, 2) compare spawning mortality in urban vs. non-urban streams, and 3) identify water quality and spawner condition factors that might be associated with the recurrent fish kills. The forensic investigation focused on conventional water quality parameters (e.g., dissolved oxygen, temperature, ammonia), fish condition, pathogen exposure and disease status, and exposures to metals, polycyclic aromatic hydrocarbons, and current use pesticides. Daily surveys of a representative urban stream (Longfellows Creek) from 2002-2005 revealed premature spawning mortality rates that ranged from 60–100% of each fall run. The comparable rate in a non-urban stream was <1% (Fortson Creek, surveyed in 2002). Conventional water quality, pesticide exposure, disease, and spawning condition showed no relationship to the syndrome. Coho salmon did show evidence of exposure to metals and petroleum hydrocarbons, both of which commonly originate from motor vehicles in urban landscapes. The weight of evidence suggests that freshwater-transitional coho are particularly vulnerable to an as-yet unidentified toxic contaminant (or contaminant mixture) in urban runoff. Stormwater may therefore place important constraints on efforts to conserve and recover coho populations in urban and urbanizing watersheds throughout the western United States.

Citation: Scholz NL, Myers MS, McCarthy SG, Labenia JS, McIntyre JK, et al. (2011) Recurrent Die-Offs of Adult Coho Salmon Returning to Spawn in Puget Sound Lowland Urban Streams. PLoS ONE 6(12): e28013. doi:10.1371/journal.pone.0028013

Editor: Howard Browman, Institute of Marine Research, Norway

Received August 5, 2011; Accepted October 29, 2011; Published December 14, 2011

This is an open-access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Funding: This project received agency funding from the National Oceanic and Atmospheric Administration (Coastal Storms Program), the U.S. Fish and Wildlife Service National Contaminants Program, the U.S. Environmental Protection Agency Region 16, and the King Conservation District. Funds were delivered via interagency agreements, so grant numbers are not applicable. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: Nathaniel.Scholz@noaa.gov

Introduction

In lowland Puget Sound, many urban streams in the vicinity of Seattle were a focus of extensive physical and biological restoration activities in the 1990s. These projects, sponsored by the City of Seattle and other regional municipalities, served multiple purposes such as the creation of public green space, the removal of culverts and other impassable barriers for fish, the placement of large woody debris and gravel substrate, the removal of noxious weeds, and the planting of native vegetation. A related aim was to evaluate the extent to which adult salmon would return to spawn in the newly available and improved habitats. This post-project effectiveness monitoring was carried out via fall spawning surveys that were conducted weekly from 1999-2001, with a primary focus on coho (Oncorhynchus kisutch), Chinook (O. tshawytscha) and chum (O. keta) salmon.

These early monitoring efforts in 1999-2001 identified an unusual syndrome of pre-spawn mortality among adult coho returning to restoration sites to spawn. Coho typically spawn in small lowland streams in October through December. Eggs incubate in gravel nests (redds) from which fry emerge in the spring (March through May). Juveniles rear in freshwater for approximately a year and then migrate to estuaries the following spring. Coho spend at least one full year in the ocean before returning to their natal watersheds to spawn, after which they die (semelparous life history). Adult migration into freshwater is triggered by fall rain events that produce transient high flows in streams. Coho spawning in Seattle-area streams are often a mix of hatchery and natural origins, with hatchery fish distinguishable by a clipped adipose fin and, less commonly, the presence of a rostral-implant coded wire tag.

Affected coho spawners observed in post-restoration effectiveness monitoring surveys showed a consistent suite of symptoms that included surface swimming, gaping, loss of equilibrium, and pectoral fin slapping (Video S1). The onset of the syndrome was rapid, and stricken fish typically died within a few hours. Pre-spawn mortality was confirmed by a near-total retention of eggs in female carcasses inspected during the surveys.
The recurrent die-off of coho in urban drainages appears to be a phenomenon distinct from other types of pre-spawn mortality that have previously been reported for other species of salmon. These include, for example, sockeye salmon in the Fraser River and watersheds of Bristol Bay, as well as Chinook salmon in the Klamath River. In these non-urban freshwater habitats, pre-spawn mortality is described as a chronic process where fish are weakened by a low energy status, poor physical condition, wasting, and eventual death. This process occurs over a protracted timeframe (i.e., weeks). The causes vary and include an abnormally early arrival on spawning grounds, thermal stress, and increased susceptibility to the myxosporean parasite *Parvicapsula microti* (Fraser River sockeye; [1,2,5]), high spawner density, low water level, high water temperature, and low dissolved oxygen levels (Bristol Bay sockeye; [4,5]; and low flows, increased water temperature, high spawner densities, and disease caused by the pathogenic ciliate *Ichthyophthirius multifiliis* and *Flavobacterium columnare*, the bacterial agent for columnaris in fish (e.g., Klamath River Chinook salmon; [6]).

Here we report the results of an eight-year investigation (2002-09) to characterize the frequency and geographical extent of coho mortality, and to identify associated water quality and spawner condition factors. We conducted daily surveys of multiple creeks to assess rates of pre-spawning mortality across the entire duration of fall coho runs. We assessed the physical condition, pathogen exposure status, and disease status of affected female coho for comparison to 1) unaffected wild adult females collected from a non-urban reference stream, 2) unaffected adult females returning to several area hatcheries, and 3) seawater-phase adults collected from Elliott Bay along the Seattle waterfront, prior to their entry to restored freshwater habitats in urban drainages. Fish from each of these locations were profiled using biomarkers of exposure to common toxic contaminants in urban runoff, including metals, homeonuse, insects, and petroleum hydrocarbons. Lastly, we monitored conventional water quality information (e.g., temperature, dissolved oxygen, specific conductance, and pH) for urban streams during adult coho die-off events.

**Materials and Methods**

A paucity of coho spawners in urban streams throughout this study placed important constrains on sample collection for the purposes of a forensic analysis. Spawner abundances were generally low and unpredictable in urban streams where the die-off phenomenon occurs. By contrast, in non-urban streams where coho are relatively abundant, spawners were unaffected. Therefore, tissue collections from coho in urban streams were ad hoc and opportunistic. The streams surveyed during the course of this study and associated samples collected are listed in Table 1.

To ensure sample integrity, tissues were not collected from the decomposing carcasses found in streams. Conversely, we did not sacrifice live, non-symptomatic fish in urban streams because these coho may or may not have survived to successfully spawn. As a consequence, some types of samples (e.g., gill, bile, PAHs; see below) had to be collected from spawners that were either overtly symptomatic or very recently dead, as evidenced by gill coloration (see below). As noted earlier, symptomatic fish progress to death rapidly. Stream surveys were generally less than two hours in a given day, and thus encounters with symptomatic fish were infrequent. This accounts for a relatively small sample size for some tissues despite an intensive overall survey effort.

**Study locations**

Daily or weekly spawner surveys (including tissue collections) were conducted on several Seattle-area streams from 2002-2009. These included Longfellow, Thornton, Piper's, Des Moines, Taylor, and Fauntleroy Creeks (Figure 1). Detailed descriptions of each of these drainages (except Des Moines) can be found in a recent City of Seattle report on urban waterways [7]. Longfellow Creek in West Seattle, the urban stream found to have the highest numbers of adult-entry coho in preliminary assessments (1999-2001), was the focus of daily surveys in each of the eight years of monitoring. Des Moines Creek to the southwest of Seattle was surveyed daily in 2004 and Piper's Creek in northwest Seattle was surveyed daily in 2006. In other years, these two urban streams were monitored approximately weekly, as were Taylor, Thornton, and Fauntleroy Creeks [8]. To assess the prevalence of pre-spawn mortality among wild coho salmon returning to spawn in a non-urban drainage, we surveyed Fortson Creek (a tributary to the North Fork Stillaguamish River north of Seattle; Figure 1) daily in the fall of 2002.

### Table 1. Summary of survey locations and associated tissue samples.

<table>
<thead>
<tr>
<th>Location</th>
<th>Category</th>
<th>Years Sampled</th>
<th>Survey Frequency</th>
<th>Water Quality</th>
<th>Tissue Sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>gills</td>
</tr>
<tr>
<td>Longfellow Creek</td>
<td>urban stream</td>
<td>2002-2009</td>
<td>daily</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piper's Creek</td>
<td>urban stream</td>
<td>2006</td>
<td>daily</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Des Moines Creek</td>
<td>urban stream</td>
<td>2004</td>
<td>daily</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fortson Creek</td>
<td>forested stream</td>
<td>2002</td>
<td>daily</td>
<td></td>
<td></td>
</tr>
<tr>
<td>University of Washington Hatchery</td>
<td>urban hatchery</td>
<td>2002</td>
<td>one day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillaguamish Hatchery</td>
<td>rural hatchery</td>
<td>2002</td>
<td>one day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Issaquah Creek Hatchery</td>
<td>urban hatchery</td>
<td>2002, 2003</td>
<td>one day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wallace River Hatchery</td>
<td>rural hatchery</td>
<td>2009, 2008</td>
<td>one day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elliot Bay</td>
<td>estuary</td>
<td>2003</td>
<td>one day</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*sampled only in 2002;  
*sampled only in 2003;  
*sampled only in 2004;  
*sampled only in 2008.
Tissue samples were also collected from regional hatcheries, including the Stillaguamish Tribal Hatchery (2002), the University of Washington Research and Teaching Hatchery (2002), the Wallace River State Hatchery (2003), and the Issaquah Creek State Hatchery (2002 & 2003). Salmon returning to both the University of Washington and Issaquah hatcheries traverse an urbanized landscape through a series of lakes separated from Puget Sound by a single set of locks. The Stillaguamish and Wallace River hatcheries are located on tributaries to the North Fork of the Stillaguamish River and the Snohomish River, respectively (Figure 1); returning coho pass through primarily rural and forested landscapes.

Adult seawater-phase coho salmon were collected in Elliott Bay (Seattle waterfront, lower Duwamish waterway) prior to freshwater entry. Animals were captured by gillnet in coordination with Muckleshoot tribal fishing operations in the early fall (September 11) of 2003. Adult coho were transferred live or recently dead (<3 hrs) to the NOAA research vessel Harold W. Strauss for immediate necropsy and sample storage.

Spawner survey procedures
Coho typically return to Puget Sound urban streams in the early fall (i.e., early October), depending on the timing and intensity of rain events. Coho returned to the non-urban tributary of the
North Fork Stillaguamish River (Fortson Creek) later in the fall relative to coho returning to urban creeks. Thus, daily surveys on Fortson Creek extended into late December in 2002 (approximately three weeks later than for surveys on urban creeks). Daily surveys involved a visual inspection of most of the accessible freshwater habitat within a given stream. In some instances, poor visibility due to turbidity, high flows, or deep pools and wetlands precluded visible access. Surveyors began at the bottom of the reach and moved upstream, inspecting the stream channel for live adult salmon and carcasses. Where possible, the stream banks were also searched for carcasses that may have been dragged into the riparian zone by predators (predominantly river otters or scavengers). Urban stream surveys spanned the entirety of the available spawning habitat within a given drainage. In some systems such as Longfellow Creek, impassable barriers restricted returning coho to spawning sites in the lower reaches of the stream.

The location and general or atypical behavior of live salmon were recorded. For all dead or moribund fish, information on collection location, species, gender, fork length, weight (with and without ovaries for females), condition (e.g., signs of physical injury), and egg retention (females only) were recorded. Because males may spawn multiple times (or not at all) within a season, a determination of pre-spawn mortality was only made for females, and the reported rates of coho mortality for different streams are based on data from females only. Although we classified all female carcasses with >50% egg retention as pre-spawn mortalities, in most cases retention was closer to 100% (representative female in Figure 2). After examination, carcasses were left in the stream and marked with dated flagging tape. Even during high flow events, the day-to-day movement of flagged carcasses within streams was minimal (a few meters at most), and there was no evidence that pre- and post-spawn carcasses were disproportionately recoverable.

We collected tissue samples only from animals that were either overtly symptomatic or freshly dead (i.e., <3 hrs post-harvest or gill coloration was red to pink). Due to the limited availability of animals in some years, we collected tissues from symptomatic males as well as females.

Throughout the study we observed a mix of marked and unmarked fish returning to urban streams. In 2002, marked coho were identified by the absence of adipose fins. From 2003 through 2009, carcasses were scanned with a handheld coded wire tag scanner (Northwest Marine Technology, Inc., Shaw Island, WA). To determine source hatcheries, retrieved tags were processed by U.S. Fish and Wildlife Service staff in the Fisheries Division of the Washington Fish and Wildlife Office (Lacey, WA).

Fish condition
Condition factor was determined for pre- and post-spawn female coho salmon collected from Longfellow Creek (2002-09), Des Moines Creek (2004), and Fortson Creek (2002) as well as for female coho from the University of Washington and Stillaguamish Hatcheries (2002). Pre-spawn mortalities were weighed, the gonads removed, and then the fish were weighed again. All post-spawn and hatchery fish were weighed without gonads. Weight of the fish without gonads was used as a standard index to compare condition factor between pre- and post-spawn mortalities and hatchery fish. Condition factor was calculated using Fulton’s condition factor (K = [weight (g)/length (cm)^3]×100; [9]).

Fish histopathology
Tissues were collected for histopathology in 2003 and 2004 from affected coho on Longfellow Creek (N = 21 animals) and Des Moines Creek (N = 22), healthy coho returning to the Wallace River (N = 20) and Issaquah (N = 24) hatcheries, and pre-freshwater entry coho from the Muckleshoot tribal fishery in Elliott Bay (N = 27). Samples of liver, head and trunk kidney, exocrine pancreas, pyloric caeca, small or upper intestine, large or lower intestine, stomach, heart, spleen, gonad, brain, and gill were preserved in Davidson’s fixative [10]. Portions of each organ (5–7 mm in thickness) were excised from salmon bodies in situ and placed in 200 ml bottles filled with approximately 120 ml of fixative. Field-collected samples were transferred to the Northwest Fisheries Science Center, trimmed to a 3–4 mm thickness, and placed in tissue cassettes labeled with a unique fish identification number. Tissues were processed according to routine methods for paraffin embedding in Polyn (Triangle Biomedical Sciences, Durham, NC) using a Shandon Hypercenter XP automated tissue processor (Shandon Lipshaw, Pittsburg, PA). Embedded sections were then cut to a 4–5 μm thickness, stained with hematoxylin and eosin-philoxine [11], and examined by light microscopy. Histopathologic diagnoses were coded as published previously [12].

The Fisher’s Exact Test [13] was used to test whether the prevalences of certain histopathologic conditions in coho exhibiting pre-spawning mortality syndrome from the urban creeks were significantly higher than those for normal spawners from either the Wallace River or Issaquah hatcheries, or the pre-freshwater entry animals collected by gillnet from Elliott Bay. The critical level of significance was set at p≤0.05.

Pathogen detection
Fish collected in 2003 and 2004 were screened for infectious non-viral pathogens commonly observed in Pacific salmon, especially those pathogens affecting osmoregulatory tissues such as trunk kidney and gill. In 2003, fish were analyzed for the myxosporean parasite *Parascaris spumilicola* (gill and kidney), the larval digenetic trematode parasite *Nauplyptus salmincola* (kidney), the bacterium *Radieslacterium salmoninarum* (kidney), the myxosporean parasite *Tetraoctocula hysaelosum* (gill), the microsporidian parasite *Lama salmonae* (gill), and the myxosporean parasite *Ceratomyxa shasta* (posterior-most large intestine). In 2004, fish were analyzed for *P. minchicornis*, *R. salmoninarum*, *T. hysaelosum*, and *L. salmonae*. 
Metal exposure

Gill tissue was collected opportunistically from affected coho from Longfellow Creek and Des Moines Creek in the fall of 2004 and from Wallace River Hatchery spawners in the fall of 2008. Samples were collected with plastic forceps and titanium scissors to avoid metal contamination. Upon collection, gill tissue was placed in plastic bags on ice in coolers and transported to the King County Environmental Laboratory (KCEL). Samples were stored at −20°C until analysis. Prior to analysis, samples were homogenized in blenders that were rinsed with methanol and wiped down prior to and between samples.

All samples were analyzed for arsenic, cadmium, chromium, copper, lead, nickel, and zinc. Total metals were measured by inductively coupled plasma-mass spectrometry (ICP-MS) using KCEL standard operating procedures. Tissue was digested with nitric acid in conjunction with hydrogen peroxide to remove the analytes from the sample matrix and then further digested in nitric and sulfuric acid in the presence of potassium permanganate and potassium persulfate. Sodium chloride hydroxylamine hydrochloride was added after digestion to reduce the sample and stannous chloride was added immediately before analysis.

Measures for quality assurance/quality control (QA/QC) included checking measurement accuracy against certified reference materials such as DORM-2 (dogfish muscle) from the Institute for National Measurement Standards (Ottawa, Canada). Further QA/QC procedures included the measurement of background metal levels with method blanks, monitoring variability with duplicate laboratory samples, and measuring recovery of total metals from spiked samples (spike blank) and with matrix spikes (the sample matrix). Accepted variability for laboratory duplicates was 20%, ±15% for spike blanks, and ±25% for matrix spikes.

Gill tissue metal concentrations were normalized using a log10 transformation. For each metal, differences in concentrations due to location were analyzed using a one-way ANOVA and Tukey-Kramer HSD posthoc test with the level of significance set at p≤0.05.

Polycyclic aromatic hydrocarbon exposure

Bile was collected from the gallbladders of returning adult coho during the 2002–2004 field seasons and analyzed for metabolites of polycyclic aromatic hydrocarbons (PAHs) using established methods [24]. Samples were collected from fish in an urban stream (Longfellow Creek in 2002 and 2005), a non-urban stream (Fortson Creek in 2002), a non-urban hatchery (Wallace River Hatchery in 2002), and seawater-phase fish prior to their entry into Seattle-area urban streams (Elliott Bay in 2003). Briefly, bile was injected directly onto a C18 reverse-phase column (Phenomenex Synergi Hydro, Torrance, CA) and eluted with a linear gradient from 100% water (containing a trace amount of acetic acid) to 100% methanol at a flow of 1.0 mL/min. Chromatograms were recorded at two fluorescence wavelength pairs: 1) 260/380 nm where several 3–4 ring compounds [e.g., phenanthrene (PHN)] fluoresce and 2) 580/430 nm where many 4–5 ring PACs [e.g., benzo[a]pyrene (BaP)] fluoresce. The concentrations of fluorescent aromatic compounds in bile were determined using PHN or BaP as external standards and converting the fluorescence response of bile to phenanthrene (ng PHN equivalents/g bile or ng PHN equivalents/mg protein) or benzo[a]pyrene (ng BaP equivalents/g bile or ng BaP equivalents/mg protein) equivalents. Total biliary protein was determined using the method of Fryer et al. [25]. Copper sulfate (in alkaline solution) and Folin reagent were added to each diluted bile sample (1:1000 v/v with distilled water). The absorbance of each sample
was measured at 620 nm using a plate-reader spectrophotometer and was compared to the absorbance of bovine serum albumin measured at this wavelength. Total biliary protein values are reported as mg protein/mL bile.

The HPLC/fluorescence system was calibrated prior to analyzing field samples by analyzing a PHN/BaP calibration standard numerous times (N=5) until a relative standard deviation <15% was obtained for each PAC as previously described [26]. As part of the QA plan, a method blank and a fish bile control sample (bile from Atlantic salmon exposed to 25 μg of Monterey crude oil per mL of water for 48 hours) were analyzed with each salmon bile sample set.

Concentrations of PHN and BaP equivalents, as well as protein values, were log_{10} transformed to increase the homogeneity of variances. Analysis of variance (ANOVA) and the Tukey-Kramer HSD test were used to determine if mean concentrations of PHN and BaP equivalents and protein content of bile varied among collection years or collection sites. The level of significance was set at p<0.05.

Conventional water quality monitoring

Field meters were used to continuously monitor conventional water quality parameters during the fall of 2003 on both Longfellow Creek and Des Moines Creek. A 4a Minisonde™ (Hydrolab, Austin, TX) was installed by the City of Seattle in Longfellow Creek, in a pond at the terminus of the survey reach on this stream (just below an impassable culvert). A YSI 6600 multi-sonde unit (YSI Inc., Yellow Springs, OH) was installed by King County on Des Moines Creek. The unit was located in the stream channel below a footbridge in a community park, about 500 feet above the point at which the stream flows directly into Puget Sound. Both meters were programmed to measure and record water temperature, pH, dissolved oxygen, and specific conductance at 15-minute intervals. The Hydrolab was serviced and calibrated during the deployment period according to U.S. Geological Survey protocols [27]. The YSI meter was serviced weekly according to King County Environmental Lab standard operating procedures.

Mortality in relation to rainfall patterns

We surveyed Longfellow Creek over eight consecutive years in part to evaluate the influence of rainfall on spawner mortality within and between fall coho runs. Daily and total rainfall data were collected as the sum of 1-minute interval detections from the nearest City of Seattle rain gauge (Rain Gauge 17, or RG17), located approximately 5 km southeast of the upstream terminus of the surveyed portion of the stream. Rainfall was quantified from one week prior to encountering the first live fish in Longfellow Creek until the day the last carcass was found. When data were not available at RG17 they were transposed from the next nearest rain gauge (RG18, distance approximately 13 km).

The relationship between inter-annual spawner mortality and total rainfall was assessed using binary logistic regression. For the correlation coefficient, the natural log of the odds ratio [% pre-spawn/(1 - % pre-spawn)] for each year was weighted by sample size and regressed (simple linear regression) against total rainfall. In 2006, only 4 females were encountered, and data from this year (100% mortality) were excluded from the linear regression because they produced an undefined odds ratio. Both analyses were performed using JMP version 8 (SAS Institute Inc., Cary, NC).

Results

Behavior, condition, and origin of affected coho spawners

Consistent with initial observations of overtly symptomatic fish during early surveys in 1999–2001, we observed the same suite of behaviors in affected spawners during daily surveys from 2002–2009. These included circular surface swimming (loss of orientation), gaping, pectoral fin flapping, and loss of equilibrium (Video S2, S3). Symptomatic coho encountered during the course of a survey usually died by the end of the survey (i.e., within 1–2 hrs). Those that were still alive were found as pre-spawn carcasses the next day. Symptoms were displayed by both male and female spawners, were consistent from year to year, and were consistent across urban drainages.

Numerous adult coho carcasses were found in all monitored streams (Figure 1 and Table 2). For the urban streams, the frequency of egg retention among dead females (Figure 2) was high. For example, for Longfellow Creek, pre-spawn mortality ranged between 70–90% of the overall run in years where returning coho were relatively abundant (Table 2).

The size and condition of affected fish from urban streams were comparable to those of wild coho returning to Fortson Creek and

| Table 2. Rates of coho pre-spawn mortality in Puget Sound lowland streams surveyed daily from 2002–2009. |

<table>
<thead>
<tr>
<th>Creek</th>
<th>Year</th>
<th>N*</th>
<th>% Wild**</th>
<th>% Pre-Spawn Mortality Wild</th>
<th>% Pre-Spawn Mortality Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longfellow</td>
<td>2002</td>
<td>57</td>
<td>4</td>
<td>100</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>18</td>
<td>28</td>
<td>20</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>2004</td>
<td>9</td>
<td>89</td>
<td>88</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>2005</td>
<td>75</td>
<td>72</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>4</td>
<td>75</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>41</td>
<td>10</td>
<td>75</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>12</td>
<td>0</td>
<td>n.a</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>44</td>
<td>0</td>
<td>n.a</td>
<td>79</td>
</tr>
<tr>
<td>Piper's</td>
<td>2006</td>
<td>9</td>
<td>78</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Des Moines</td>
<td>2004</td>
<td>30</td>
<td>33</td>
<td>60</td>
<td>63</td>
</tr>
<tr>
<td>Fortson (non-urban)</td>
<td>2002</td>
<td>114</td>
<td>100</td>
<td>0.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*Sample size reflects female coho of known spawning condition, with no signs of predation.
**Presumed wild origin based on presence of adipose fin and absence of a coded wire tag.
unaffected hatchery coho returning to regional hatcheries (Table 3). For example, the condition factor for affected fish from Longfellow Creek in 2002 was not significantly different than the condition factor for wild coho from the non-urban reference stream (Fortson Creek; t-test, p = 0.12) and it was higher than the condition factors for the unaffected hatchery fish (e.g., Issaquah Hatchery; Table 3).

The spawner mortality syndrome appears to be specific to coho in urban drainages. We observed no symptoms and less than 1% pre-spawn mortality among wild coho returning to spawn in the non-urban reference stream in 2002 (Fortson Creek; Table 2). This is consistent with a widely reported absence of the syndrome among coho spawners from non-urban catchments. For example, in 2003-2004, Washington Trout (now the Wild Fish Conservancy) surveyed 29 Washington Department of Fish and Wildlife index reaches (<10% developed land cover) for coho spawner success in the Snohomish River basin north of Seattle. Of more than 1,000 intact adult female carcasses inspected, less than 0.5% died with an egg retention rate of >50% [28].

We did not observe corresponding die-offs of resident fish in urban streams (e.g., sticklebacks, sculpins, or cutthroat trout), nor did we find the syndrome in other species of migratory salmon return to these same urban streams to spawn in the fall. Also, the phenomenon appears to be specific to adult coho. In 2003, water from Longfellow Creek was diverted into a flow-through streamside shed facility with juvenile coho housed individually in separate aquaria (N=24). The juveniles were fed daily and monitored throughout the duration of the fall spawner run. Despite the presence of symptomatic adults in the adjacent stream, juveniles exposed to the same surface flows showed no overt symptoms, with 100% survival across the experimental group (data not shown).

Throughout the study, the general dearth of coho salmon returning to Seattle-area urban streams posed a challenge in terms of collecting tissues for forensic analyses. Longfellow Creek was chosen as a site for long-term monitoring in part because of the proportionally higher number of coho that typically enter this drainage relative to the other urban creeks in Seattle. Coded wire tag analysis of >50 tags collected from coho in Longfellow Creek (2003-2008) showed that many of these fish are hatchery strays originating from a net pen facility operated in Elliott Bay by the Muckleshoot and Suquamish Tribes. This facility serves to transition approximately 500,000 juvenile coho each year from the Soos Creek hatchery (Washington State Department of Wildlife) to the saltwater environment. Importantly, however, for certain high-return years (e.g., 2002 and 2005; Table 2), most of the stricken coho spawners were unmarked and presumably of wild origin. The mortality syndrome therefore appears to affect wild and hatchery coho alike.

### Table 3. Spawner condition for female coho salmon collected from urban and non-urban locations.

<table>
<thead>
<tr>
<th>Site</th>
<th>Year</th>
<th>N</th>
<th>Mean Condition</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fortson Creek</td>
<td>2002</td>
<td>20</td>
<td>0.893</td>
<td>0.111</td>
</tr>
<tr>
<td>University of Washington Hatchery</td>
<td>2002</td>
<td>21</td>
<td>0.816</td>
<td>0.103</td>
</tr>
<tr>
<td>Stillaguamish Hatchery</td>
<td>2002</td>
<td>5</td>
<td>0.840</td>
<td>0.047</td>
</tr>
<tr>
<td>Issaquah Hatchery</td>
<td>2002</td>
<td>21</td>
<td>0.814</td>
<td>0.06</td>
</tr>
<tr>
<td>Longfellow Creek</td>
<td>2002</td>
<td>47</td>
<td>0.856</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>10</td>
<td>0.920</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td>2004</td>
<td>8</td>
<td>1.018</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>2005</td>
<td>54</td>
<td>1.057</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>4</td>
<td>1.084</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>21</td>
<td>0.995</td>
<td>0.153</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>7</td>
<td>1.032</td>
<td>0.122</td>
</tr>
<tr>
<td></td>
<td>2009</td>
<td>30</td>
<td>0.930</td>
<td>0.235</td>
</tr>
<tr>
<td>Des Moines Creek</td>
<td>2004</td>
<td>19</td>
<td>1.109</td>
<td>0.258</td>
</tr>
<tr>
<td>Piper's Creek</td>
<td>2006</td>
<td>9</td>
<td>1.055</td>
<td>0.107</td>
</tr>
</tbody>
</table>

*Condition Factor was Fulton's K = (weight/length^3)*100. Weights were for gravid females (i.e., with ovaries containing eggs). doi:10.1371/journal.pone.0028013.t003

### Coho mortality is not correlated with common pathogen-associated disease or noninfectious lesions

A systemic survey of histopathological conditions in pre-spawn carcasses (urban streams) and unaffected fish from Elliott Bay and two regional hatcheries (Wallace River and Issaquah Creek) was conducted in 2003 and 2004. Various infectious, parasitic and idiopathic (of unknown etiology) diseases were detected in the gill, heart, trunk kidney, gastrointestinal tract and liver of adult spawners. These findings are summarized in Table S1 and Text S1. No significant lesions were detected in the gonad, brain, spleen or exocrine pancreas of adult coho regardless of origin. None of the observed disease conditions were specific to animals that succumbed to the mortality syndrome, nor were they unique to the urban streams where die-off rates were high.

Among the six pathogens screened by PCR or microscopy, *L. salmonis* in the gill and *R. salmoninarum* in the kidney were detected among fish from all sampling sites, with the highest prevalence observed among Longfellow Creek fish (*L. salmonis*) or Des Moines Creek fish (*R. salmoninarum*) (Table S1). In contrast, *P. miniichromis* in the gill or kidney was detected among fish from all sites except Des Moines Creek. Prevalence of *T. pyrogenes* in kidney, *N. salmioncola* in kidney, and *C. sputa* in lower intestine varied widely among the sites, ranging from 0% to 48%. Although no single pathogen was consistently associated with pre-spawn mortality, infection or infestation by multiple pathogens occurred more frequently among Longfellow Creek fish. In 2003 fish from Longfellow Creek exhibited a significantly higher number of infections or infestations per fish (median = 3) than fish from Elliott Bay (median = 2), Issaquah Hatchery (median = 1), or Wallace River Hatchery (median = 1) (Fisher Exact Probability, p<0.0001; Figure S1).

Prematurely dying spawners show no indication of exposure to common insecticides

A reduction in the enzymatic rate of brain acetylcholinesterase is a biomarker of exposure to common carbamate and organophosphate insecticides. As shown in Figure 3, measured brain enzyme activity affected coho spawners from Longfellow Creek was not significantly lower than corresponding brain enzyme activities in fish from a non-urban reference stream (Fortson Creek) or from regional hatcheries. Rather, AChE activity in the brains of affected fish was slightly but significantly higher than for coho spawners from Fortson Creek and the Stillaguamish Tribal Hatchery (one-way ANOVA; p<0.05).

Affected coho have elevated levels of metals in gill tissues

The measured concentrations of arsenic, cadmium, chromium, copper, lead, nickel, and zinc in the gill tissue of adult salmon collected from two urban sites (Des Moines and Longfellow

December 2011 | Volume 6 | Issue 12 | e28013
Coho Spawner Mortality in Urban Watersheds

Figure 3. Prematurely dying spawners do not show evidence of neurotoxic pesticide exposure. Shown are relative rates of brain acetylcholinesterase (AChE) activity, a target enzyme for common homeowner use insecticides, in adult coho salmon. The brain enzyme activities of affected fish from an urban stream (Longfellow Creek; LF) were not significantly inhibited relative to unaffected fish from a non-urban stream (Fortson Creek; FT) and three regional hatcheries; Issaquah (05-S-H), University of Washington (UW-H), and Stillaguamish (ST-H). Error bars are 1 standard error of the mean. Sample size is indicated in parentheses and letters indicate significant differences between locations (one-way ANOVA, Tukey’s HSD; p<0.05). doi:10.1371/journal.pone.0028034.g003

Creeks) and one non-urban site (Wallace River Hatchery) are shown in Figure 4. There were significant differences among the three sites for cadmium, lead, nickel, and zinc (one-way ANOVA and Tukey-Kramer HSD; p<0.05). Fish from the two urban streams had similar levels of cadmium, lead, and nickel, and these were significantly higher than corresponding levels in the gills of coho from the non-urban location. For zinc, fish from the non-urban hatchery had slightly but significantly higher gill concentrations relative to fish from one of the two urban streams (Des Moines Creek).

Bile analysis indicates elevated exposure to petroleum hydrocarbons in fish from urban streams

In the fall of 2002, bile was collected from symptomatic and recently dead coho from Longfellow Creek, as well as from adults returning to spawn in the non-urban reference stream (Fortson Creek). Relative exposures to polycyclic aromatic hydrocarbons (PAHs) were quantified by measuring mean concentrations of phenanthrene (PHN) and benzo(a)pyene (BaP) metabolites in bile. As shown in Figure 5, affected fish from the urban stream had significantly higher biliary levels of both PHN and BaP equivalents (one way ANOVA, Tukey-Kramer HSD, p<0.05).

In 2003, the biliary levels of PHN in fish from Longfellow Creek were compared to PAH levels in seawater-phase adults collected from a gillnet fishery in Elliott Bay, prior to freshwater entry, and adults returning to a non-urban hatchery (Wallace River Hatchery). As in 2002, fish from the urban stream showed significantly higher exposures to both PHN and BaP relative to the non-urban sampling location (Figure 5; one way ANOVA, Tukey-Kramer HSD, p<0.05). PAH levels in the bile of seawater-phase coho collected from Elliott Bay were slightly but not significantly elevated relative to the non-urban location.

Stream temperature, dissolved oxygen, and other conventional water quality parameters do not appear to be causal factors for the mortality syndrome

Monitoring results for conventional surface water quality parameters in urban drainages where premature coho mortality is prevalent have been published previously [7]. During the fall months, urban streams were cool and well mixed. For example, fixed station surface temperature monitoring on Longfellow Creek between October and December in 2002 (86% pre-spawn and mortality across the entire run that year; Table 2) revealed maximum daily temperatures ranging from about 6–11°C. Over the same interval, surface water concentrations of dissolved oxygen ranged from about 9–11 mg/L. This fall and winter pattern of relatively cool, oxygen-rich surface flows is also typical of other urban streams where coho die-offs commonly occur (e.g., Piper’s Creek; [7]). Monitored conditions for other conventional water quality parameters were also favorable for salmon health and survival. The average level of ammonia-N in water samples collected from Longfellow Creek during storm events (0.04 mg/L) was more than an order of magnitude below the pH-adjusted benchmark criterion for chronic ammonia toxicity (0.43–2.1 mg/L). Moreover, pH levels were normal (pH 6.5–6.5) for Longfellow Creek in the survey years included in this study [7].

Mortality is qualitatively but not quantitatively influenced by rainfall

During the first year of annual surveys on Longfellow Creek (2002), fall coho returns were several weeks late due to an unusually dry October and early November. The first significant rains in the second week of November triggered a large influx of spawners. As the rains continued over the next two weeks, every fish entering the drainage succumbed to the mortality syndrome, with many observations of overt symptomology during daily surveys (Figure 6). Fish only survived to spawn in the weeks following the mid-November storms.

Based on the apparent strength of this association between rainfall and mortality in 2002, we continued with daily
Coho Spawner Mortality in Urban Watersheds

Figure 5. Analysis of bile from affected coho spawners indicates exposure to polycyclic aromatic hydrocarbons (PAHs). Concentrations of fluorescent PAH metabolites (as phenanthrene [PHN] and benzo-a-pyrene [Bap] equivalents) in the bile of coho collected in an urban stream (Longfellow Creek; LF) and a non-urban hatchery (Wallace River Hatchery; WR-H). In 2003, seawater-phase coho (pre-freshwater entry) were also sampled from urban Elliott Bay (EB). The bile data demonstrate a significant increase in PAH exposure after coho spawners transition from a highly urbanized estuary to freshwater spawning habitats. Sample sizes, the same for PHN and BaP, are indicated over each bar. Error bars are 1 standard error of the mean. doi:10.1371/journal.pone.0028013.g005

surveys on Longfellow Creek in successive years (2003–2009). The relationship between inter-annual variation in total rainfall and the severity of spawner mortality was evaluated using binary logistic regression. The results suggest a pattern of higher coho survival in wetter years where more water moves through the watershed before many of the adults arrive on the spawning grounds. However, there was a large amount of inter-annual variability in both rainfall (timing and amount) and coho return (timing and number). As a consequence, the logistic regression was not significant at p<0.05 (not shown; χ²(1) = 1.70, p = 0.19). The slope for the regression was −0.021 (se = 0.016, p = 0.19) and the intercept was 1.674 (se = 0.403, p<0.0001). Across years, rainfall explained 29% of the variability in the spawner mortality syndrome (log odds ratio weighted linear regression, r² = 0.298).

Notably, in each of the eight survey years, the first carcasses found was always a pre-spawn mortality. Conversely, in six of the seven years in which at least one fish survived to spawn, the last carcass found was a successful spawner.

Discussion

We have documented a distinct mortality phenomenon among adult coho salmon returning to spawn in urban watersheds of central Puget Sound. The syndrome has been recurrent for more than a decade, with a consistent symptomology across years and survey locations. The annual die-offs have claimed a large proportion of the fall runs in the drainages monitored during the course of this study. These high mortality rates (e.g., 90–100%) are likely to preclude sustainable natural production in urban drainages more generally [29], and the coho we monitored during the course of this study were fish that appear to have strayed into ecological traps [30] in search of spawning habitat. Our findings fit a general pattern for Puget Sound, in which adult coho are very few in number in watersheds where the mortality syndrome has been observed. Conversely, in non-urbanized watersheds where coho spawners are relatively abundant, they appear to be unaffected.

Factors that are known to cause spawner mortalities in other species of salmon do not appear to be involved in the coho pre-spawn mortality syndrome that we have explored here. The temperatures and dissolved oxygen content of urban streams during mortality events were not unusually high or low, respectively. Although all fish of the fish we examined showed evidence of infection with common pathogens, there was no correlation with the high rates of mortality in urban drainages or the observed symptomology. Lastly, the stricken coho were generally in good physical condition, and we found no evidence that origin (i.e., wild or hatchery) influences an animal’s susceptibility.

The weight of evidence therefore suggests that adult coho salmon are unusually vulnerable to the toxic effects of one or more chemical contaminants, most likely delivered to urban spawning habitats via stormwater runoff. The rapid progression of the syndrome and the specific nature of the symptoms are consistent with acute cardiopulmonary toxicity. Our current findings support this hypothesis, albeit indirectly by ruling out alternative, non-chemical explanations.

We found that affected coho show elevated exposure to metals and petroleum hydrocarbons, the latter after spawners transition to freshwater from a highly urbanized estuary (Elliott Bay). Evidence of exposure to metals and PAHs does not imply causality, but future studies should address these toxins, as they are specifically known to disrupt respiratory, osmoregulatory, and cardiovascular physiology in fish. The abrasion of vehicle tires and brake pads releases aluminum, barium, cadmium, cobalt, copper, lead, nickel, zinc, and other elements onto impervious surfaces [31]. Copper and other metals disrupt ionoregulation by binding to ligands in the fish gill [32] without causing overt cytotoxicity. Moreover, metals are generally more bioavailable and thus more toxic to fish in soft waters such as stormwater [33]. Motor vehicles are also sources of PAHs via exhaust and leaking crankcase oil. Certain PAHs are cardiotoxic to fish [34], including specifically the tricyclic phenanthrene and dibenzothiophene (e.g., [35]).
Coho Spawner Mortality in Urban Watersheds

Figure 6. Pre-spawn mortality and survival to spawn in relation to rainfall. Shown are the results of daily stream surveys throughout the 2002 coho spawning season in Longfellow Creek in relation to daily rainfall. Asterisks (*) indicate days when stream flows were too high to survey the creek. doi:10.1371/journal.pone.0028013.g006

It is important to note, however, that the toxicological context (i.e., established literature) for anticipating possible acute lethal toxic effects of stormwater contaminants on coho spawners is practically nonexistent. On the one hand, urban runoff typically contains organic chemicals and metals in the low parts per billion to parts per trillion range (e.g., [36,37]), well below levels that would be expected to cause fish kills based on established median lethal (LC50) concentrations for rainbow trout and other salmonids. On the other hand, to our knowledge, there have been no toxicological studies on freshwater-transitional adult coho. When adults return from saltwater to freshwater in preparation for spawning, they undergo osmoregulatory adjustments that include shifts in plasma osmolality, gill sodium-potassium ATPase activity, and the density of chloride cells in the gill (e.g., [38]), as well as changes in the circulation of stress and reproductive hormones [2]. These changes may render adult animals particularly vulnerable to toxics that interfere with the physiological processes that underlie freshwater acclimation. Coho have recently been shown to be considerably more vulnerable to chemical toxicity when they make the opposite transition from freshwater to saltwater [39].

Sensitivity related to freshwater transition might explain our observations of affected adults and unaffected juveniles exposed to the same surface waters, but not our observations of affected coho spawners side-by-side with unaffected spawners of other salmon species. For example, in 2006 there were temporally overlapping runs of coho and chum spawners in Piper’s Creek. Whereas all of the coho succumbed, the egg retention rate for chum carcasses was <4% (5 of 135 females; data not shown). Moreover, symptomatic coho were observed in the stream side-by-side with healthy chum, the latter actively digging and defending redds (Video S4). The underlying reason for the interspecific difference in sensitivity remains to be determined.

More work is also needed to define the influence of rainfall on the spawner mortality syndrome. The clearest indication that rainfall plays a role was the 2002 survey results for Longfellow Creek. That year was characterized by an unusually long antecedent dry interval (presumably allowing a proportionally greater accumulation of pollutants on impervious surfaces within the watershed), a relative abundance of returning spawners, and consistent rainfall for approximately two weeks at the beginning of the compressed run. As in 2002, in subsequent years we observed a general tendency towards higher survival later in the run, after multiple fall rain events. However, the relationship was not statistically significant across the survey years, due in part to highly variable rainfall patterns, longer run durations, and very low spawner numbers in some years. Notably, the mortality syndrome is not a simple first-flush phenomenon, as spawned and unspawned carcasses were usually intermixed throughout the duration of each run in 2003–2009.

Additional evidence implicating urban runoff was recently provided by a spatial land use analysis of the watersheds surveyed during the course of this study. Feist et al. [40] found that inter-watershed rates of coho spawner mortality correlate closely and positively with the relative proportion of local roads, impervious surfaces, and commercial property within a basin. These and other correlations were then used to predict areas of possible coho spawner die-offs in unmonitored drainages throughout central Puget Sound [40]. The link to roads and other impervious surfaces further implicates motor vehicles as the likely source of causal toxics, but this remains to be demonstrated directly – e.g., by reproducing the mortality syndrome in otherwise healthy adult coho via exposures to environmentally relevant mixtures of metals and PAHs in freshwater.

Recent population-scale modeling has shown the potential for rapid local declines in coho population abundance across the range of spawner mortality rates observed in urban drainages during the course of this study [29]. Regional human population growth and land use changes that increase the proportion of impervious surfaces within watersheds may therefore pose an important future threat to wild coho populations if 1) toxic urban runoff is the underlying cause of the mortality phenomenon, and 2) wild coho are similar in their vulnerability to the hatchery and unmarked (and presumably wild) coho that were found unspawned in 2002–2009. This is above and beyond the established and widely documented stormwater-driven threats to aquatic habitats (e.g., [41,42,43]).

In closing, past efforts to restore salmon habitats in Seattle-area urban watersheds have revealed unexpected challenges for improving coho spawner abundance and survival. These restoration projects have been successful in numerous other ways, including revitalizing urban green spaces, extending watershed connectivity, enhancing public education and involvement, and improving habitat conditions for otters, waterfowl, amphibians, stream invertebrates, native plants, and other fish species.Restored urban streams have also provided an experimental setting to study what may become a very important threat to wild
coho populations in the decades ahead as some healthy stream networks gradually acquire the land cover characteristics of the Longellower Creek system and similar urban drainage systems. The next generation of urban watershed improvements is now underway, including the catchment-scale implementation of natural drainage systems (using green infrastructure and other emerging technologies), floodplain restoration, and new pollution mitigation activities such as vacuum sweeping of roadways. Moreover, Washington recently became the first state in the U.S. to legislatively mandate a phased reduction of metals in vehicle brake pads and other friction materials (SB6557). Future improvements in the survival of adult coho in urban streams will be an important indicator of success for these and other pollution reduction strategies.

Supporting Information

Text S1 A detailed description of the histopathology results from tissue samples collected during the study from urban and non-urban sites. (DOC)

Figure S1 Number of pathogens per fish detected by pathogen screening methods for fish collected in 2003. Horizontal bar is positioned at the median. Longellower Creek fish differ from fish from all other locations (Chi-square test, p<0.0001). (TIFF)

Table S1 Prevalence of infectious (parasitic/bacterial) and idiopathic conditions detected by histopathology and by pathogen screening (molecular and microscopic) in adult coho salmon sampled from several creeks and hatcheries in the Puget Sound region in 2003 and 2004. H = histopathology; PS = pathogen screening methods; -- = analysis not performed. (DOC)

Video S1 Symptomatic female coho salmon in Piper's Creek in 2000. The fish appears to be in good physical condition, with ocean-bright (silver) coloration. Characteristic symptoms include loss of equilibrium, gaping, and pectoral fin spreading. (MP4)

References


Coho Spawner Mortality in Urban Watersheds


Sublethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish

Corinne E. Hicken, Tiffany L. Linbo, David H. Baldwin, Maryjean L. Willis, Mark S. Myers, Larry Holland, Marie Larsen, Michael S. Stekoll, Stanley D. Rice, Tracy K. Collier, Nathaniel L. Schofield, and John P. Incardona

*University of Alaska-Fairbanks Fisheries Division, University of Alaska-Fairbanks Juneau Center, Juneau, AK 99801; 1Environmental Conservation Division, Northwest Fisheries Science Center, National Oceanic and Atmospheric Administration, Seattle, WA 98112; and 2Alaska Fisheries Science Center, National Oceanic and Atmospheric Administration, Juneau, AK 99801

Edited by Greg Goss, University of Alberta, Edmonton, AB, Canada, and accepted by the Editorial Board March 21, 2011 (received for review December 17, 2010)

Exposure to high concentrations of crude oil produces a lethal syndrome of heart failure in fish embryos. Mortality is caused by cardiotoxic polycyclic aromatic hydrocarbons (PAHs), ubiquitous components of petroleum. Here, we show that transient embryonic exposure to very low concentrations of oil causes toxicity that is sublethal, delayed, and not counteracted by the protective effects of cytochrome P450 induction. Nearly a year after embryonic oil exposure, adult zebrafish showed subtle changes in heart shape and a significant reduction in swimming performance, indicative of reduced cardiac output. These delayed physiological impacts on cardiovascular performance at later life stages provide a potential mechanism linking reduced individual survival to population-level ecosystem responses of fish species to chronic, low-level oil pollution.

cardiac toxicity | fish populations | heart development | oil spills

Oil spills such as the 1989 Exxon Valdez spill and the 2010 Deepwater Horizon disaster pose major threats to fish health and population viability. Both spills have catalyzed research efforts to discern how to detect fish injury (1). The population effects on pink salmon (Oncorhynchus gorbuscha) and Pacific herring (Clupea pallasi) after Exxon Valdez are cause for concern regarding the effects of any major spill, including the Deepwater Horizon. Evidence of population effects is strongest for pink salmon, for which studies of embryos in spawning gravels in the intertidal zone of streams crossing oil beaches demonstrated elevated mortality for at least 4 years after the spill (2–4). Laboratory studies subsequently showed that water contaminated with PAHs in the low 10–100 parts per billion (ppb or μg/L) dissolved from oil gravel produced a characteristic syndrome of edema in both species (5–8). Moreover, a series of mark and recapture studies with pink salmon found that morphologically normal juveniles that survived embryonic exposure to water containing <20 μg/L total PAHs had elevated rates of postrelease mortality in the marine environment, with an average reduction in adult survival by 36% (6, 9, 10). Growth rate depression was also measured in juveniles several months after exposure ceased, but cellular or tissue mechanisms were not investigated (10). There is evidence for similar effects in Pacific herring after the spill (11, 12), but comparable studies for this species were not conducted.

Because of the logistical difficulties of identifying mechanisms of toxicity in wild species, we use the zebrafish (Danio rerio) model to explore the long-term impacts of sublethal oil exposure. Oil exposure studies using zebrafish embryos demonstrated a heart failure syndrome that is lethal to larvae (13, 14), findings that were validated in Pacific herring (15). As oil weathered, the proportional composition of dissolved-phase PAHs becomes dominated by the tricyclic fluorenes, dibenzoanthracenes, and phenanthrenes (5, 6, 16), which were shown to be directly cardiotoxic (13, 14). In herring embryos, cardiotoxicity occurred at tricyclic PAH concentrations in the tissue as low as 0.8 nmol/kg (150 ppb) wet weight, indicating a specific, high-affinity cellular target (15). Individual nonalkylated tricyclic PAHs caused atrioventricular conduction arrhythmias indistinguishable from those caused by drugs known to block potassium channels required for the repolarization phase of cardiac action potentials (13, 14). PAH mixtures from weathered crude oil caused more complex cardiac dysfunction, suggestive of additional targets, including pacemaker currents and plasma membrane or sarcoplasmic calcium channels (14, 15). Consistent with genetic analyses of cardiac form and function in zebrafish (17, 18), this oil-induced cardiac dysfunction affects later morphogenetic steps, such as looping of the atrial and ventricular chambers into their normal side-by-side positioning (13, 14). Although these morphological defects are lethal, these aggregate findings raise the question of whether milder, transient cardiac dysfunction caused by low doses of PAHs can have subtle impacts on cardiac form that could ultimately influence physiological performance later in life and, in turn, reduce survival.

We hypothesized that low levels of embryonic oil exposure might influence ventricular shape and, ultimately, cardiac output in adult animals because previous studies showed that intermediate PAH concentrations caused a compensatory dilation of the cardiac chambers in larvae (13). Ventricular shape is linked to maximum cardiac output as demonstrated by critical swimming speed (Ucrit) studies (19, 20). Continuously swimming species such as salmon or herring have pyramidal ventricles (21, 22), and fish with rounded ventricles (reduced length/width ratio) are slower swimmers with reduced cardiac output (23). Zebrafish are an appropriate model because they have pyramidal ventricles (24) and are among the highest measured critical swimming speeds (13 body lengths per s at 28 °C) (25).

Results

Zebrafish embryos were exposed to low PAH concentrations for a few hours after fertilization to just before the hatching stage (48 h) in the effluent of a continuously flowing oiled or clean (control) gravel generator column (14), which mimics the...
natural weathering of an oiled shoreline (16) (Materials and Methods). We assessed the critical swimming speed and cardiac anatomy of adult survivors after rearing them in clean water for 10–11 mo. Contaminated effluent initially contained 60 ppb $\Sigma$PAH causing lethal pericardial edema in 100% of exposed embryos (e.g., at day 0; Fig. 1A). Clutches of embryos from common parents were divided in half and added to either control or oiled effluent every several days, and after 3 wk of weathering, $\Sigma$PAH dropped in oiled gravel effluent to a point where most embryos appeared normal. Approximately 100 adult fish were reared from each half-clutch incubated in either oiled or clean gravel effluent during three independent replicate exposures. The oiled-gravel exposures had an overlapping range of $\Sigma$PAH concentrations (24–36 ppb), and total tricyclic PAHs (16–14 ppb; Fig. 1A). The composition of PAHs was similar among the doses (Fig. S1), although the first exposure had slightly higher levels of noncardiotoxic alkyl-naphthalenes (13).

Oil exposure led to reduced larval survival (ANOVA, $P < 0.01$), and larvae survival for each clutch was not significantly different within treatments (ANOVA, $P > 0.05$; Fig. 1B) despite slightly different $\Sigma$PAH in the exposed group. On average, 95% of control embryos survived the larval–juvenile transition, whereas 80–87% of the exposed embryos survived (Fig. 1B). Embryos with edema generally failed to feed as larvae and did not survive metamorphosis. The increased mortality observed here was higher than that reported for pink salmon embryos exposed to somewhat lower $\Sigma$PAH (18–20 ppb) (6). Although pink salmon had a mortality rate 1.2 times higher than unexposed controls (from 29.6 to 35%), here the average mortality for zebrafish embryos was 3.4 times higher (from 5 to 17%) after exposure to $\Sigma$PAH in the range of 24–36 ppb.

Subsamples of embryos were assayed for induction of cytochrome P450IA (CYP1A) immediately after exposure. CYP1A is the primary detoxification enzyme for PAHs (14) and a biomarker of PAH exposure. Whereas control embryos showed only background immunofluorescence, oil-exposed embryos showed a similar pattern of immunofluorescence consistent with previous oil-graded column studies (14) (Fig. S2).

We exposed another clutch of embryos to 9 ppb $\Sigma$PAH at day 97 (Fig. 1A) to examine the effectiveness of CYP1A in protecting embryos. This exposure included a subset of embryos injected with cypla antisense morpholino to knock down expression of CYP1A (14). Few oil-exposed uninjected (3% or 1/31) or control-injected embryos (0/17) exhibited pericardial edema and failed cardiac looping compared with 92% (33/36) of CYP1A-blocked morphants (Fig. S3). No edema was observed in control embryos exposed to clean gravel effluent (0/29 for uninjected, 0/17 for standard control morpholino, and 0/33 for cypla morphants). Therefore, CYP1A induction clearly plays a protective role rather than contributing to toxicity of petrogenic PAHs in early life history stages of fish. The sensitivity of zebrafish to PAH is similar to that of salmonids and other marine species (5, 6), in contrast to other classes of contaminants, such as the dioxins, to which zebrafish are orders of magnitude more resistant (26). The metabolic capacity of CYP1A enzymes for petrogenic PAH substrates in different fish species may account for variation in oil toxicity.

Although we did not measure growth rates during the juvenile period, sublethal oil exposure did not result in dramatically different final growth trajectories. There were some significant differences in final size measures for some groups of female fish, but there was not a consistent relationship with exposure (Table S1). Females from both oil-exposed day 33 and day 42 clutches were slightly longer than their paired control groups. Females from the oil-exposed day 33 clutch were significantly heavier than all other groups except the oil-exposed day 42 females, whereas the latter had a condition factor significantly higher than all other groups. There were no significant differences for male fish between treatment groups within or across all clutches for length and weight (Table S1). Two-way ANOVA indicated an effect of clutch on male condition factor, with clutch 3 fish (oil-exposed and clean) having a slightly larger $K$ value. However, there was no interaction between oil exposure and clutch.

After growing to adulthood in clean water (>10 mo), exposed zebrafish had reduced $U_{crit}$, indicative of reduced cardiac output (19, 23, 27). For each clutch, $U_{crit}$ was measured by using a standard swim tunnel for equally sized fish from each of five replicate rearing tanks (Table 1; two-way ANOVA, $P > 0.2$). All oil-exposed groups showed reduced $U_{crit}$ compared with controls from the same clutch, whether determined by absolute speed (cm/s; Fig. 2A) or relative to body length (BL per s; Fig. 2B). For oil-exposed groups, swimming speed was reduced by 17%, 22%, and 15%, respectively. Oil exposure affected both absolute ($P < 0.01$, two-way ANOVA) and relative $U_{crit}$ ($P < 0.0001$), but there was no effect of clutch, nor an interaction between oil exposure and clutch. Hence, the three clutches can be treated as replicates, with oil exposure producing a reduction in mean relative $U_{crit}$ by 18 ± 4%.

Changes in ventricular shape correlated with the reduced swimming performance from oil-treated fish. We measured ventricular shape as the length-to-width ratio of the heart by using digital images. We determined length as the distance between the apex of the heart and the center of the ventriculobulbar valve, and width as the widest distance perpendicular to the length (Fig. 3A, arrows). Oil-exposed fish (14–20 days) had rounder hearts, indicated by a length-to-width ratio of 1.38 ± 0.04.
Fig. 2. Reduced swimming performance in adult fish exposed to oiled gravel effluent as embryos. $U_{cml}$ was measured as described in Materials and Methods. Mean $U_{cml}$ (±SE) for fish embryonically exposed to effluent from clean gravel (light gray bars, $n = 5$) or oiled gravel (dark gray bars, $n = 9$) is given as an absolute speed (A, cm/s) or relative to body length (B, BL/A). Statistical analysis is discussed in the text.

Discussion

Observations of reduced swimming performance and change in ventricular shape are consistent with our predictions based on the cardiac toxicity of crude oil. The developing heart is one of the first organs to become functional during organogenesis. In zebrafish (and other species), a regular heart rate is established during the tubular stage when both chambers have walls that are a single cell layer. Subsequently, looping brings the chambers into an adjacent arrangement, an atrioventricular conduction pathway is established, valves are formed, and the ventricular myocardium proliferates to become multilayered (24). The genetics of heart development in zebrafish has established the inseparable relationship between cardiac form and function during early stages of cardiac morphogenesis (17): Mutations affecting heart structure impact its function, whereas mutants with impaired function concomitantly experience impacts to the form of the chambers or valve structure. At sufficient concentrations, the tri cyclic PAH compounds in petroleum produce severe arrhythmias mimicking those cardiac function mutants. These arrhythmias are lethal owing to circulatory failure. Moreover, sublethal exposure to PAHs induces subtle changes in heart shape (e.g., a 9% decrease in length-to-width ratio) that translate into larger impacts on aerobic performance (a reduction of $U_{cml}$ by 18%).

Sustained swimming ($U_{cml}$) is a relevant indicator of individual fitness for pelagic planktivores (e.g., herring) and migratory salmonids (28). Even for larval fishes, swimming performance has ecologically relevant implications for predator avoidance (29). Moreover, a rounded ventricle is associated with increased stress-induced mortality (30). Survivorship depends on optimizing physiological performance. We show a direct link between oil exposure during embryonic development and delayed effects on physical capacity of adults.

Our finding that transient embryonic oil exposure affects the performance of adult zebrafish, together with the previously documented population-scale effects of pink salmon exposed to Exxon Valdez oil during early life stages, strongly suggests a physiological mechanism linking individual-based toxicity and population-level response. A similar mechanism could be the basis for the population impacts of other cardiotoxic pollutants such as the dioxins and planar polychlorinated biphenyls (31). With the high degree of evolutionary conservation among vertebrate hearts, these findings in zebrafish also have implications beyond both fish populations and contaminants, relating to environmental impacts on heart shape and cardiac output in humans. Similar relationships exist in the human heart (32), and physical factors that influence intracardiac forces during fetal development can result in rounder hearts with reduced output (33). Without being overtly teratogenic, chemicals with the ability to affect embryonic or fetal cardiac function could potentially produce morphological changes that could underlie some of the variation in human cardiac performance.

Materials and Methods

Zebrafish Culture and Exposures. Zebrafish (D. rerio) wild-type AB broodstock were maintained in a modular system on a 14-h light/10-h dark cycle at 28.5 °C using methods detailed (34). Fish spawning, embryo exposures, and adult zebrafish maintenance were all carried out with system water (reverse osmosis water with instant Ocean sea salt added to adjust it to a conductivity of n=1,500 μS/cm and a pH between 7 and 8). Spawning and egg collection

Table 1. Length and condition factor of adult fish used in swim test

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>$n$</th>
<th>Length, cm</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clutch 1 clean</td>
<td>25</td>
<td>2.91 ± 0.03</td>
<td>2.01 ± 0.07</td>
</tr>
<tr>
<td>Clutch 1 oiled</td>
<td>45</td>
<td>2.99 ± 0.03</td>
<td>2.12 ± 0.06</td>
</tr>
<tr>
<td>Clutch 2 clean</td>
<td>25</td>
<td>2.89 ± 0.05</td>
<td>1.97 ± 0.06</td>
</tr>
<tr>
<td>Clutch 2 oiled</td>
<td>45</td>
<td>3.07 ± 0.03</td>
<td>2.10 ± 0.06</td>
</tr>
<tr>
<td>Clutch 3 clean</td>
<td>25</td>
<td>2.92 ± 0.05</td>
<td>2.36 ± 0.17</td>
</tr>
<tr>
<td>Clutch 3 oiled</td>
<td>45</td>
<td>2.99 ± 0.03</td>
<td>2.12 ± 0.06</td>
</tr>
</tbody>
</table>

K, condition factor.
was carried out as described in detail (34). Embryos used in the exposures were all between the four- and eight-cell stage at the start of the exposure. Embryos were exposed for 48 h to partially weathered Alaskan North Slope (ANS) crude oil by using oiled gravel columns, as described (14). System water was passed through columns (2-L glass beakers) containing oiled (6 g/kg) and control (unooled) gravel, using centrifugal pumps to maintain a constant flow of 10 mL/min. The resulting effluent was collected in rectangular 23 × 33 cm glass baking dishes, which were set at a slight angle to allow the water to overflow out the far end. Embryos were placed in open 60 × 15 mm diameter glass Petri dish replicates (n = 5, 30 embryos per dish) set in the baking dishes. The temperature in each baking dish was maintained between 27 and 28.5 °C with submersible heaters, and recorded up to three times per day. System water was pumped continuously through both the oiled and control gravel columns 24 h a day, 7 days a week, for 97 days. During this time, new embryos were exposed every 3 to 4 d. After each 48-h exposure, embryos were examined for pericardial edema with a Nikon SMZ800 stereo microscope at magnification and photographed in addition to the wet-streak analysis. Embryos from clutches whose exposure started on column days 23, 33, and 44 were transferred to clean water in 150-mm Petri dishes and incubated in an incubator at 28 °C for an additional 48 h. At this point, they were transferred down to a temperature-controlled, dark zebrafish facility and reared to 12 d after fertilization (dpf) in 1-L larvae tanks on a diet of paramecia, newly hatched Artemia, and finely powdered dry food (14). Mortality was assessed daily through 12 dpf, and surviving larvae from each clutch were reared to adulthood in five subgroups in the modular colony, with each subgroup treated as a replicate to control for potential tank effects.

CYP1A Immunofluorescence and Confocal Microscopy. CYP1A immunofluorescence processing was carried out as described (35). In general, embryos were fixed overnight in 4% phosphate-buffered paraformaldehyde (pH 7.4) and then transferred to methanol plus 10% DMSO for storage. Primary antibodies used were monoclonal anti-CYP1A C107 (Cayman Chemical) and anti-mouse heavy chain monoclonal MF20 (Developmental Studies Hybridoma Bank) (36). Secondary antibodies used were Alexa Fluor 488-conjugated goat anti-mouse IgG (S46) and Alexa Fluor 588-conjugated goat anti-mouse IgG2a (MF20), both from Invitrogen/Molecular Probes. Mounted immunolabeled embryos were imaged by using a Zeiss LSM 5 Pascal confocal system with Ar and HeNe lasers (Carl Zeiss Advanced Imaging Microscopy).

PAH Analysis. Water samples (200 mL) were taken at the beginning and end of each exposure from both the control and oiled gravel columns. Each sample was stored in a 1-L amber glass bottle with addition of 20 mL of 4% methane in a 4 °C refrigerator until extraction. After deuterated internal standards were added, each sample was extracted twice with 25 mL of dichloromethane. The extracts were stored in a −20 °C freezer until further processing to remove any residual water. The solvent dichloromethane was replaced with hexane via boiling evaporation. Boiling stones were added to each sample extract, and the extracts were placed on a 40 °C hot plate and concentrated to 10 mL, and then 1 mL of hexane was added. The extract was further boiled down to 2 mL at this point, the extract was transferred to an amber sample vial, to which more boiling stones and 0.5 mL of hexane was added, and the vial was placed on a 70 °C hot plate. The extract was boiled down to 1 mL and removed from the heat in preparation for GC/MS analysis. The samples were processed for and analyzed by GC/MSD and GC/FID as described (37).

Swimming Performance Assay. Sustained critical swimming speed (Ucrit) of adult zebrafish (treated as embryos to control or oiled effluent 10 mo earlier) was determined by a rectangular-shaped swim tunnel, using a modification of a previous design (38). A water current was generated by a magnetic drive pump, and the flow was controlled by a knife gate valve located immediately downstream from the pump. A flow meter installed in the swim tunnel measured flow in liters per minute. An electric barrier was installed immediately downstream from the testing chamber to discourage fish from resting against the downstream screen.

Five fish (mixed males and females ages 10–11 mo) were used in each swim trial. Fish were acclimated to be as close size as possible. They were introduced into the swim tunnel and allowed to swim to the testing chamber, after which the screened knife gate was lowered to prevent escape. The fish were allowed to acclimate for 10 min before a low flow (6.2 cm/s) was introduced. The flow was maintained at 6.2 cm/s for 30 min to allow the fish to orient themselves to the flow direction. After 30 min, the flow was increased to 10.3 cm/s and maintained there for 20 min. The flow was increased in 10.3 cm/s increments every 10 min until the first fish was fatigued. Fatigue was defined as when a fish fell against the downstream screen and could not recover despite being pushed back from the electric barrier. Once fatigue was determined, the trial was ended and the fish removed from the swim tunnel. All fish were anesthetized after each trial, and their lengths and weights were recorded.

Histology. Live adult zebrafish (formerly exposed as embryos to control or oiled effluent) selected for assessment of histopathological changes were killed by being placed on ice. Any external abnormalities (e.g., frayed fins, cloudy eyes, ulcers, skin discolorations, parasitae) were assessed visually. The gills were similarly examined after cutting away the operculum, and internal organs were also examined after making a midline incision on the belly from the anus to the pectoral girdle, and assessed for ascites, hemorrhage, or other abnormalities. The visceral cavity was then further exposed by removing a small section of the abdominal wall, and the tail was excised posterior to the anus. Fish for histology were preserved whole in Dietrich’s fixative solution for histology, at 1:120 (v/v) solution to tissue to fixative, typically requiring 15 mL of fixative for 1–2 zebrafish. To ensure uniform and complete fixation, tissues were fixed for 3 d on a rocking platform (10 rpm). Fish were then rinsed in two or three changes of water, and placed in 70% ethanol. The fish were then bisected along their length by using a fresh razor blade, making the cut parallel to and on the left side of the spinal cord, and the fish were then processed. The bisected fish were then loaded into cassettes for processing, using a VIP Tissue-Tek enclosed processor (Sakura Finete). The tissue processing protocol followed that specified by the Zebrafish International Research Center, University of Oregon (http://zebrafish.org/technotes/WorldwideMaterials.php). Processed tissues were then embedded in Surgipath Formula “R” paraffin and cut, either in step or serial sagittal sections, as needed, at 5- to 7-μm thickness with a high-profile disposable blade. If the fish were difficult to cut, the block face was soaked in ice-cold 0.05% Tween 20. To focus on the morphology of the heart, serial sections of each fish were cut until it was possible to view a full section of the ventricle, atrium, and bulbus arteriosus, with the key feature being a full longitudinal section of the bulbus arteriosus. This approach standardized the orientation and sectioning plane of the three heart chambers as much as possible for imaging and measurements.

Sections of gills, spinal cord, and all internal organs were taken to ensure proper pathological evaluation. Because the fish were cut to one side of the spinal cord, sections were cut toward the midline on one half of the fish, and away, from the midline on the other. This procedure produced a rib of sections containing gills (one bisected half) and spinal cord (the other bisected half), along with a complete sampling of internal organs, while continuing to focus on the plane of section with the heart.

Sections were routinely stained by hematoxylin and eosin, using protocols described (39). Sections were then screened by using light microscopy for proper heart visualization and orientation and imaged on a Nikon E600 compound microscope by using a Spot RT camera and Spot 4.5.S.9 software (Diagnostic Instruments). Images of the hearts were subjected to image analysis to evaluate cardiac area. Significant differences due to oil exposure were observed in the pooled clutch 1 and 2 fish, animals from clutch 3 were not sectioned. There were no significant differences in condition factor between oil-exposed and control fish among the clutch 1 and clutch 2 treatment groups as a whole (Table S1).

Statistical Analysis. Statistical analyses were performed with JMP 6.0.2 for Mac (SAS Institute). Common measurements made on all three clutches (mortality, length, weight, condition factor, Ucrit) were analyzed by two-way ANOVA with treatment and clutch as independent variables. In cases where effects of or interactions between variables were detected, post hoc means comparisons were performed by using either Tukey-Kramer Honestly Significant Differences test or Student’s t test, depending on the number of groups.
ACKNOWLEDGMENTS. We thank Barbara Block, Mark Carls, Ron Heinritz, and two anonymous referees for critical reviews of the manuscript. These studies were funded in part by the NOAA Coastal Storms Program and Oceans and Human Initiative; a grant from the California Department of Fish and Game's Oil

Natural sunlight and residual fuel oils are an acutely lethal combination for fish embryos

Kristin Katlen, Catherine A. Sloan, Douglas G. Burrows, Tracy K. Collier, Nathaniel L. Scholz, John P. Incardona*

Environmental Conservation Division, Northwest Fisheries Science Center, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd E, Seattle, WA 98112, USA

ABSTRACT

The majority of studies characterizing the mechanisms of oil toxicity in fish embryos and larvae have focused largely on unrefined crude oil. Few studies have addressed the toxicity of modern bunker fuels, which contain residual oils that are the highly processed and chemically distinct remains of the crude oil refinement process. Here we use zebrafish embryos to investigate potential toxicological differences between unrefined crude and residual fuel oils, and test the effects of sunlight as an additional stressor. Using mechanically dispersed oil preparations, the embryotoxicity of two bunker oils was compared to a standard crude oil from the Alaska North Slope. In the absence of sunlight, all three oils produced the stereotypical cardiac toxicity that has been linked to the fraction of tricyclic aromatic compounds in an oil mixture. However, the cardiotoxicity of bunker oils did not correlate strictly with the concentrations of tricyclic compounds. Moreover, when embryos were sequentially exposed to oil and natural sunlight, the bunker oils produced a rapid onset cell-lethal toxicity not observed with crude oil. To investigate the chemical basis of this differential toxicity, a GC/MS full scan analysis was used to identify a range of compounds that were enriched in the bunker oils. The much higher photoxic potential of chemically distinct bunker oils observed here suggests that this mode of action should be considered in the assessment of bunker oil spill impacts, and indicates the need for a broader approach to understanding the aquatic toxicity of different oils.

Published by Elsevier B.V.

1. Introduction

The largest oil spills typically result from accidents involving the transport of large volumes of crude oil. Prominent examples include the 1989 Exxon Valdez oil spill in Alaska (37,000 tons of oil) and the 2007 Hebei Spirit oil spill in South Korea (10,800 tons). The Exxon Valdez spill in particular spurred two decades of ecotoxology research on crude oil, with an emphasis on petrogenic polycyclic aromatic compounds (PACs). As a result, the Exxon Valdez spill is by several measures the most intensively studied oil spill in history, and there is now a large literature focusing on the toxicity of Alaska North Slope crude oil (ANSCO) carried by that ship (Peterson et al., 2003). Many of these studies focused primarily on the impacts to the early life history stages of fish and the underlying toxic mechanisms, identifying subsets of PACs with specific toxicodynamic properties linked to the most obvious responses in fish embryos to ANSCO exposure (Carls and Meador, 2005). Much less is known, however, about the toxic mechanisms that may be associated with oils that are chemically distinct from ANSCO. This includes, for example, the toxicity of residual fuel oils that are burned to power large vessels (“bunker” oils). Given the relatively high frequency of vessel accidents and losses around the world, bunker oil toxicity warrants greater study, despite the smaller scale of most bunker oil spills.

Studies on ANSCO have led to a model in which component PACs are the primary consideration for an oil’s toxicity. However, a handful of studies on residual fuel oils have provided evidence of toxic activities unattributable to constituent PACs alone (Barron et al., 1999; González-Dongel et al., 2008; Neff et al., 2000). Due to the nature of modern refinery practices, residual fuel oils have chemical compositions that are distinct from those of unrefined crude oils, and even from those of bunker oils of previous decades (Uhler et al., 2007). Residual fuel oils are by definition the end-products of the refining process, and often have been subjected to conversion processes such as catalytic cracking (Matar and Hatch, 2001). Bunker fuels, the generic term applied to fuel stored onboard ships, typically consist of a highly viscous heavy residual fuel oil that is mixed with a lighter fuel (typically diesel), to facilitate pumping and flow. For example, one of the most common bunker fuel classes is intermediate fuel oil (IFO) 380, which is a residual oil cut

Abbreviations: ANSCO, Alaska North Slope crude oil; IFO, intermediate fuel oil; MDO, mechanically dispersed oil; PACs, polycyclic aromatic compounds.

* Corresponding author. Tel.: +1 206 880 3347; fax: +1 206 880 3335. E-mail address: john.incardona@noaa.gov (J.P. Incardona).

0166-445X/– see front matter. Published by Elsevier B.V.

015632
with roughly 3% marine gasoil (equivalent to no. 2 diesel) to produce a viscosity of 380 centistokes. Many chemical and elemental components of crude oil are much more highly concentrated in residual oils (Clark and Brown, 1977; Uhler et al., 2007; Wang et al., 2003). Residual fuel oils and mixed products such as IFO 380 have a higher percentage of aromatic compounds, a higher total mass of PACs and, importantly, fractions of uncharacterized polar compounds (an "unresolved complex mixture") that can approach 30% of the mass (Clark and Brown, 1977). Residual fuel oils also are often enriched with a higher content of metals such as nickel and vanadium (Matar and Hatch, 2001). Moreover, there is an overall high degree of chemical heterogeneity among modern residual fuel oils from different sources (Uhler et al., 2007).

We previously explored mechanisms underlying the toxic effects of ANSCO and individual PACs in a series of studies using zebrafish embryos (Carls et al., 2008; Incardona et al., 2004, 2005, 2006). These studies showed that various waterborne preparations of ANSCO containing only dissolved constituents, or dissolved constituents together with whole oil droplets or particulate oil, produced a characteristic and highly reproducible syndrome of developmental abnormalities in fish embryos. This "canonical" crude oil toxicity is marked primarily by pericardial and yolk sac edema, which results from cardiac dysfunction due to directly cardioactive constituents consistent with the activities of the most abundant PACs in ANSCO, specifically, the tricyclic fluorenes, dibenzothiophenes, and phenanthrenes (Carls et al., 2008; Incardona et al., 2004, 2005, 2009). Other toxic effects in zebrafish embryos resulting from ANSCO exposure included intracranial hemorrhage and blister-like malformations of the finfolds (Incardona et al., 2005), but the precise etiology of these effects is unknown. These findings reflect the inherent toxicity of ANSCO in the absence of other stressors.

There is also large literature demonstrating that certain PACs are capable of producing cellular phototoxicity through the UV-mediated activation of bioaccumulated compounds and subsequent generation of reactive oxygen species and membrane damage (Arftsen et al., 1996; Yu, 2002). This has been raised as a mechanism that is putatively important in the environment, due to the potential susceptibility of unpigmented organisms to UV exposure from solar radiation in shallow waters (Barron and Ka ihune, 2001). Most of the studies on PAC phototoxicity in aquatic systems have focused on planktonic invertebrates e.g. (Cleveland et al., 2000; Duesterloh et al., 2002; Pelletier et al., 1997), while only a few have focused on phototoxicity of individual PACs and ANSCO preparations in fish early life history stages (Barron et al., 2003; Diamond et al., 2006; Farwell et al., 2006; Farwell et al., 1988). To date there is no systematic comparative toxicology study in fish early life history stages that has included modern marine residual fuel oils with and without additional stressors such as natural sunlight.

Here we begin to elucidate potential toxic differences among oil types by directly comparing the effects of exposing zebrafish embryos to similar preparations of ANSCO and two IFO 380 originating from different ports, with and without the influence of natural sunlight. The PAC fractions of the whole oils were analyzed and compared to the waterborne PAC composition of mechanically dispersed oil (MDO) preparations (Carls et al., 2008) used in exposures. In addition, a number of waterborne compounds were identified that were markedly enriched in the IFO 380s relative to ANSCO.

2. Materials and methods

2.1. Mechanically dispersed oil (MDO) preparations

The ANSCO was obtained from the NOAA Auke Bay Laboratory as previously described (Carls et al., 2008; Incardona et al., 2005, 2009). The crude oil was subjected to partial artificial weathering by heating at 65 °C until reduced volumetrically by 20% (by mass). One of the IFO 380s, IFO1, was obtained from the California Department of Fish and Game's Office of Spill Prevention and Response. The other IFO 380, IFO2, was obtained from Olympic Tug and Barge in the Port of Seattle. Originating from different suppliers, these two IFO 380s were likely derived from different crude oil stocks, with IFO2 probably derived from an Alaska North Slope product. MDOs are in essence small-scale (e.g. 100-ml) preparations comparable to a high energy water accommodated dispersed fuel (WEAF), in which oil and water are agitated to disperse the oil, followed by a one-hour separation period allowing coalescence of droplets on the surface. The resulting water beneath the surface carries a mixture of dissolved oil components as well as some remaining uncoalesced droplets or microdroplets of whole oil. MDOs prepared by manual shaking in separatory funnels (Carls et al., 2008) have been shown to produce comparable water chemistry to large-scale (e.g. 40-l) high energy WAFs (Barron et al., 2003; Incardona et al., 2005). For these studies, a small mass of oil was added to 100 ml of zebrasib system water (Linbo, 2009) in a separatory funnel and shaken to disperse the oil into fine droplets or particles. Due to the difficulty of volumetrically delivering microliter quantities of viscous oil, micropipets were calibrated to reproducibly deliver a drop of desired mass, either 2 mg or 5 mg. Several MDOs were prepared for each exposure by delivering a 2- or 5-mg drop of oil to the surface of 100 ml zebrasib system water in a 1-1 glass separatory funnel. For PAC analytical chemistry, MDOs were prepared by weighing 12.5 mg oil onto a glass coverslip. The coverslip was then placed into the separatory funnel with 100 ml zebrasib system water. All MDOs were systematically prepared by shaking vigorously for 5 min, followed by 1 h of separation. Whereas visual inspection of the ANSCO and IFO 380 MDOs revealed oil adhered to the coverslip, adhered to the vessel side with a remaining fraction still visible at the end of shaking. All MDOs had entrained droplets/particles of oil as indicated by microscopic inspection (data not shown). The reproducibility of MDO preparation for the two IFO 380s was determined biologically (see below) and chemically (Supplementary Fig. S1).

2.2. Analytical chemistry

PACs were analyzed for all MDOs from two experiments; additional compounds were identified in the MDOs from one of these. Aliquots from each MDO were removed for embryo exposures and then the remaining MDO portions were each filtered through no. 1 Whatman paper to partially remove particulates. The filterate was then stored in brown glass bottles containing 20 ml dichloromethane until analysis. For quality assurance, a blank water sample and a spiked blank water sample were prepared in brown glass bottles, both containing 250 ml of zebrasib system water and 20 ml of dichloromethane, with a known mixture of PACs (3000 ng each analyte) added to the spiked blank. After addition of surrogate standards (1080 ng each of D10-acenaphthene, D10-acenaphthene and D12-benzo[a]pyrene in 600 µl of isooctane), each sample was transferred to a 1-separatory funnel. Samples were extracted by shaking with dichloromethane for 2 min followed by 2-min separation time. After collecting the dichloromethane phase, the samples were extracted a second time with another 20 ml of dichloromethane and the two extracts were combined. Sodium sulfate (5 g) was added to each sample, the samples were allowed to sit overnight, then more sodium sulfate (5 g) was added. To quantify selected PACs, a 3,3-ml aliquot of each extract was exchanged from dichloromethane to isooctane while being concentrated to 100 µl, then analyzed using gas chromatography/mass spectrometry (GC/MS) selected ion monitoring as described previously (Sloan et al., 2005) with additional monitoring for alkylated
PACs. Analyte concentrations in the water (μg/l) were calculated using surrogate standards and six concentration levels of GC/MS calibration standards ranging from 0.004 to 1.0 ng/μl for each analyte. For each analyte, the concentration evoking the peak response in the lowest level standard was used to calculate the lower limit of quantitation (LOQ). LOQs ranged from approximately 0.02 to 0.2 μg/l. Surrogate standard recoveries ranged from 83 to 94%, and the spiked blank recoveries ranged from 106 to 124%. The method blank contained 0.72 ng/ml total low molecular weight PACs (napthalenes, fluorenes and phenanthrenes), whereas the high molecular weight PACs in this sample were all below the LOQs.

For the identification of additional oil-related compounds, 1 ml aliquot of each extract was exchanged from dichloromethane to isooctane while being concentrated to 100 μl, then analyzed using GC/MS scanning at 50–600 Da. The GC/MS system included an Agilent 5973 Mass Selective Detector, an Agilent 5890 GC with on-column injection and a J&W 60 m × 250 μm DB-5 column (0.25-μm film-thickness). Software used for data acquisition and compound identification included Agilent Chemstation, the Automated Mass Spectral Deconvolution and Identification System (AMDIS). For each visible peak on the chromatogram, the spectrum from the nearest baseline was subtracted from the top of the peak. The resultant background-subtracted spectrum was compared to the NIST library for the most appropriate match. When a member of an alkylic aromatic family of compounds was identified (e.g. 1-carbazole), the chromatogram was examined for additional homologs belonging to that family (e.g. 2-carbazole, 3-carbazole, etc.). Because the full range of n-alkanes could be found in all samples, Kovats indices (Kováts, 1958, 1965) were calculated for all identified peaks. (The calculation of the Kovats retention index, I_k, of a compound X is:

\[ I_k = 100 \times \frac{T_n - T_x}{T_{n+1} - T_x} + 100 \]

where \( T_x \) is the retention time of compound X, \( T_n \) and \( T_{n+1} \) are the retention times of the n-alkanes with carbon numbers n and n+1 which bracket the compound X.)

The chromatograms of the extracts were searched for additional compounds with known Kovats indices that might be found in the water-soluble fraction of oil. Once a compound was detected in an extract chromatogram, the chromatograms of the other extracts were screened for the same compound. Without standards, it was not possible to precisely determine the concentrations of these compounds. Instead, a "quantitation" ion was chosen for each analyte (usually the molecular ion or the largest ion) and the integrated peak area for that ion was measured. This peak area was divided by the peak area of the surrogate standard D10-acenaphthene in that extract to produce a normalized ion area. Compounds that were present in both the sample blank and oil water extracts at approximately the same level (or lower) for the latter were excluded from further analysis.

Whole oil samples were prepared for quantifying selected PACs by diluting 100 mg of oil plus surrogate standards (2700 ng each of D8-naphthalene, D10-acenaphthene and D12-benzo[al]pyrene in 1.5 ml of isooctane) to 10 ml in dichloromethane. A 200 μl aliquot of each oil sample was further diluted to 3.35 ml in dichloromethane and then cleaned up by gravity-flow silica/alumina chromatography columns and size-exclusion high performance liquid chromatography as previously described (Sloan et al., 2005). The samples were analyzed by GC/MS as described above for the oil water extracts. Analyte concentrations in the oil ng/μg) were calculated using the surrogate standards and eight concentration levels of GC/MS calibration standards ranging from 0.004 to 10 ng each analyte μl. Surrogate standard recoveries, taking into account the portion of sample analyzed, ranged from 85 to 124%.

2.3. Zebrafish embryo exposure

MDO preparations were made for eight independent exposure experiments conducted between late July and mid-December; five of these included phototoxicity assays. Adult zebrafish were maintained and embryos collected as described elsewhere (Linde, 2009). To assess a potential protective role of pigment in sunlight exposures, some experiments utilized nacre embryos, which have a marked reduction in melanophores (Lister et al., 1999). Duplicate or triplicate groups of 20 embryos were exposed beginning 4-6 h postfertilization (hpf) in pre-cleaned 30-mm glass Petri dishes. Aliquots of MDOs (15 ml) were added to the Petri dishes, and embryos were then transferred into treatment solutions in a small volume (~100 μl) of zebrafish system water. Exposures were carried out in a laboratory incubator without light at 28.5°C, and embryos incubated up to 72 hpf without renewal of exposure water. Controls were incubated in clean zebrafish system water in the same incubator. Pericardial edema was scored at 48 hpf or 72 hpf. Subsamples of developing embryos were removed at 24, 48, and 72 hpf for exposure to natural sunlight. There were two types of negative control for sunlight exposure: one exposed to clean water and sunlight, and one exposed to oil and ambient outdoor temperature shaded from sunlight. Typically 10-15 embryos were removed from each replicate exposure dish and placed in a new Petri dish with clean system water and then transported outdoors in a shallow plastic tray on a hardcart. Petri dishes were left uncovered for the duration of sunlight exposure, and gently agitated occasionally to evenly disperse the embryos. Negative controls for sunlight exposure experienced the same outdoor ambient temperature, covered loosely with aluminum foil and shaded on the lower shelf of the cart; water temperature was not recorded. In order to compare experiments performed under different weather conditions, nominal UV exposure was estimated using a Spectronic UV203 radiometer (Advanced Photonics International, Inc., Fairfield, CT). The radiometer was used in average mode to obtain average UV-A and UV-B levels for the entire sunlight exposure duration. UV-A levels were measured for the first half of the exposure, and UV-B levels during the second half. Sunlight exposure ranged from 20 min on partly cloudy summer days to 2 h on overcast winter days (Table 1). Two of the phototoxicity experiments utilized the nacre mutant with

Table 1

<table>
<thead>
<tr>
<th>Date (experiment no.)</th>
<th>Weather</th>
<th>UV-A (mW/m2)</th>
<th>UV-B (mW/m2)</th>
<th>Duration (min)</th>
<th>UV-A/UV-B dose (μW h/m2)</th>
<th>Nominal oil dose (ppm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 July (experiment 1)</td>
<td>Sunny</td>
<td>18,670</td>
<td>1805</td>
<td>10</td>
<td>311/30</td>
<td>20</td>
</tr>
<tr>
<td>1 October (experiment 2)</td>
<td>Cloudy</td>
<td>8950</td>
<td>294</td>
<td>20</td>
<td>286/10</td>
<td>20</td>
</tr>
<tr>
<td>7 October (experiment 3)</td>
<td>Rainy</td>
<td>4611</td>
<td>469</td>
<td>120</td>
<td>922/94</td>
<td>50</td>
</tr>
<tr>
<td>22 October (experiment 4)</td>
<td>Sunny</td>
<td>8971</td>
<td>402</td>
<td>120</td>
<td>1794/894</td>
<td>50</td>
</tr>
<tr>
<td>4 December (experiment 5)</td>
<td>Cloudy</td>
<td>3421</td>
<td>ND</td>
<td>120</td>
<td>684/ND</td>
<td>125</td>
</tr>
</tbody>
</table>

ND = not determined.

* By mass of whole oil dispersed in water.
Table 2
PAC concentrations and composition of MDO preparations.*

<table>
<thead>
<tr>
<th>OIL</th>
<th>Assay</th>
<th>$\sum$ PACs</th>
<th>$\sum$ NPHs</th>
<th>$\sum$ PUs</th>
<th>$\sum$ DBTs</th>
<th>$\sum$ PHNs</th>
<th>$\sum$ FLAs</th>
<th>$\sum$ CHRs</th>
<th>$\sum$ S-5-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANSCO</td>
<td>Edema + phototoxicity</td>
<td>210</td>
<td>154.0</td>
<td>11.7</td>
<td>16.9</td>
<td>24.1</td>
<td>0.6</td>
<td>3.0</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Concentration (µg/l)</td>
<td>73.5</td>
<td>5.6</td>
<td>2.0</td>
<td>13.5</td>
<td>3.0</td>
<td>1.0</td>
<td>1.4</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Composition (% of $\sum$ PACs)</td>
<td>73.5</td>
<td>5.6</td>
<td>2.0</td>
<td>13.5</td>
<td>3.0</td>
<td>1.0</td>
<td>1.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Edema</td>
<td>Concentration (µg/l)</td>
<td>340</td>
<td>249.0</td>
<td>19.1</td>
<td>20.7</td>
<td>40.1</td>
<td>1.1</td>
<td>5.3</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Composition (% of $\sum$ PACs)</td>
<td>73.2</td>
<td>5.6</td>
<td>2.0</td>
<td>13.5</td>
<td>3.0</td>
<td>1.0</td>
<td>1.4</td>
<td>0.06</td>
</tr>
<tr>
<td>IFO1</td>
<td>Edema + phototoxicity</td>
<td>240</td>
<td>160.0</td>
<td>10.7</td>
<td>11.4</td>
<td>40.8</td>
<td>3.4</td>
<td>8.6</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Concentration (µg/l)</td>
<td>66.7</td>
<td>4.5</td>
<td>4.8</td>
<td>17.0</td>
<td>1.4</td>
<td>3.6</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Composition (% of $\sum$ PACs)</td>
<td>65.2</td>
<td>4.8</td>
<td>4.7</td>
<td>17.1</td>
<td>1.3</td>
<td>3.7</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Edema</td>
<td>Concentration (µg/l)</td>
<td>100</td>
<td>65.2</td>
<td>4.8</td>
<td>4.7</td>
<td>17.1</td>
<td>1.3</td>
<td>3.7</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Composition (% of $\sum$ PACs)</td>
<td>65.2</td>
<td>4.8</td>
<td>4.7</td>
<td>17.1</td>
<td>1.3</td>
<td>3.7</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>IFO2</td>
<td>Edema + phototoxicity</td>
<td>440</td>
<td>318.6</td>
<td>17.1</td>
<td>20.3</td>
<td>53.6</td>
<td>4.2</td>
<td>13.4</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Concentration (µg/l)</td>
<td>72.3</td>
<td>3.9</td>
<td>4.6</td>
<td>12.3</td>
<td>0.9</td>
<td>3.0</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Composition (% of $\sum$ PACs)</td>
<td>72.3</td>
<td>3.9</td>
<td>4.6</td>
<td>12.3</td>
<td>0.9</td>
<td>3.0</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Edema</td>
<td>Concentration (µg/l)</td>
<td>310</td>
<td>229.0</td>
<td>13.2</td>
<td>14.4</td>
<td>38.5</td>
<td>2.7</td>
<td>8.6</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Composition (% of $\sum$ PACs)</td>
<td>73.9</td>
<td>4.3</td>
<td>4.6</td>
<td>12.4</td>
<td>0.9</td>
<td>2.8</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

* Data are for MDOs from experiments 7 and 8. $\sum$ PACs is the sum of all 39 analytes (shown in Figure S1). $\sum$ NPHs, etc. are the sums of the parent compound and each alkylated homologue, and $\sum$ S-5-6 is the sum of seven 5-ring and one 6-ring analytes. NPHs, naphthalenes; PUs, fluoranthenes; DBTs, dibenzothiophenes; PHNs, phenanthenes; FLAs, fluoranthenes; CHRs, chrysenes.

3. Results

3.1. Chemical composition of MDO preparations

Aqueous concentrations of selected PACs were analytically determined for MDO preparations used for two experiments (Table 2 and Supplementary Fig. S1), and assayed for edema and phototoxicity, and edema only, respectively. The MDO filtrates would have contained some oil microdplets (Carls et al., 2008; Payne et al., 1999), and based on these previous studies, we estimate that dissolved phase compounds represented roughly 20% of the total measured PACs. Consistent with this, each MDO analysis showed a relative enrichment with compounds that were more water-soluble relative to the source oils (Fig. S1). These included the naphthalenes as well as the parent and lower alkylated fluorenes, dibenzothiophenes and phenanthenes. For example, the ratios of phenanthrene to C4-phenanthrene in the source oils were 1.0, 0.45, and 0.52 for ANSCO, IFO1, and IFO2, respectively, while the mean ratios in the respective MDO preparations were 2.3, 1.1, and 1.8. There was also a relative reduction in the higher molecular weight chrysenes. The PAC composition was highly reproducible between different MDO preparations using the same oil (Table 2, Fig. S1). Although the concentrations of PAC subclasses and summed PAC ($\sum$ PACs) were more variable between preparations constituted on different days, all of the preparations fell within a range of 100–440 µg/L $\sum$ PACs (Table 2).

As an initial step towards identifying differences between the unrefined ANSCO and the residual IFOs, a GC/MS full scan analysis was carried out on extracts of MDO preparations from one phototoxicity experiment (#7, Table 1). Identified compounds are listed individually in Supplementary Table S1. These consisted of 45 alkanes, 378 aromatic hydrocarbons with their alkylated families, 35 nitrogen heterocycles with their alkylated families (primarily carbazoles), 32 various oxygen containing compounds, 81 sulfur heterocycles and their alkylated families, and 4 large unidentified peaks. Because of the lack of available quantification standards for these compounds, we were unable to calculate actual concentrations. Rather, it was only possible to compare the normalized ion peaks of major compounds.
Table 3
Summary of compounds of interest in the full scan analysis of oiled water extracts.

<table>
<thead>
<tr>
<th>Chemical group</th>
<th>Compound</th>
<th>Number of isomers</th>
<th>IFO1 to ANSCO peak area ratio</th>
<th>IFO2 to ANSCO peak area ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic hydrocarbons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzenes</td>
<td>1,1'-Bis(di)phenylene-bis-benzene</td>
<td>1</td>
<td>19.015</td>
<td>39.216</td>
</tr>
<tr>
<td></td>
<td>(tentative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C3 benzene</td>
<td>5</td>
<td>3.954</td>
<td>2.555</td>
</tr>
<tr>
<td>Indans</td>
<td>Indian</td>
<td>1</td>
<td>4.582</td>
<td>4.672</td>
</tr>
<tr>
<td></td>
<td>C1 indan</td>
<td>4</td>
<td>2.201</td>
<td>3.262</td>
</tr>
<tr>
<td>Indenes</td>
<td>Indene</td>
<td>1</td>
<td>43.402</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>C1 indene</td>
<td>2</td>
<td>48.868</td>
<td>8.961</td>
</tr>
<tr>
<td></td>
<td>C2 indene/C1 dihydrophenanthrene</td>
<td>7</td>
<td>9.405</td>
<td>4.054</td>
</tr>
<tr>
<td></td>
<td>C3 indene</td>
<td>3</td>
<td>6.371</td>
<td>6.008</td>
</tr>
<tr>
<td>Naphthalenes</td>
<td>C2 alkenyl naphthalene</td>
<td>5</td>
<td>5.121</td>
<td>8.183</td>
</tr>
<tr>
<td></td>
<td>1,2-Dihydropnaphthalene</td>
<td>1</td>
<td>14.613</td>
<td>0.000</td>
</tr>
<tr>
<td>Phenyl naphthalenes</td>
<td>C1 phenyl naphthalene/C3 alkenyl</td>
<td>6</td>
<td>2.754</td>
<td>3.400</td>
</tr>
<tr>
<td></td>
<td>phenanthrene/anthracene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C2 phenyl naphthalene/C4 alkenyl</td>
<td>6</td>
<td>9.092</td>
<td>18.334</td>
</tr>
<tr>
<td></td>
<td>phenanthrene/anthracene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acenaphthene</td>
<td>Acenaphthene</td>
<td>1</td>
<td>5.086</td>
<td>0.943</td>
</tr>
<tr>
<td>Phenanthrene/anthracene</td>
<td>C4-phenanthrene/anthracene</td>
<td>15</td>
<td>3.259</td>
<td>3.528</td>
</tr>
<tr>
<td></td>
<td>Dihydrophenanthrene</td>
<td>2</td>
<td>2.338</td>
<td>4.361</td>
</tr>
<tr>
<td></td>
<td>Dihydronaphthalene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoranthene/pyrenes</td>
<td>Fluoranthene/pyrene</td>
<td>2</td>
<td>8.325</td>
<td>7.206</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>Benzo(b)fluoranthene</td>
<td>1</td>
<td>6.853</td>
<td>6.937</td>
</tr>
<tr>
<td></td>
<td>C1 fluoranthene/pyrene/benzofluoranthene</td>
<td>6</td>
<td>6.325</td>
<td>7.844</td>
</tr>
<tr>
<td></td>
<td>C2 fluoranthene/pyrene/benzofluoranthene</td>
<td>14</td>
<td>6.505</td>
<td>16.912</td>
</tr>
<tr>
<td></td>
<td>C3 fluoranthene/pyrene/benzofluoranthene</td>
<td>11</td>
<td>12.207</td>
<td>16.420</td>
</tr>
<tr>
<td>Benzo(a)anthracenes/chrysenes</td>
<td>C1 benzo(a)anthracene/chrysenes</td>
<td>7</td>
<td>3.313</td>
<td>5.635</td>
</tr>
<tr>
<td></td>
<td>C2 benzo(a)anthracene/chrysenes</td>
<td>10</td>
<td>3.615</td>
<td>6.003</td>
</tr>
<tr>
<td>Nitrogen heterocycles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoles</td>
<td>C2 indole (tentative)</td>
<td>1</td>
<td>1.881</td>
<td>3.771</td>
</tr>
<tr>
<td>Carbazoles</td>
<td>Carbazole</td>
<td>1</td>
<td>6.526</td>
<td>10.163</td>
</tr>
<tr>
<td></td>
<td>C1 carbazole</td>
<td>4</td>
<td>7.651</td>
<td>13.049</td>
</tr>
<tr>
<td></td>
<td>C2 carbazole</td>
<td>11</td>
<td>8.608</td>
<td>11.122</td>
</tr>
<tr>
<td></td>
<td>C3 carbazole</td>
<td>12</td>
<td>5.334</td>
<td>7.582</td>
</tr>
<tr>
<td></td>
<td>C4 carbazole</td>
<td>6</td>
<td>3.833</td>
<td>3.148</td>
</tr>
<tr>
<td>Oxygen containing hydrocarbons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo(b)aldehydes</td>
<td>C4 benzaldehyde (tentative)</td>
<td>1</td>
<td>0.356</td>
<td>1.144</td>
</tr>
<tr>
<td></td>
<td>Pentamethylbenzaldehyde</td>
<td>1</td>
<td>1.634</td>
<td>1.037</td>
</tr>
<tr>
<td>Phenols</td>
<td>C1 phenol</td>
<td>3</td>
<td>5.619</td>
<td>21.223</td>
</tr>
<tr>
<td></td>
<td>C2 phenol</td>
<td>5</td>
<td>3.389</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>C3 phenol</td>
<td>3</td>
<td>3.949</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>C11 phenol</td>
<td>2</td>
<td>107.945</td>
<td>4.135</td>
</tr>
<tr>
<td>Alcohols</td>
<td>Phenylethyl alcohol</td>
<td>1</td>
<td>28.486</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Phenoxycapropanol</td>
<td>1</td>
<td>28.681</td>
<td>0.000</td>
</tr>
<tr>
<td>Ethers</td>
<td>Diphenyldiethyl ether</td>
<td>2</td>
<td>58.171</td>
<td>0.000</td>
</tr>
<tr>
<td>Ketones</td>
<td>Benzoylacetone (tentative)</td>
<td>1</td>
<td>5.550</td>
<td>0.000</td>
</tr>
<tr>
<td>Sulfur heterocycles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo(1)thiophenes</td>
<td>Benzo(1)thiophene</td>
<td>1</td>
<td>43.008</td>
<td>40.804</td>
</tr>
<tr>
<td></td>
<td>C1 benzo(1)thiophene</td>
<td>4</td>
<td>22.426</td>
<td>29.403</td>
</tr>
<tr>
<td></td>
<td>C2 benzo(1)thiophene</td>
<td>5</td>
<td>5.498</td>
<td>7.111</td>
</tr>
<tr>
<td></td>
<td>C3 benzo(1)thiophene or C7 benzene</td>
<td>15</td>
<td>2.517</td>
<td>5.528</td>
</tr>
<tr>
<td>Benzonaphthothiophenes</td>
<td>Benzonaphthothiophene</td>
<td>3</td>
<td>1.353</td>
<td>4.042</td>
</tr>
<tr>
<td>Unidentified compounds</td>
<td>Unknown (191 ion)</td>
<td>1</td>
<td>3.608</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Unknown (247 ion; MW 318; maybe C16 phenol)</td>
<td>1</td>
<td>41.654</td>
<td>1.001</td>
</tr>
<tr>
<td></td>
<td>Unknown (247 ion; possibly MW 318)</td>
<td>1</td>
<td>90.799</td>
<td>2.793</td>
</tr>
<tr>
<td></td>
<td>Unknown (303 ion; MW 374)</td>
<td>1</td>
<td>77.027</td>
<td>1.866</td>
</tr>
</tbody>
</table>

* Ratio of normalized peak areas in extract compared to ANSCO normalized peak areas.
Fig. 2. Morphological effects of exposure to different oils. Embryos were exposed to the indicated MOQO preparation from 6 to 72 hpf in the dark, exposed to sunlight or outdoor ambient temperature in the dark for 10 min, then returned indoors and imaged (experiment 1, 31 July). The top image in each panel shows the entire larva with the head to the left, while the lower left and right images show higher magnification views of the tail and pericardial region, respectively. Arrows indicate pericardial edema in oil-exposed embryos (B–E). Scale bars are 0.5 mm (top images) and 100 μm (lower and right images). (A) Larva exposed to clean water and sunlight. (B) Larva exposed to ANSCO without sunlight. Caudal finfold shows reduced growth (unfilled arrowheads). (C) Larva exposed to ANSCO followed by sunlight; note absence of tail necrosis. (D) Larva exposed to IPO1 without sunlight. Caudal finfold shows reduced growth and notch on distal side (arrowhead). (E) Larva exposed to IPO1 followed by sunlight; note striking loss of tail tissue to cytolsis. Images are representative of results from all experiments.
areas of a particular compound between the different extracts. The relative ion areas of the two IFO 380s compared to ANSCO are summarized for each family of compounds in Table 3. Notably, the analytical method used was likely unsuitable for the detection of polar compounds.

We focused primarily on the chromatographic peaks that were substantially larger (5 times or more) in the IFO1 and/or IFO2 extracts compared to the ANSCO extract. These compounds are summarized in Table S2. Some of the most prominent differences between the crude oil and IFOs included the following compounds, with normalized ion area sums displayed in Fig. 1: carbazole and C1 to C3 carboxylic acids (C0–C3 CBZs), C1 to C3 fluoranthene/pyrenes (C1–C3 FLAs), benzo[b]thiophene and C1 to C3 benzothiophenes (C0–C3 BTPs), C7 phenyl naphthalenes (C8 PhNPHs), C6 to C7 indenes (C1–C3 IDEs) and C1 phenols (C1 PhOhs).

3.2. Comparable and novel sublethal toxicities of IFOs relative to ANSCO in the absence of sunlight

Overall, all three oils produced cardiovascular toxicity after incubation in the dark (Table 4). For all oil exposures in the absence of sunlight, embryos developed a high prevalence of pericardial edema by 48 hpf (data not shown, Table 4 and Fig. 2). Edema was qualitatively assessed in all experiments, and quantified in 4 experiments, where the frequency ranged from 81 to 100% (mean 88%) after exposure to ANSCO and 37–100% (mean 68%) for IFO1, and was 100% for IFO2 in all experiments. In the experiments for which PACs were analyzed (Table 2), edema was present in a lower percentage of embryos exposed to ANSCO relative to IFO1 despite a comparable or higher total PAC concentration. Although the edema response was qualitatively similar between ANSCO and IFO1, the corresponding concentrations of tricylic PACs differed by a factor of three (87 μg/l vs. 27 μg/l respectively). Similarly, the IFO2 MDO from experiment 8 had total tricylic PACs (66 μg/l) slightly lower than the ANSCO MDO (87 μg/l), but produced greater cardiac toxicity. However, both IFO MDOs had a greater diversity of 4- and 5-ring PACs, with the IFO2 MDO having concentrations of fluoranthene and total 5-ring PACs (e.g. benzo[a]pyrene) that were 2 times as high as those of the ANSCO MDO. These PACs are capable of contributing to developmental abnormalities of the heart through mechanisms that are different than those that mediate the cardiotoxicity of tricyclic compounds (see Section 4).

One novel type of sublethal toxicity consistently observed with dark exposures to IFO1 was an effect on tail development. Exposure to ANSCO typically causes a delay or reduced outgrowth of the finsfolds (Fig. 18 and Incardona et al., 2005), which later manifests as finfold blisters (Icardona et al., 2005). In addition to reduced outgrowth of the entire caudal finfold, embryos exposed to IFO1 displayed at 72 hpf a high frequency (>50%) of a different defect characterized by a notch on its dorsal side (Fig. 2D). This type of defect was not observed with IFO2.

3.3. Sunlightinitiates acute cell-letal toxicity following exposure to IFOs but not ANSCO

The potential for photoinduced toxicity from sunlight (i.e., UV) exposure was tested in 5 experiments under very different weather regimes from July to December 2008 (Table 1). In each experiment there was a dramatic and acute lethal toxicity induced by subsequent sunlight exposure with the 2 residual IFOs (Figs. 2, 3 and 52 and Table 4), but not the unrefined ANSCO. Embryonic exposure to either IFO1 (Fig. 2) or IFO2 (Fig. 52), followed by sunlight exposure at either 48 hpf or 72 hpf, resulted in a rapid and severe lytic deterioration of the caudal finfold, sometimes involving the entire tail (Fig. 2E, 72 hpf). Finfold cytology was discernible within minutes of terminating the sunlight exposure, and in some cases had evidently commenced prior to the end of sunlight exposure. This effect was observed in all 5 experiments, and differed only in degree of severity, but was absent in ANSCO-exposed embryos (Fig. 2C).

Exposure to normal zebrafish embryos develop large numbers of black pigment-containing melanophores in the head and trunk, but not the finsfolds. To determine if light-absorbing natural skin pigments provided protection from photoinduced oil toxicity, we tested either 24hpf embryos (prior to melanophore development) or 48hpf nacre larvae (a mutant with markedly reduced melanophores). Despite shorter exposure to MDOs, cytoytic effects were much more severe in 24hpf embryos exposed to IFO1 (Fig. 3D and E), and the lytic response to both IFO MDOs in nacre larvae at 48hpf often involved tissues in the head and trunk (Fig. 52 and data not shown). While sunlight did not produce phototoxicity in 24hpf embryos exposed to ANSCO (Fig. 3B), with IFO1 exposure, large portions of the trunk (Fig. 3D) and sometimes the entire embryo (Fig. 3E) would undergo cytology.

In the MDO preparations where quantification of phototoxicity was paired with analyses for PACs, the PAC levels and composition were comparable (Table 2). The ANSCO and IFO1 MDOs had PAC of 210 and 240 μg/l respectively, while the IFO2 MDO had 440 μg/l largely due to a 2-fold higher level of naphthalenes. Nevertheless, the cytoytic response to sunlight exposure was absent in embryos exposed to ANSCO (Table 4). In this experiment, the most marked differences between MDO preparations of ANSCO and the
residual fuel oils among the conventionally measured PACs were the higher concentrations of 4- and 5-ring compounds. In particular, the total concentrations of fluoranthenes and chrysenes were 3–4 times higher in the IFO MDOs (12 and 18 μg/l vs. 4 μg/l). However, the IFO1 MDO produced slightly more severe tail damage, despite having lower concentrations of 4- and 5-ring compounds than the IFO2 MDO.

4. Discussion

As would be expected from the overall PAH compositions, the canonical cardiogenic edema response was observed after exposure to each of the three oil types in the absence of light. However, there were important differences between the magnitude of the response in relation to the PAC concentrations and composition of the corresponding MDO preparations, especially the concentrations of tricyclic PACs. For example, although IFO1 and ANSCO both produced edema in ~90% of embryos in 1 experiment, the concentrations of ∑ PACs and tricyclic PACs in IFO1 were ~17–44% of those in ANSCO and IFO2. This suggests that compounds other than the tricyclic PACs contributed to the edema response from IFO1. The higher molecular weight PACs that are generally more abundant in the IFOs are a likely candidate, including benz[a]anthracene, fluoranthene, and benzo[a]pyrene, as they also are capable of causing pericardial edema through a different mechanism (Icardona et al., 2006; Matson et al., 2008; Wills et al., 2009; our unpublished observations). Similarly, the relatively abundant carbazoles in the IFOs are likely act synergistically to increase the embroyotoxicity of other PACs (Wassenberg et al., 2005). The pericardial edema resulting from IFO exposure most likely has a more complicated etiology than the response from ANSCO exposure, consistent with the greater complexity of compounds. The etiology of the novel tail defect associated with exposure to IFO1 is unclear, because most of the compounds identified as enriched in IFO1 relative to ANSCO were common to both IFOs. However, our chemical analysis was not comprehensive, and we did not characterize polar and other potentially toxic compounds that may also be present in the residual fuel oils. Because the overall toxicity and chemistry of the 2 IFO 380s tested here were grossly similar, it is likely that inclusion of a larger number residual fuel oils would reveal even greater toxicological heterogeneity.

In general, only sub lethal effects (e.g. pericardial edema, fin malformation) are observed as an acute response in fish embryos exposed to oiled water preparations (as an individual stressor) that have ∑ PAC concentrations in the ranges reported here. In contrast, the addition of sunlight as a co-stressor converted the toxicity of the 2 IFOs but not ANSCO to produce rapid and overt lethality through a cytotoxic mechanism. The rapid cell death observed in the finfold and tail tissue within minutes to an hour after sunlight exposure is most consistent with a photoinduced cytolytic response due to plasma membrane damage, rather than an apoptotic mechanism.

This is particularly supported by our finding that this enhanced oil toxicity is ameliorated by the presence of light-absorbing, melanin-containing pigment cells in the skin of zebrafish embryos. However, the precise mechanisms leading to membrane damage cannot be determined from these data. Several studies in both cell lines and in intact animals have shown that photoactivation of PACs leads to general membrane damage through lipid peroxidation (Kagan et al., 1987; McCloskey and Oris, 1993; Weinstein et al., 1997). While membrane lipid peroxidation is a well-known action of other non-PAC phototoxic compounds (Girotti and Deziel, 1983), some compounds associated with photoinduced cytolsis may act through oxidation of membrane proteins (Cardenas et al., 1992).

In a previous study, ANSCO was found to produce lethal phototoxicity when combined with sunlight or artificial UV exposure in herring larvae, but not embryos (Barron et al., 2003). Our study was not designed to replicate these results, and there are key differences. First, our results with zebrafish embryos and ANSCO are consistent with the results with herring embryos, and may relate to the toxicokinetic differences between early embryos and larvae. Second, the UV-A doses from sunlight in the previous study ranged from 2160 to 3600 μW·h/cm², while in this study the range was 250–1790 μW·h/cm². Finally, lethality was quantified over a number of days in the former study, while here acute lethality was assessed only immediately after sunlight exposure. The marked differences between the two IFO 380s and ANSCO in phototoxic potential observed here is most likely due to distinct chemical differences between residual fuel oils and crude oil.

While our preliminary analysis of the IFO 380 MDO preparations identified a large number of additional compounds, these particular compounds may or may not be responsible for the markedly different phototoxicity. Although the elevated phototoxicity of the IFOs could be due to the higher concentrations of fluoranthenes and chrysenes alone, this seems unlikely. The differences in total fluoranthenes and chrysenes (and 5-ring compounds) between the various MDOs were not large (e.g. only 3 times greater in IFO1 vs. ANSCO). If these compounds alone were responsible for the dramatic phototoxicity of the IFOs, then there must be a very sharp threshold that is not far above the concentrations present in the ANSCO MDOs. The other heterocyclic PACs identified in the IFOs (e.g. the benzothiophenes and carbazoles) are unlikely to be phototoxic alone (Kocheva et al., 1982). A more parsimonious explanation is that these PACs contribute to the phototoxicity in combination with some of the other identified or unidentified compounds that are present at higher concentrations in the IFOs. Despite the wide range of compounds included in our analysis, it is likely that an even more comprehensive analysis is necessary to identify the compounds responsible for the potent phototoxicity of these IFOs.

These results indicate that, compared to crude oil, residual fuel oils can produce different sub lethal toxicity (e.g. tail defects), and that at least 2 individual IFOs have the potential to produce dramat-
ically lethal toxicity among translucent animals in the presence of sunlight. Moreover, the canonical response of cutaneous edema is likely to arise through more than one mechanism, depending on the relative ratios of PAs that act through different (e.g., aryl hydrocarbon receptor-independent and -dependent) mechanisms. Our findings identify a number of compounds beyond the conventionally studied PAs that should be targeted for further toxicity studies, in particular the heterocyclic compounds which are enriched in residual fuel oils. Finally, this study highlights the need for a broader approach to understanding the toxicity of diverse oil types to better assess the ecological impacts of oil spills, especially in areas where additional stressors such as intense solar radiation may exist.

Acknowledgements

The authors thank Susan Sugarman (OSP) for providing the IFO1 sample and Sven Christiansen (Olympic Tug and Barge) for providing the IFO2 sample, Mark Carls for providing the radiometer, Tiffany Linbo for zebrasib husbandry, Gina Viltalo, Jim Meador, and Jana Labenia for critical reviews of the manuscript. This work was supported by NOAA Fisheries programmatic funds and the authors are solely responsible for the experimental design, implementation, data analysis and interpretation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.aquatox.2010.04.002.

References

Attenuation of polycyclic aromatic hydrocarbons from urban stormwater runoff by wood filters

Thomas B. Boving a,*, Kevin Neary b,1

a Department of Geosciences University of Rhode Island, Woodward Hall, Rm. 315 Kingston, RI 02881, United States
b State of Connecticut. Department of Environmental Protection, 79 Elms Street, Hartford, CT 06106-5127, United States

Received 11 December 2005; accepted 2 August 2006
Available online 27 November 2006

Abstract

A significant amount of contamination enters water bodies via stormwater runoff and, to reduce the amount of pollution, retention ponds are installed at many locations. While effective for treating suspended solids, retention ponds do not effectively remove dissolved constituents, such as polycyclic aromatic hydrocarbons (PAH). Previous laboratory studies demonstrates that aspen wood cuttings can be utilized to enhance the removal of dissolved contaminants. The objective of this pilot-scale field test was to determine if wood filters could effectively remove dissolved PAH from the runoff under field conditions. Four wood filter tests were conducted, lasting from 1 to 9 weeks, to determine the degree of PAH attenuation from the aqueous phase as a function of wood mass, residence times, and seasonal changes. The prototype wood filters removed on average between 18.5% and 35.6% (up to 66.5%) of the dissolved PAH contaminants. The PAH removal effectiveness of the wood was not affected by changes in water temperature or pH. The filter effectiveness increased with filter size and was highest in continuously submerged parts of the filter system. Also, heavier molecular weight PAH compounds (e.g. chrysene) were more effectively removed than lighter molecular weight compounds. Dissociation of weakly particle-bound PAH from the filter was identified as the most likely cause for a temporary drop of the wood filter’s PAH load during intense storms. Simple filter design changes are likely to double the filter effectiveness and alleviate the disassociation problem.

© 2006 Published by Elsevier B.V.

Keywords: Urban hydrology; Polycyclic aromatic hydrocarbons (PAH); Best management practice (BMP); Pollution remediation; Biofilter; Nonpoint source pollution

Abbreviations: BMP, best management practices; GC-FID, gas chromatography-flame ionization detector; HMW, high molecular weight; LMW, low molecular weight; PAH, polycyclic aromatic hydrocarbons; WFT, wood filter tests.

* Corresponding author. Tel.: +1 401 874 7053, fax: +1 401 874 2190.
E-mail addresses: boving@uri.edu (T.B. Boving), kevin.neary@po.state.ct.us (K. Neary).
1 Tel.: +1 860 424 3947.

0169-7722/$ - see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.jconhyd.2006.08.009
1. Introduction

Particularly in urban areas, runoff from impervious surfaces, such as roads and parking lots, contributes significant quantities of pollutants to surrounding surface water bodies (Drapper et al., 2000; Krein and Schorer, 2000; Smith et al., 2000). Stormwater runoff can contain high levels of anthropogenic contaminants, including polycyclic aromatic hydrocarbons (PAH) and heavy metals (US EPA, 1983; Stanley, 1996; Pitt et al., 2004; Mahler et al., 2005). Upon entering the water, organic and inorganic contaminants can remain in the environment for long periods (Sanders et al., 1993), posing a threat to human health and the environment (Tuhačková et al., 2001; Gryniewicz et al., 2002). Especially PAH originate from numerous, mostly anthropogenic sources including combustion of fossil fuels, vehicle emissions, and industrial effluent. For instance, Mahler et al. (2005) identified parking lot sealcoats as a significant source of PAH in urban runoff waters. PAH concentrations in urban environments have risen over the last two decades due to higher traffic flows and increased consumption of fossil fuel (Van Metre et al., 2000).

Most PAH are genotoxic (Harvey, 1997) and the abatement of PAH in road runoff is typically dealt with by so-called best management practices (BMP). Stormwater BMPs can be either structural or nonstructural to prevent, control, and treat polluted runoff. Retention ponds are an example of a structural stormwater BMP (US EPA, 1999). A retention pond is designed to allow settling of suspended sediment and to control the effluent flow rate into adjacent water bodies (Mallin et al., 2002; Krishnappan and Marsalek, 2002). Contaminants, such as PAH, are generally sorbed to the suspended sediment and co-settle in the retention pond. A fraction of the contaminants, however, persist in the dissolved phase. For instance, Hoffinan et al. (1984) determined that between 7% and 21% of PAH in parking lot runoff were not contained in the suspended load, leaving a significant amount of PAH dissolved. Another major source of dissolved contaminants is desorption from polluted sediment. As shown by Ghosh et al. (2001), who performed laboratory desorption studies with dredged silt/clay sediments and simulated the results with four different models and at changing temperature conditions, over 90% of the PAH associated with the sediment eventually desorbed and entered the dissolved phase.

One of the most promising abatement strategies for dissolved contaminants in roadway runoff relies on low-cost biofilters. For instance, Clark et al. (2000) investigated municipal leaf compost and peat moss for treating stormwater runoff and compared the effectiveness of these biofilters to that of conventional adsorbents, including activated carbon and cation-exchange resin. Although about 3 to 4 times less effective than the conventional filters, the cost of the biofilter systems was significantly lower. Other organic materials that demonstrated heavy metal treatment capabilities for stormwater runoff include sawdust, banana peels, peanut and hazelnut shells (Brown et al., 2000; Cimino et al., 2000; Schneegeurt et al., 2001; Annadurai et al., 2003). Besides for heavy metals, biofilters have been successfully used to treat dissolved pesticides, chemical dyes, orthophosphate, monoaromatics (benzene, toluene, and o-xylene) and PAH (Bras et al., 1999; Morais et al., 1999; Cimino et al., 2000; MacKay and Gschwend, 2000; Trapp et al., 2001; Palma et al., 2003; Boving and Zhang, 2004; Karthikeyan et al., 2004).

Wood in particular appears to be a promising biofilter matrix for the several reasons. First, wood is an inexpensive and renewable resource and current forest management practices produce large quantities of low quality/low value wood (US DA, 2003) that could be used for filter construction. Second, Boving and Zhang (2004) have already shown that under laboratory conditions over 90% of dissolved pyrene – one of the most prominent PAH in stormwater runoff – can be removed. Similarly high removal effectiveness was determined for three other PAH...
(naphthalene, anthracene, fluorene). Importantly, Boving and Zhang (2004) also showed that once the PAH were removed from solution, the wood greatly resisted contaminant remobilization when flushed with clean water. The capability of wood to remove and contain contaminants from the aqueous phase is related to the composition of the wood. Aspen wood (*Populus tremula*), which is the wood species used in the Boving and Zhang (2004) study, typically consists of 51% cellulose, 26% hemicellulose, 21% lignin, 1% ash, and less than 1% inorganic matter (Fengel and Wegner, 1989). Lignin is a hydrophobic polymer and is generally recognized as the principal wood sorbent (Garbarini and Lion, 1986). PAH are hydrophobic compounds and therefore expected to sorb to hydrophobic organic matter or sediment rather than remaining in aqueous solution. Wood is organic matter and because the wood lignin is also very hydrophobic, the wood is an attractive sorbent for dissolved PAH and other hydrophobic compounds (Chubets et al., 2000; Salloum et al., 2002). Based on these considerations, placing wood in stormwater runoff is expected to enhance the contaminant removal efficiency of existing BMPs.

The goal of this study was to field test wood filters under pilot-scale conditions. The main objective was to evaluate the effectiveness of the wood filter for removing dissolved PAH from roadway runoff. Multiple filters of different sizes were investigated and the potential effects of heavy precipitation, pH, and temperature changes due to seasonal fluctuations were monitored. Potential spatial and temporal variations of PAH removal effectiveness in the filter matrix were also studied. Because of the large number of factors considered and their inherent variability no modeling of the results was attempted in this study.

2. Materials and methods

2.1. Site selection

The field site was located off Interstate 195, exit 3 (Gano Street), in Providence, RI. The catchment area was approximately 5.26 ha and the traffic count averages 150,000 vehicles per day (RI DOT, 1999). The retention system was built in 1999 and consisted of three ponds (from inflow to outflow): settling pond, constructed wetland, and micro-pool. The wood filters were installed in the concrete-lined micro-pool because it was the deepest of the three ponds (up to 0.8 m in the center, depending on flood stage), relatively narrow, and closest to the stormwater discharge point. The maximum storage capacity of this pond was 344 m³. Based on a rain intensity of 2.5 cm/h, the average runoff residence time was 16 min. Prior to the installation of the wood filters, the contaminant flux through this retention pond system was investigated (Boving, 2002).

2.2. Filter construction

The wood filters were constructed from Aspen wood shavings, i.e. string fibers 1 to 2 mm thick and at least 15 cm long. The wood (Curlex Brand®) was obtained from *American Excelior Company* and used as received. The wood was contained in bags ("modules") made from nylon netting. Each of these modules measured about 1.5 m by 1 m by 5 to 10 cm (L x H x W, Fig. 1) with a dry weight of 10 kg to 20 kg (wet weight: between 45 kg and 60 kg depending on density of packing). The filter modules were suspended from a 2.5 cm steel pipe across the pond and perpendicular to the flow direction. The coverage of the flow-through cross-section was identical for all tests and independent of the wood mass used for constructing the filter. The modules were anchored to the bottom of the pond. The wood filters were fully submerged during storms and partially submerged during dry periods. The water
level in the retention pond fluctuated by about ±0.5 m. During storms, the pond elevation was controlled by a concrete weir and an emergency overflow, while evaporation caused the water level to drop during dry periods. Water flowed through the pond only during storm and the emergency overflow stage was never reached during the study period.

Four wood filter tests (WFT) were conducted during the yearlong experiment (Spring 2003 through Winter 2004). The tests covered all four seasons and varying weather conditions, including moderate droughts, frost, snow, and thunderstorms. WFTs lasted from 6 to 64 days (Table 1). The first and shortest wood filter test (WFT1) served as a proof of concept study. The amount of wood used for constructing the filters was systematically increased from 36 kg (WFT1) to 115 kg (WFT4). The duration of each experiment was primarily controlled by the mass of wood used in the filter (i.e., filters with a greater mass remained in the pond longer) and by seasonal influence. Seasonal influences decreased the water level in the pond, exposing much of the filter during droughts or prevented sampling when the pond was frozen over. Freezing occurred during most of January and February 2004. Temperature and precipitation data were obtained from the nearby National Weather Service weather station at the T.F. Green Airport.

2.3. Analytical methods

All chemicals and supplies were purchased from Fisher Scientific Inc. or Aldrich Inc. if not indicated otherwise. Aqueous samples from about 1 m up- and down-gradient of the wood filter were collected on a regular basis and analyzed for ten PAH compounds. These ten PAH are naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene and benzo(a)pyrene and are referred to as ΣPAH in the following discussion. Sampling was more frequent during the first week of each test to capture possible

<table>
<thead>
<tr>
<th>Test</th>
<th>Wood mass (kg)</th>
<th>Date installed</th>
<th>Date removed</th>
<th>Test duration (days)</th>
<th>No. samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFT1</td>
<td>36</td>
<td>03/28/03</td>
<td>04/02/03</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>WFT2</td>
<td>55</td>
<td>05/05/03</td>
<td>05/28/03</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>WFT3</td>
<td>90</td>
<td>07/22/03</td>
<td>09/23/03</td>
<td>64</td>
<td>16</td>
</tr>
<tr>
<td>WFT4</td>
<td>115</td>
<td>11/18/03</td>
<td>01/06/04</td>
<td>49</td>
<td>18</td>
</tr>
</tbody>
</table>
short-term variations. Later, an about once-per-week sampling frequency was adopted. Also, wood samples were collected from the filter matrix to determine the contaminant loading of the wood. Dissolved oxygen (DO), electrical conductivity (EC), pH, and temperature were measured regularly in the field.

Sample preparation and analysis were conducted immediately after returning from the field. The dissolved PAH were extracted using a liquid–liquid extraction method modified from MacKay and Gschwend (2001). “Dissolved” was operationally defined as the aqueous fraction that passes through a 0.7 μm Whatman 90 mm GF/F glass microfiber filter. After filtration, an internal standard (100 μg/L 2-fluorobiphenyl 96%) and 100 mL methylene chloride (HPLC grade) were added to 1 L of aqueous sample and mixed for 24 h. The methylene chloride was separated from the aqueous phase using a 1000 mL Squibb separatory funnel. This extraction step was repeated twice, but without adding additional internal standard. The 300 mL methylene chloride extract was distilled down to 10 mL, filtered through 2 g of activated silica gel (Selecto Scientific) in an Alltech Silica Extra Clean column (60 Å porosity). After purification with 5 mL of 8:1 hexane/methylene chloride and 15 mL of 3:4:1 hexane/methylene chloride, the hexane/methylene chloride mixture was concentrated (Kuderna Danish) to less than 10 mL for analysis. A Shimadzu GC-17A FID gas chromatograph with flame ionization detector and a J&W Scientific DB-5MS glass capillary column (30 m, 0.32 mm id, 0.25 μm film thickness) was used for the analysis. The column temperature program was modified after EPA method 610. An external PAH standard was used to calibrate the GC-FID (EPA method 610 PAH mixture; Ultra Scientific Inc.). The external standard was also used to spike selected samples and compare them with unspiked runs of the same sample. Although this approach does not completely substitute for a confirmatory GC-MS analysis (or similar), it at least provided high confidence in the correct identification of individual PAH compounds. The detection limit of the GC-FID was 1 μg/L. To ensure quality assurance, control blanks, sample duplicates, and standard repeats were analyzed frequently.

The PAH from the wood fibers were extracted after EPA method 3540 (Soxhlet extraction). Any visible debris was removed from the wood samples. The wet wood was cut into millimeter size fractions, with 15 to 20 g (equating 3 to 4 g dry) placed into a 30 × 80 mm Whatman

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Average, minimum, and maximum values for pH, EC, DO, temperature, and precipitation observed during test period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WFT1</td>
</tr>
<tr>
<td>Average pH</td>
<td>8.0</td>
</tr>
<tr>
<td>Maximum pH</td>
<td>8.5</td>
</tr>
<tr>
<td>Minimum pH</td>
<td>7.4</td>
</tr>
<tr>
<td>Average EC (μS/cm)</td>
<td>na</td>
</tr>
<tr>
<td>Maximum EC</td>
<td>na</td>
</tr>
<tr>
<td>Minimum EC</td>
<td>na</td>
</tr>
<tr>
<td>Average DO (mg/L)</td>
<td>7.5</td>
</tr>
<tr>
<td>Maximum DO</td>
<td>8.4</td>
</tr>
<tr>
<td>Minimum DO</td>
<td>6.4</td>
</tr>
<tr>
<td>Average temperature (°C)</td>
<td>10.5</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>13.9</td>
</tr>
<tr>
<td>Minimum temperature</td>
<td>7.5</td>
</tr>
<tr>
<td>Precipitation total (mm)</td>
<td>54.6</td>
</tr>
<tr>
<td>Single largest rain event (mm)</td>
<td>40.4</td>
</tr>
</tbody>
</table>

na = not analyzed.
extraction thimble. The PAH were extracted with 300 mL methylene chloride for 24 h. The extract was concentrated to less than 5 mL (Kuderna Danish evaporative concentrator) and analyzed by the aforementioned gas chromatographic method.

<table>
<thead>
<tr>
<th></th>
<th>WFT1</th>
<th>WFT2</th>
<th>WFT3</th>
<th>WFT4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Naphthalene</strong> (128.16 g/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness</td>
<td>-22.5%</td>
<td>nd</td>
<td>9.7%</td>
<td>16.0%</td>
</tr>
<tr>
<td>Average concentration of up-gradient</td>
<td>1.9</td>
<td>nd</td>
<td>0.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Average concentration of down-gradient</td>
<td>2.3</td>
<td>nd</td>
<td>0.4</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Acenaphthylene</strong> (152.21 g/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness</td>
<td>-17.9%</td>
<td>42.3%</td>
<td>19.1%</td>
<td>35.7%</td>
</tr>
<tr>
<td>Average concentration of up-gradient</td>
<td>1.3</td>
<td>1.0</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Average concentration of down-gradient</td>
<td>1.6</td>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Acenaphthene</strong> (154.25 g/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness</td>
<td>21.7%</td>
<td>39.6%</td>
<td>14.9%</td>
<td>21.4%</td>
</tr>
<tr>
<td>Average concentration of up-gradient</td>
<td>1.1</td>
<td>1.5</td>
<td>0.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Average concentration of down-gradient</td>
<td>0.9</td>
<td>0.9</td>
<td>0.3</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Fluorene</strong> (166.23 g/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness</td>
<td>27.0%</td>
<td>-7.4%</td>
<td>34.4%</td>
<td>46.1%</td>
</tr>
<tr>
<td>Average concentration of up-gradient</td>
<td>3.7</td>
<td>2.9</td>
<td>2.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Average concentration of down-gradient</td>
<td>2.7</td>
<td>3.1</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Phenanthrene</strong> (178.24 g/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness</td>
<td>25.4%</td>
<td>44.7%</td>
<td>28.2%</td>
<td>38.9%</td>
</tr>
<tr>
<td>Average concentration of up-gradient</td>
<td>1.0</td>
<td>4.5</td>
<td>1.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Average concentration of down-gradient</td>
<td>0.7</td>
<td>2.5</td>
<td>1.1</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>Anthracene</strong> (178.24 g/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness</td>
<td>19.4%</td>
<td>47.8%</td>
<td>37.1%</td>
<td>17.1%</td>
</tr>
<tr>
<td>Average concentration of up-gradient</td>
<td>2.9</td>
<td>4.9</td>
<td>1.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Average concentration of down-gradient</td>
<td>2.3</td>
<td>2.6</td>
<td>1.2</td>
<td>2.8</td>
</tr>
<tr>
<td><strong>Fluoranthenne</strong> (202.26 g/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness</td>
<td>40.4%</td>
<td>3.9%</td>
<td>20.4%</td>
<td>47.4%</td>
</tr>
<tr>
<td>Average concentration of up-gradient</td>
<td>2.4</td>
<td>1.4</td>
<td>0.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Average concentration of down-gradient</td>
<td>1.5</td>
<td>1.3</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Pyrene</strong> (202.26 g/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness</td>
<td>34.1%</td>
<td>10.9%</td>
<td>45.1%</td>
<td>36.9%</td>
</tr>
<tr>
<td>Average concentration of up-gradient</td>
<td>4.3</td>
<td>1.8</td>
<td>1.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Average concentration of down-gradient</td>
<td>2.9</td>
<td>1.6</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Chrysene</strong> (228.30 g/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness</td>
<td>11.1%</td>
<td>25.8%</td>
<td>40.6%</td>
<td>66.5%</td>
</tr>
<tr>
<td>Average concentration of up-gradient</td>
<td>1.3</td>
<td>1.9</td>
<td>1.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Average concentration of down-gradient</td>
<td>1.3</td>
<td>1.4</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Benz(a)pyrene</strong> (252.32 g/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness</td>
<td>0.3%</td>
<td>2.4%</td>
<td>20.6%</td>
<td>19.8%</td>
</tr>
<tr>
<td>Average concentration of up-gradient</td>
<td>1.7</td>
<td>2.8</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Average concentration of down-gradient</td>
<td>1.7</td>
<td>2.7</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Total PAH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effectiveness</td>
<td>18.5%</td>
<td>26.3%</td>
<td>30.8%</td>
<td>35.6%</td>
</tr>
<tr>
<td>Average concentration of up-gradient</td>
<td>21.4</td>
<td>22.6</td>
<td>11.8</td>
<td>21.8</td>
</tr>
<tr>
<td>Average concentration of down-gradient</td>
<td>17.6</td>
<td>16.6</td>
<td>8.1</td>
<td>13.8</td>
</tr>
</tbody>
</table>

All concentrations are in μg/L. Number next to compound name is the PAH molecular weight (na = not analyzed; nd: not detected).
3. Results and discussion

3.1. Field parameters

Table 2 summarizes the average pH, EC, DO, temperature, and total amount of precipitation measured during each of the four tests. The average annual pH for storm water entering the detention pond system was 7.3, but fluctuated over the seasons. During the height of summer the pH was more acidic (6.3); while during the rest of the year the pH was close to or slightly higher than neutral (7.0 to 8.0). Similar seasonal changes were observed in electric conductivity. For instance, average EC was the highest during winter (164 μS/cm) because of the presence of de-icing salt. As expected, the highest temperatures and the lowest dissolved oxygen (DO) values were measured during the summer test WFT3. Although no significant impact of pH on the PAH attenuation in natural water was expected nor observed, there were significant temperature changes during the four field tests (amplitude: 3.2 °C to 25.3 °C). Generally, sorption is temperature dependent, i.e. it decreases with increasing temperature (Sleep and McClure, 2001). The results of this study, however, did not show significant changes in PAH removal with temperature. Any of such temperature effects, if existing, were probably masked by other factors such as variable contaminant influent concentrations or alterations of the wood filter surface.

3.2. PAH removal

Table 3 summarizes the average aqueous concentration in filter influent and effluent for each of the ten PAH compounds during tests WFT1 through WFT4. Actual ∑PAH influent and effluent concentrations together with the corresponding removal effectiveness are shown in Fig. 2A through D. Effectiveness is defined as the percentage difference between influent (up-gradient) and effluent (down-gradient) concentration relative to the influent concentration. Hence, a positive value indicates removal, while a negative value indicates release.

As summarized in Table 3, the average aqueous ∑PAH concentrations up-gradient from the filter ranged from 11.8 μg/L to 22.6 μg/L and from 8.1 μg/L to 17.6 μg/L down-gradient. Individual PAH concentrations (fluoranthene, pyrene, and phenanthrene) were similar to those found in average stormwater runoff (Pitt et al., 2004). ∑PAH concentrations were lowest during summer, i.e. at a time when algae bloom and lush vegetation began growing in the pond system. With more organic material for PAH to partition to, less PAH remained in solution, which is the working principle of constructed wetlands (US EPA, 2000). At the field site, runoff had to flow through a constructed wetland (Pond 2) first before entering the pond in which the wood filters were installed. Hence, the decrease in ∑PAH concentration during summer was attributed to partial removal by vegetation up-gradient from the wood filter. From Fig. 2A through D, the PAH removal effectiveness appeared to be independent of the absolute PAH influent concentrations. For instance, the filter effectiveness was not necessarily higher at lower PAH concentrations. Comparing filter influent and effluent concentrations, the ∑PAH removal effectiveness was as high as 66.5% (Chrysene; WFT4). On one occasion during test WFT2, a 26.3% increase in PAH concentration down-gradient from the filter was observed. With no unusual environmental factors (e.g. heavy rain) to consider, this unique increase was presumably a sampling error. Overall, the average filter effectiveness for ∑PAH ranged from 18.5% to 35.6% with the filter effectiveness systematically increasing with increasing wood mass (Fig. 3).

Assessment of the removal of individual PAH compounds per unit weight of wood revealed more effective removal of high molecular weight (HMW) PAH (e.g. pyrene) compared to low
molecular weight (LMW) PAH (e.g. naphthalene). The preferential removal of HMW PAH by the wood indicated – not unexpectedly – that partitioning of PAH to the wood must be a function of molecular weight. This is illustrated in Fig. 4, which shows an apparently linear relationship.
between PAH molecular weight and the mass concentration of PAH extracted from the wood filter at the end of test WFT2. Because molecular weight is closely related to the degree of hydrophobicity of a PAH, that is, high molecular weight PAH are typically more hydrophobic
(Schwarzenbach et al., 1993), the wood filter proved most effective for HMW PAH removal. This has advantageous implications for treating stormwater runoff, as the most toxic compounds (e.g. benzo(a)pyrene) are also those with high molecular weight.

Fig. 4. Total PAH uptake per gram of dry wood after test WFT2. PAH compounds are arranged by increasing molecular weight. Wood samples were collected from the center bottom section of the filter.
Table 4
Individual PAH concentrations per gram of dry wood during tests WFT2 through WFT4

<table>
<thead>
<tr>
<th>PAH compound</th>
<th>WFT2 (µg/g)</th>
<th>WFT3 (µg/g)</th>
<th>WFT4 (µg/g)</th>
<th>Average (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>1.03</td>
<td>1.42</td>
<td>1.05</td>
<td>1.17</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>0.95</td>
<td>1.96</td>
<td>1.24</td>
<td>1.38</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>1.48</td>
<td>2.08</td>
<td>0.74</td>
<td>1.43</td>
</tr>
<tr>
<td>Fluorene</td>
<td>2.10</td>
<td>3.27</td>
<td>1.62</td>
<td>2.33</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>3.03</td>
<td>7.83</td>
<td>8.86</td>
<td>6.57</td>
</tr>
<tr>
<td>Anthracene</td>
<td>4.88</td>
<td>6.92</td>
<td>3.19</td>
<td>5.00</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>6.27</td>
<td>3.73</td>
<td>6.11</td>
<td>5.37</td>
</tr>
<tr>
<td>Pyrene</td>
<td>5.71</td>
<td>3.94</td>
<td>6.63</td>
<td>5.63</td>
</tr>
<tr>
<td>Chrysene</td>
<td>13.03</td>
<td>5.86</td>
<td>5.99</td>
<td>7.99</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>10.27</td>
<td>4.72</td>
<td>5.13</td>
<td>6.71</td>
</tr>
<tr>
<td>LMW PAH total</td>
<td>5.56</td>
<td>8.74</td>
<td>4.66</td>
<td>6.32</td>
</tr>
<tr>
<td>HMW PAH total</td>
<td>43.19</td>
<td>33.00</td>
<td>35.02</td>
<td>37.07</td>
</tr>
<tr>
<td>Total PAH</td>
<td>48.75</td>
<td>41.73</td>
<td>39.67</td>
<td>43.39</td>
</tr>
</tbody>
</table>

All samples were collected from parts of the wood filter that were submerged at all times (center bottom). LMW: low molecular weight, HMW: high molecular weight.

The average contaminant uptake per gram of dry wood for all four tests combined was 5.4 µg/g pyrene, 5.0 µg/g anthracene, and 2.3 µg/g fluorene (Table 4). The PAH loadings on the filter measured during this pilot-scale filter tests were lower than values reported by Boving and Zhang (2004). In their column experiments, contaminant uptakes of 74 µg/g pyrene, 67 µg/g anthracene, and 25.5 µg/g fluorene were determined. However, Boving and Zhang (2004) conducted their experiments with single compound solutions in deionized water. Hence, the difference between the field and laboratory observations may be caused by competitive sorption of various organic and inorganic contaminants that are present in actual stormwater runoff. It is also possible that sediment deposition on the wood filter or wood decay decreased the filter removal effectiveness in the field. These aspects were not investigated in this study; further experiments are needed to evaluate their importance.

Besides alterations of the wood matrix, the discrepancy between laboratory and field results may be related to significant spatial variations in PAH uptake by the wood filter. For instance, as summarized in Table 5, the highest total PAH concentration per gram of wood (39.7 µg/g to 48.8 µg/g) was measured in samples from the continuously submerged center bottom part of the filter (Fig. 1). Less than half of these concentrations (12.8 µg/g to 21.7 µg/g) were detected in those parts of the filter that were submerged only during storm events. This result indicates that the filter effectiveness could have been about twice as high if the entire filter had remained in contact with the runoff all the times. Also, from Table 4 it is evident that the PAH mass removal

Table 5
Mass of PAH removed during tests WFT2 through WFT4 (mass loadings were not determined during WFT1)

<table>
<thead>
<tr>
<th>Test name</th>
<th>Center bottom (µg/g)</th>
<th>Center top (µg/g)</th>
<th>Left periphery (µg/g)</th>
<th>Right periphery (µg/g)</th>
<th>Average of entire filter (µg/g)</th>
<th>Total PAH removed (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFT2</td>
<td>48.8</td>
<td>16.2</td>
<td>16.3</td>
<td>20.2</td>
<td>25.4</td>
<td>1.4</td>
</tr>
<tr>
<td>WFT3</td>
<td>41.7</td>
<td>15.7</td>
<td>18.0</td>
<td>21.7</td>
<td>24.3</td>
<td>2.2</td>
</tr>
<tr>
<td>WFT4</td>
<td>39.7</td>
<td>12.8</td>
<td>NA</td>
<td>NA</td>
<td>26.2</td>
<td>3.0</td>
</tr>
</tbody>
</table>

na: not analyzed.
per gram of wood was not constant, i.e. actually decreased with increasing wood filter mass. Findings by Huang et al. (2006) indicate that the sorption of PAH by aspen wood is nonlinear, which could explain this observation. However, further studies are needed.

In total, 6.6 g of $\Sigma$PAH were removed by 260 kg of wood during tests WFT2 through WFT4 (no data were gathered during WFT1). Boving (2002) calculated that 118.8 g of dissolved $\Sigma$PAH flows through the retention pond system each year. Based on the removal efficiencies reported in Boving and Zhang (2004), it was estimated that about 1000 kg wood per year would be necessary to remove the entire PAH load from the stormwater. This pilot-scale field test showed that with the current filter design about 4680 kg/year of wood would be needed (4.7 times the estimated amount and 18 times the amount of wood used during this pilot-scale test). If, however, the required amount of wood was calculated based on the effectiveness of the continuously submerged part of the filter, about 2400 kg/year wood would have been sufficient.

Temporal changes in the filter effectiveness were monitored during test WFT4. For this, wood samples were collected from the center bottom throughout the test duration. As shown in Fig. 5, the amount of $\Sigma$PAH mass taken up by the wood increased sharply immediately after filter installation. Over the following 3 weeks, $\Sigma$PAH concentration increased steadily to 37.6 $\mu$g/g and then remained essentially unchanged. Between day 24 and 29, PAH were released from the wood. Afterwards and until the end of the test, $\Sigma$PAH concentration once again increased steadily. The drop in PAH loading was related to the precipitation history during the test (Fig. 5). While comparably small rain events (<15 mm) had no significant effect on the filter, a series of four exceptionally heavy rain storms that lasted from December 11 to 15, 2003 (74.6 mm of precipitation) coincided with a 50% drop in the amount of PAH bound to the wood filter (from 37.6 $\mu$g/g to 18.7 $\mu$g/g). Although the flow velocity was not measured during any storm event, observations in the field clearly point to much faster flow conditions during these strong storms. The response of the wood filter to these particularly strong storms indicated that at least some of the PAH bonded.

![Graph](image_url)

**Fig. 5.** Total PAH loading of the wood filter ($\mu$g/g) compared to the precipitation amounts received during test WFT4. Error bars represent the cumulative error associated with sample preparation and analysis.
weakly to the wood, leading to disassociation when flow through the filter was very high for an extended length of time. This remobilized fraction may represent PAH initially associated with colloidal matter deposited on the filter or biofilm that formed on the wood. Notably, the particles and PAH that washed off the filter during these storm events did not cause a spike in the dissolved PAH concentration down-gradient from the filter (Fig. 2D). In fact, up and down-gradient dissolved PAH concentrations were almost identical or even indicate some residual removal effectiveness of the filter. Hence, it appears that initially small particles formed larger aggregates while adhering to the wood filter. When these aggregates and the contaminants associated with them washed off the filter, they were large enough to be retained by the 0.7-μm filter used in sample preparation (i.e., became part of the suspended load). Independent of the cause, these observations indicate that the PAH removal may not entirely be attributed to the wood, but other processes (biofilm formation, deposition of fine sediment, formation of larger aggregates) may contribute to the filter performance under field conditions.

4. Conclusion

The assessment of the wood filter removal efficiency for dissolved PAH under field conditions was the principle research objective of the pilot-scale tests. The results demonstrate that the wood filter effectiveness did not change with seasons. Particularly, there was no evidence of increasing temperatures causing decreasing PAH uptake or vice versa. Other factors, such as pH or EC, did not significantly influence the filter effectiveness either. Further, the PAH removal effectiveness was independent of the absolute PAH influent concentrations. The average filter effectiveness for \( \Sigma \) PAH ranged from 18.5% to 35.6% and was as high as 66.5% (Table 3). The filter effectiveness systematically increased with increasing wood mass. Also, HMW PAH were more effectively removed from the stormwater than LMW PAH and an apparently linear relationship between PAH molecular weight and wood filter effectiveness exists. Because the most toxic PAH in stormwater are HMW PAH (e.g., benzo(a)pyrene), wood filter are therefore be especially well suited to address these contaminants.

The wood filter tests varied in length from 1 to 9 weeks. For all PAH compounds investigated, the lifetime (i.e., maximum capacity before filter becomes inefficient) could not be determined, i.e., was longer than the longest test (WFT3). Even longer tests are therefore needed to study if a significant decrease in wood filter contaminant removal takes place over time. The PAH loadings on the wood filter measured during these field tests were lower than laboratory values reported by Boving and Zhang (2004), i.e., with the current filter design about 4.7 times more wood would have been necessary than estimated to remove the entire annual PAH load. Besides the factors that are not investigated in this field study (e.g., alterations of the wood filter matrix), the discrepancy between laboratory and field results appeared related to significant spatial variations in PAH uptake by the wood filter. For instance, the filter effectiveness was about twice as high in parts of the filter that were always submerged compared to those parts that were under water only during storm events. Hence, a better gauge of the filter effectiveness is the amount of PAH removed by the wood from the always submerged center bottom part of the filter. Based on this measure, simple filter design changes, such as ensuring that the entire wood filter is submerged at all time, could reduce by 50% the amount of wood needed for treating the PAH load.

Another challenge is the minimization of contaminant remobilization during strong storms. It was observed that the PAH load of the wood filter decreased when flow through the pond was very high. The underlying cause(s) for the contaminant remobilization was not further investigated, but is most likely related to erosion of particles that accumulated on the wood
This possibility points to other non-sorptive, rather mechanical filter mechanisms that are independent of the wood, but contribute to the wood filter performance. Hence, future experiments must examine how the contaminants are bound to the wood filter, including studying the role of particle-bound contaminant deposition onto the wood filter and effects of biofilm formation on the wood matrix. In addition, the filter design has to be optimized in order to minimize contaminant remobilization under high flow conditions, for instance by allowing bypass flow during intense storms or adding measures to reduce the flow velocity. These design changes are likely to significantly enhance the filter effectiveness and may alleviate the contaminant disassociation problem.

Acknowledgements

This study was made possible by a grant from the University of Rhode Island Transportation Center. Additional support was provided by the Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET). Mr. Tony Johnson, American Excelsior Inc., generously supported wood cuttings and technical advice.

References


RI DOT Rhode Island Department of Transportation, 1999. Traffic flow map: average daily traffic. State Highway Map Prepared by RIDOT and USDOT/FHWA.


Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons

John P. Incardona,* Tracy K. Collier, and Nathaniel L. Scholz

Eco toxico logic and Environmental Fish Health Program, Environmental Conservation Division, Northwest Fisheries Science Center, National Oceanic and Atmospheric Administration, Seattle, WA 98112, USA

Received 19 August 2003; accepted 6 November 2003

Abstract

Fish embryos exposed to complex mixtures of polycyclic aromatic hydrocarbons (PAHs) from petrogenic sources show a characteristic suite of abnormalities, including cardiac dysfunction, edema, spinal curvature, and reduction in the size of the jaw and other craniofacial structures. To elucidate the toxic mechanisms underlying these different defects, we exposed zebrafish (Danio rerio) embryos to seven non-alkylated PAHs, including five two- to four-ring compounds that are abundant in crude oil and two compounds less abundant in oil but informative for structure–activity relationships. We also analyzed two PAH mixtures that approximate the composition of crude oil at different stages of weathering. Exposure to the three-ring PAH dibenzothiophene and phenanthrene alone was sufficient to induce the characteristic suite of defects, as was genetic ablation of cardiac function using a cardiac troponin T antisense morpholino oligonucleotide. The primary etiology of defects induced by dibenzothiophene or phenanthrene appears to be direct effects on cardiac conduction, which have secondary consequences for late stages of cardiac morphogenesis, kidney development, neural tube structure, and formation of the craniofacial skeleton. The relative toxicity of the different mixtures was directly proportional to the amount of phenanthrene, or the dibenzothiophene-phenanthrene total in the mixture. Pyrene, a four-ring PAH, induced a different syndrome of anemia, peripheral vascular defects, and neuronal cell death, similar to the effects previously described for potent aryl hydrocarbon receptor ligands. Therefore, different PAH compounds have distinct and specific effects on fish at early life history stages.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Petroleum; Oil spill; Fish development; Heart morphogenesis; silent heart gene; CYP1A

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are pervasive contaminants in rivers, lakes, and nearshore marine habitats. This is particularly true of urbanized areas, where anthropogenic inputs are derived predominantly from the consumption of petroleum. The largest fraction enters marine waters as land-based runoff or atmospheric deposition (National Research Council, 2003). While PAH levels in urban watersheds declined during the 1970s and 1980s due to reductions in coal burning and industrial emissions (Heit et al., 1988; Hites et al., 1980), the last decade has produced new increases in aquatic PAH accumulation due to automobile use associated with urban sprawl (Van Metre et al., 2000) or diesel combustion by heavy vehicles (Lima et al., 2002). Moreover, new evidence suggests that petroleum hydrocarbons from oil spills can persist in nearshore sediments for decades or longer (Reddy et al., 2002). Residential populations are increasing in coastal areas of the US (Barrett et al., 2000) and, as a result, an increase in nearshore contamination by PAHs is expected. This issue is particularly important for regions such as the Pacific Northwest, where major recovery efforts are currently underway for several threatened and endangered fish species.

While there is an extensive literature describing the effects of PAHs on adult or juvenile animals, few studies have addressed the effects of PAHs on embryonic and early larval development in fish. These early life history stages may be particularly susceptible to PAH exposure,
especially for species that spawn or rear near human settlements. The 1989 Exxon Valdez oil spill contaminat-
ed nearshore and intertidal spawning grounds for Pacific herring (Clupea pallasi) and pink salmon (Oncorhynchus gorbuscha) with Alaska North Slope crude oil, prompting a series of field and laboratory studies that examined the effects of PAHs on herring and salmon development (Brown et al., 1996; Carls et al., 1999; Heintz et al., 1999; Hose et al., 1996; Kocan et al., 1996; Marty et al., 1997; McGurk and Brown, 1996; Middaugh et al., 1998; Norcross et al., 1996; Spies et al., 1996). Together with recent studies on other species (Couillard, 2002: Middaugh et al., 1996, 2002; Pollino and Holdway, 2002) and an analysis of the effects of PAH-rich creosote on herring development (Vines et al., 2000), these collective studies documented what appears to be a common suite of developmental defects in teleost embryos exposed to petroleum-derived PAH mixtures. Gross malformations resulting from PAH exposure included pericardial and yolk sac edema, jaw reductions, and presumptive skeletal defects described as lordosis or scoliosis (dorsal curvature). Reductions in larval heart rate (bradycardia) and cardiac arrhythmia were also observed. Increased weath-
ing of crude oil, which shifts the composition from predominantly two-ring (naphthalenes) to three-ring PAHs (e.g., phenanthrenes), resulted in a greater toxic potency and a higher frequency of malformations (Carls et al., 1999; Heintz et al., 1999). Significant sublethal effects were also observed in the absence of malformations. For example, pink salmon that were exposed to weathered crude oil as embryos and then released as smolts returned from the sea as adults in significantly fewer numbers (Heintz et al., 2000). Despite these careful analyses and the consistent findings across species, the precise mecha-
nisms leading to PAH-associated malformations and sublethal effects are unknown. Moreover, all of the previous studies used complex mixtures of PAHs, and it is unclear from the results whether individual components act through distinct mechanisms or share a common mode of action.

To address these issues, we evaluated the effects of model PAH compounds on the embryonic and early larval development of the zebrafish Danio rerio. In zebrafish, hundreds of genes involved in the formation of virtually every organ system have been identified by large-scale sustripenesis screening (Haffler et al., 1996). Consequently, the phenotypes resulting from loss of gene function through mutation can be compared to malformations resulting from embryonic exposure to contaminants. This "chemical genetic" approach has been used recently to identify specific mechanisms of developmental toxicity (Peterson et al., 2000, 2001). Moreover, the bioaccumulation of PAHs by zebrafish embryos and larvae is similar to that for temperate marine species (Petersen and Kristensen, 1998). In the present study, we analyze the effects of non-alkylated PAHs containing 2–4 rings. Our primary objectives were to (1) determine if individual PAHs representing the most abundant homologous series in petroleum were capable of inducing developmental defects in laboratory zebrafish like those described for embryos of wild fish species exposed to petrogenic PAH mixtures, and (2) gain insight into the mechanism(s) underlying any PAH-induced developmental defects.

Methods

Chemicals. Naphthalene (purity >99%), fluorene (99%), dibenzoanthracene (>99%), phenanthrene (>99.5%), anthracene (>99%), pyrene (>99%), and chrysene (98%) were obtained from Sigma-Aldrich, St. Louis, MO. Stock PAH solutions were made in dimethyl sulfoxide (tissue culture grade, Sigma) at 10 mg/ml, except anthracene (5 mg/ml) and chrysene (1 mg/ml).

Zebrafish exposures. A zebrafish breeding colony (wild-type AB strain) was maintained using routine procedures (Westerfield, 2000). Maintenance of adult fish, collection of fertilized eggs, and PAH exposures were all performed in water adjusted to a conductivity of approximately 1.000 µS/cm, pH 7.5–8 with Instant Ocean salts ("system water"). Fertilized eggs were collected and washed with system water within 2 h of spawning and conventionally staged (Kimmel et al., 1995). In a series of experiments, exposure regimens were initiated at times ranging from 4 to 8 h post-fertilization (hpf). Exposures were carried out in 12-well plastic tissue culture plates with 5–10 embryos per well in 2.5 ml at 28.5 °C. Several experiments carried out in volumes up to 10 ml in 100 mm plastic dishes produced similar results (data not shown). Exposure water was exchanged at 18– to 24-h intervals. In general, the exposures utilized nominal concentrations of all PAHs at or above their solubility limits and actual aqueous concentrations were not measured. It was empirically determined (data not shown) that high nominal concentrations were required to maintain steady aqueous levels due to the small exposure volumes and high PAH-binding capacity of the tissue culture plastic.

Morpholino antisense "knockdown". The morpholino oligonucleotide (5’-CATGTTTGCTCTGATCTGACGCA-3’; GeneTools, Philomath, OR) spans the cardiac troponin T translation start site and flanking 5’ sequence (Sehnert et al., 2002). Embryos were injected with approximately 4 ng oligonucleotide at the 1–4 cell stage using an MPPI-2 pressure microinjector (Applied Scientific Instrumentation, Eugene, OR), and incubated in system water for examination of phenotype over the following 6 days.

Imaging of live embryos or larvae. Embryos generally were examined with a Nikon SMZ800 stereomicroscope and by differential interference contrast (DIC) microscopy.
using a Nikon E600 compound microscope. Before hatching stages embryos were analyzed without anesthesia and were manually dechorionated for DIC microscopy. For analysis of post-hatching stages, larvae were anesthetized with MS-222 (Sigma) and were mounted in 3% methyl cellulose (Sigma) in system water for DIC microscopy. Digital still images were acquired from both microscopes with a Spot RT camera and Spot 3.2.6 software (Diagnostic Instruments, Inc., Sterling Heights, MI), and video analysis was performed with a Fire i400 digital camera (Unibrain, San Ramon, CA) and BTV Pro Carbon 5.4 software. Heart rates were counted over 15- to 30-s intervals in unanesthetized animals viewed with the stereoscope after acclimation to room temperature for at least 10 min.

**Immunofluorescence and Alcian blue staining.** For analysis of Na⁺/K⁺ ATPase labeling in the pronephros with monoclonal antibody α6F hybridoma supernatant (Takeyasu et al., 1988) (Developmental Studies Hybridoma Bank, University of Iowa), 75–80 hpf embryos were fixed in methanol–DMSO and processed as described elsewhere (Dent et al., 1989; Drummond et al., 1998), except the fixed embryos were not treated with hydrogen peroxide. Cardiac morphogenesis was examined in embryos fixed in 4% phosphate-buffered paraformaldehyde with monoclonal antibodies MF20 against myosin heavy chain and atrium-specific S46 (Yelon et al., 1999). Embryos were permeabilized by washing in water followed by 1-h incubation in blocking solution (PBS, 0.2% Triton X-100, 1% DMSO, 5% normal goat serum). Embryos were incubated overnight at 4 °C with agitation in α6F or MF20 and S46 hybridoma supernatants diluted 1:10 in blocking solution. After three 1-h washes in PBS + 0.2% Triton X-100, embryos were incubated several hours at room temperature in secondary antibodies diluted 1:1000 in blocking solution. Secondary antibodies (Molecular Probes, Eugene, OR) were AlexaFluor488-conjugated goat anti-mouse IgG (α6F) or goat-anti-mouse IgG1, (S46), and AlexaFluor568-conjugated goat anti-mouse IgG2b (MF20). After three 1-h washes in PBS + 0.2% Triton X-100, embryos were mounted in 50% glycerol in PBS and imaged with a Nikon E600 compound microscope and Spot RT camera. Alcian blue staining of the cartilaginous skeleton was performed as described (Kimmel et al., 1998) on 5–6 dpf larvae. Head skeletons were dissected after treatment with trypsin and flat-mounted for DIC microscopy.

**Results**

**Differential toxicity of PAH compounds during embryonic and early larval development**

We tested the effects of seven non-alkylated PAH compounds containing 2–4 rings (Fig. 1 and Table 1) on zebrafish development. To obtain data potentially comparable to those from studies prompted by the Exxon Valdez spill, we focused primarily on PAHs representing the homologous series most abundant in weathered Alaska North Slope crude oil (naphthalene, fluorene, dibenzothiophene, phenanthrene, and chrysenene). We also included anthracene and pyrene to determine if similar defects could also be induced in fish embryos by these PAHs that are far less abundant in weathered Alaska crude oil and have identical ring numbers but different structure as phenanthrene and chrysenene, respectively. Using static renewal exposure conditions, zebrafish embryos were incubated in water containing PAH compounds or vehicle (≤0.1% DMSO) beginning at 4–8 h post-fertilization (hpf), with a final replacement of exposure water at 72–78 hpf. To allow for high-throughput screening using zebrafish embryos reared in very small volumes, we used nominal exposure concentrations that were higher than the levels reported in the natural environment and generally well above their solubility limits. Although not measured, the actual PAH aqueous concentrations were probably much lower, in part because of the high affinity of plastic for PAHs. Representative examples of hatching-stage larvae at 4 days post-fertilization (dpf) are shown in Fig. 2. Larvae treated throughout embryogenesis with naphthalene (Fig. 2B), anthracene (Fig. 2F), or chrysenene (Fig. 2H) had grossly normal anatomic features. In contrast, larvae treated with fluorene (Fig. 2C), dibenzothiophene (Fig. 2D), or phenanthrene (Fig. 2E) displayed dorsal curvature of the trunk and tail and significant growth reduction, particularly of the head. In addition, dibenzothiophene- and phenanthrene-treated...
embryos showed severe pericardial and yolk-sac edema, while fluorene treatment resulted in mild pericardial edema. Treatment with pyrene (Fig. 2G) resulted in mild pericardial edema and a less-pronounced dorsal curvature, and other distinct defects described in more detail below. Larvae treated with 0.2% DMSO (Fig. 2A), the highest level of solvent carrier achieved in the PAH exposures, were indistinguishable from larvae grown in system water only (data not shown). No lethality was observed with any PAH treatment by 96 hpf, despite the severe edema induced by some compounds. After placement in clean water and continued rearing to 6 or 7 dpf, 100% mortality was observed for fish treated with dibenzothiophene, phenanthrene, or pyrene, but not the other PAHs. Despite grossly normal development in the presence of naphthalene, anthracene, or chrysene, all PAH-exposed embryos showed delayed or failed inflation of the swim bladder.

<table>
<thead>
<tr>
<th>PAH</th>
<th>MW</th>
<th>Water solubility (μM)</th>
<th>Mean log K&lt;sub&gt;ow&lt;/sub&gt;*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>128.2</td>
<td>247</td>
<td>3.34</td>
</tr>
<tr>
<td>Fluorene</td>
<td>166.2</td>
<td>12</td>
<td>4.22</td>
</tr>
<tr>
<td>Dibenzothiophene</td>
<td>184.3</td>
<td>7.9</td>
<td>4.44</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>178.2</td>
<td>7.2</td>
<td>4.53</td>
</tr>
<tr>
<td>Anthracene</td>
<td>178.2</td>
<td>0.4</td>
<td>4.53</td>
</tr>
<tr>
<td>Pyrene</td>
<td>202.2</td>
<td>0.67</td>
<td>5.07</td>
</tr>
<tr>
<td>Chrysene</td>
<td>228.3</td>
<td>0.009</td>
<td>5.77</td>
</tr>
</tbody>
</table>


Fig. 2. Effects of individual non-alkylated PAHs on zebrafish development. Shown are 4 dpf larvae after exposure to (A) 0.2% DMSO, (B) 78 μM naphthalene, (C) 60 μM fluorene, (D) 34 μM dibenzothiophene, (E) 56 μM phenanthrene, (F) 56 μM anthracene, (G) 5 μM pyrene, and (H) 8.8 μM chrysene. Swim bladder (sb) is indicated in A. Scale bar is 0.5 mm. Larvae shown are representative of at least three replicate experiments and approximately 25–100 treated embryos.
Three-ring PAH compounds selectively disrupt embryonic cardiac function

In embryos treated with the three-ring PAHs dibenzothiophene and phenanthrene, the earliest observed defect was loss or reduction in circulation at approximately 36 hpf (see below). We therefore compared PAH treatment to genetic disruption of cardiac function to determine whether cardiac dysfunction and loss of circulation were responsible for the suite of observed developmental abnormalities. The zebrafish mutant silent heart (sih) fails to develop a heartbeat due to severely reduced expression of cardiac troponin T, an essential component of the sarcomere in cardiomyocytes (Sehnert et al., 2002). Targeted "knockdown" of cardiac troponin T expression by injection of sih antisense morpholino faithfully reproduces the sih phenotype (Sehnert et al., 2002) and resulted in a suite of defects nearly identical to those induced by three-ring PAHs (Fig. 3). At 48 hpf, embryos treated with 56 μM phenanthrene or injected with sih morpholino exhibited mild pericardial edema (not shown) and by 3 dpf began to show dorsal curvature of the body axis (Figs. 3A and B). In both cases, dorsal curvature was more severe by 4 dpf as edema accumulated (Figs. 3C and D). Notably, eye and jaw growths were similarly reduced by phenanthrene treatment or sih morpholino injection (Figs. 3E and F). This indicates that cardiac dysfunction alone is sufficient to cause the predominant developmental defects induced by three-ring PAHs.

A closer examination of the timing of phenanthrene- or dibenzothiophene-induced defects indicated that these compounds affect cardiac function and not primary cardiac morphogenesis. The onset of the heartbeat occurred normally in PAH-treated embryos at about 24 hpf (16–20 h of exposure; data not shown), but several hours thereafter embryos displayed bradycardia and arrhythmias characteristic of atrioventricular (AV) conduction block (see digital movies in Supplemental Material for the Web). Phenanthrene (Fig. 4B) or dibenzothiophene (data not shown) produced dose-dependent reductions in heart rate with AV conduction block occurring at the highest doses, which, in turn, was associated with gross morphological abnormalities at later developmental stages. At intermediate doses by 30–35 hpf (22–28 h of PAH exposure), both phenanthrene

![Image](image_url)

Fig. 3. Malformations induced by three-ring PAHs are indistinguishable from those associated with specific genetic disruption of heart development by sih antisense morpholino injection. Phenanthrene-treated (A, C, and E) and sih morpholino-injected (B, D, and F) are shown at the same stages of development, 3 dpf (A and B) and 4 dpf (C and D). (E and F) Higher magnifications of 4 dpf larvae. Scale bars are 0.2 mm.
Fig. 4. Three-ring PAHs induce bradycardia and atrioventricular conduction block. Heart rates were counted without anesthesia in clutches of five larvae at 3 dpf after exposure to the indicated concentrations of PAHs. Atrial rates are indicated by circles and ventricular rates by triangles. Non-overlap of circles and triangles indicates an animal with 2:1 conduction block, or complete block (triangle with zero rate). Dose responses shown for (A) fluorene, (B) phenanthrene, (C) less-weathered oil mixture (LWO), and (D) more-weathered oil mixture (MWO). Pie chart insets indicate relative proportions of naphthalene (NPH), fluorene (FLU), dibenzothiophene (DBT), and phenanthrene (PHN) in the mixtures.

(28 μM) and dibenzothiophene (27 μM) induced a 2:1 AV conduction block, where the atrium beats twice for every ventricular contraction (Web Movies 1 and 2), although blood circulation was maintained. At higher doses, complete AV conduction block occurred, resulting in a more rapidly contracting atrium, a silent ventricle (Web Movie 3), and circulatory failure. These effects were reversible; heart rate began to increase within several hours of transition to clean water. The AV conduction block also resolved in a few hours to overnight depending on the initial severity of the block (Web Movies 4–6). However, for embryos that were continuously exposed to doses ≥50 μM, cardiac activity ceased by 3 dpf. This resulted in the characteristic suite of morphologic abnormalities. In contrast, 60 μM fluorene did not induce complete AV block, although exposed embryos showed significant bradycardia (Fig. 4A), occasional 2:1 AV block, and a slowing of circulation (data not shown). Naphthalene induced a mild bradycardia (5–6% reduction) at 39 or 78 μM that was not dose-dependent, and anthracene and chrysene had no significant effect on heart rate (Table 2).

To determine whether PAH exposure disrupted the developmental patterning of the heart, we examined cardiac morphology with the chamber-specific antibodies MF20 and S46, which recognize myosin heavy chain in the ventricle and atrium, respectively (Yelon et al., 1999). The teleost heart begins as a linear tube that later loops at the atrioventricular boundary (between 24 and 48 hpf in zebrafish) to bring the ventricle to the right side of the atrium. As indicated by MF20/S46 immunofluorescence (Fig. 5) and
Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>183 ± 2</td>
</tr>
<tr>
<td>Naphthalene, 39 μM</td>
<td>173.6 ± 4.5 (P = 0.056)</td>
</tr>
<tr>
<td>Naphthalene, 78 μM</td>
<td>175.2 ± 2.7 (P &lt; 0.05)</td>
</tr>
<tr>
<td>DMSO</td>
<td>165 ± 3</td>
</tr>
<tr>
<td>Chrysene, 22 μM</td>
<td>178 ± 7 (P = 0.076)</td>
</tr>
<tr>
<td>DMSO</td>
<td>201 ± 6</td>
</tr>
<tr>
<td>Anthracene, 56 μM</td>
<td>199 ± 4</td>
</tr>
</tbody>
</table>

direct light microscopic observations (not shown), formation of the cardiac chambers and initial looping of the heart occurred normally in phenanthrene- or dibenzothiophene-treated embryos. However, both cardiac chambers were dilated and had thinner walls in treated embryos (Fig. 5B). This was typically followed by a collapse and stretching of the chambers between their connections to either side of the distended pericardium (Fig. 5C). Collapse and generation of this string-like appearance of the heart is indicative of a failure to complete cardiac looping (Garrity et al., 2002). Severe AV conduction block induced by PAHs, even when reversed and circulation restored, had consequences for chamber size and later stages of cardiac looping (see Web Movie 6). These data indicate that three-ring PAHs, in particular dibenzothiophene and phenanthrene, can disrupt late stages of cardiac morphogenesis via persistent inhibition of cardiac conduction.

Narcosis is often considered a major toxic effect of PAH compounds, and narcotic compounds can affect cardiac function, although typically at much higher doses than those that produce anesthesia. To determine if the observed effects on cardiac function could be attributed to non-specific membrane effects on excitable cells, the basis for the nonpolar narcosis model (van Wezel and Oppenhuizen, 1995), we assessed mechanosensory-evoked reflexes in PAH-treated embryos. One of the earliest behaviors that develop in zebrafish is the touch response, which is a precursor to the adult escape reflex. The touch response reflects the integration of mechanosensory input from Rohom–Beard neurons in the spinal cord and motor output via the hindbrain (Granato et al., 1996; Ribera and Nusslein-Volhard, 1998; Saint-Amant and Drapeau, 1998). In fish this response is abolished by general anesthetics such as MS-222. Qualitative analysis showed the touch responses of PAH-treated embryos were largely intact (Web Movies 7 and 8). Therefore, it does not appear that PAHs are generally narcotic in early zebrafish larvae, and there is no correlation between the effects of different PAHs on cardiac conduction and sensorimotor function.

The toxicity of PAH mixtures depends on the percentage of three-ring compounds, particularly phenanthrene

We tested the effects of two PAH mixtures prepared to mimic the composition of crude oil observed at two stages of weathering which represented conditions in Prince Wil-

Fig. 5. Cardiac morphogenesis in PAH-treated embryos assessed with chamber-specific antibodies. Embryos treated with 0.2% DMSO (A), 56 μM phenanthrene (B), or 54 μM dibenzothiophene (C) were fixed at 52 hpf (44 h of exposure) and immunofluorescence performed with monoclonal antibodies S46 and ME20 to visualize the atrium (a, green) and ventricle (v, red), respectively. Lateral views are shown with anterior to the left. Scale bar is 50 μm.
respectively. Although alkylated homologues were more abundant than non-alkylated parent compounds, we tested a LWO-like mixture consisting of 44.1% naphthalene, 15.7% fluorene, 13.2% dibenzothiophene, 24.2% phenanthrene, and 0.9% chrysene; and a MWO-like mixture consisting of 13.0% naphthalene, 17.7% fluorene, 12.8% dibenzothiophene, 46.4% phenanthrene, and 4.4% chrysene. Note that the major difference between LWO and MWO is the relative proportion of naphthalene and phenanthrene. We also tested mixtures without chrysene to determine if the relatively small amount of this compound made a measurable contribution to the toxicity of the mixtures.

Using the same exposure protocol as above, we found that the LWO and MWO mixtures differed significantly in the ability to induce the malformation syndrome in a manner representative of the component three-ring PAHs. Both mixtures induced bradycardia and AV conduction blocks (Figs. 4C and D). However, at 64.5 μM, the LWO mixture induced only the partial AV block giving rise to the 2:1 rhythm and not circulatory failure (Fig. 4C), and was associated with only mild pericardial edema (data not shown) that readily resolved after transfer to clean water at 4 dpf (see Fig. 8A). In contrast, at 54 μM, the MWO mixture induced complete AV block (Fig. 4D) and the same cardiac degeneration and sequelae as observed with phenanthrene and dibenzothiophene alone (see Fig. 8B). Thus, the two PAH mixtures have markedly different effects on cardiac function at nearly the same total PAH concentration, consistent with our findings on individual PAHs and indicating that the relative amounts of specific PAHs are more important. Because the fluorene and dibenzothiophene concentrations are similar in both LWO and MWO mixtures, the toxicity of the MWO mixture can be attributed to the increase in phenanthrene (or the three-ring PAH total). Omission of chrysene in the mixtures had no apparent effect. Similar to what we observed for active three-ring compounds alone, larvae recovered cardiac function after transfer to clean water before obvious cardiac degeneration.

Three-ring PAH exposure has indirect effects on other tissues (kidney, neural tube, head skeleton) that are secondary to cardiovascular dysfunction

Because freshwater fish are hyperosmotic relative to their surroundings and must produce copious dilute urine, kidney dysfunction can lead to severe edema. The pronephric kidney of teleosts (Figs. 6A and B) consists of paired bilateral pronephric ducts joined to convoluted pronephric tubules that meet anteriorly at a midline glomerulus, which is derived by midline migration and fusion of bilateral pronephric primordia that coalesce around an ingrowing capillary. Completion of glomerular assembly in zebrafish occurs between 36 and 55 hpf and depends on hemodynamic forces provided by the early embryonic circulation (Drummond et al., 1998; Serluca et al., 2002). Assembly of the glomerulus fails in zebrafish mutants with severe cardiac dysfunction such as slik (Serluca et al., 2002). Therefore, for zebrafish embryos treated with concentrations of dibenzothiophene or phenanthrene sufficient to block cardiac func-
tion completely, the severe yolk sac edema observed by 4 dpf is likely to reflect failure of kidney morphogenesis. Embryos treated with levels of PAH sufficient to cause circulatory failure by 36 hpf showed a characteristic failure of glomerular assembly, as indicated by the appearance of bilateral pronephric cysts (data not shown) and straightening of the pronephric tubule with thinning of the epithelium (Fig. 6C). PAH-treated embryos with very weak circulation displayed marked but less severe defects in the pronephric tubular epithelium (Fig. 6D). Fluorene-treated embryos with bradycardia and mild pericardial edema did not show defects in the pronephric tubule (Fig. 6E).

Light microscopic examination of the trunk of PAH-treated (Fig. 7) and sih morpholino-injected embryos (data not shown) indicated that dorsal curvature of the body axis may be secondary to fluid imbalance in the neural tube. The lumen or central canal of the neural tube is normally only faintly discernible by DIC microscopy (arrowheads in Fig. 7A). In contrast, the central canal appeared more distinct and increasingly dilated in fish with more severe dorsal curvature (Figs. 7B and C). Dorsal curvature and distension of the central canal were observed in embryos that had a reduction of circulation that was sufficient to cause pericardial but not yolk sac edema (e.g., fluorene-treated; data not shown), suggesting that fluid balance in the neural tube is more sensitive to circulatory function than kidney morphogenesis. Examination of the skeleton in affected 6-day-old larvae by calcine staining confirmed that the dorsal curvature did not result from a primary defect in skeletogenesis (data not shown). If not too severe, the dorsal curvature was reversible with PAH deparmination (see Figs. 8B and C), consistent with a functional rather than structural etiology.

Reductions in jaw size have been observed previously in larval fish exposed to PAHs. We analyzed head skeletons in larvae at 6 dpf after treatment up to 4 dpf with individual PAHs (data not shown) or the LWO–MWO mixtures (Fig. 8). Embryos treated with the MWO mixture to 4 dpf had moderate to severe pericardial edema (Fig. 8B), but recovered after transfer to clean water (Fig. 8C), with the exception of larvae that had irreversible cardiac degeneration (Fig. 8C, arrowhead). Although larvae that recovered appeared grossly normal, skeletal elements of the head were smaller (Figs. 8F and G). The most severe effects on the head skeleton were observed in larvae with no cardiac function and severe edema (Fig. 8H). Similar results were observed in sih morpholino-injected embryos reared to 5 dpf (data not shown), indicating that edema, absent circulation, or both affect the growth of skeletal precursors in the jaws. Upon higher magnification (data not shown), the size of individual chondrocytes appeared reduced rather than the total number of cells contributing to individual skeletal elements. Moreover, the overall size of the head skeleton was reduced disproportionately to body length (data not shown). Larvae exposed to LWO exhibited a moderate but transient bradycardia and mild pericardial edema, and had slightly smaller head skeletons (Fig. 8E). Overall, these data suggest that reduction of head skeletal elements is correlated with the degree of edema accumulation secondary to even transient cardiac dysfunction.

Pyrene does not affect cardiac conduction but induces anemia, peripheral vascular defects, and neuronal cell death

Embryos exposed to pyrene showed less severe pericardial edema and slight bradycardia by 80 hpf (data not shown). By 98 hpf slight dorsal curvature was observed (Fig. 2G). However, this appeared to reflect a different underlying pathophysiology. Despite a relatively unaffected heart rate and rhythm, pyrene-treated embryos at this stage...
Fig. 8. Reduction of craniofacial skeletal elements correlates with the degree of pericardial edema. Larvae are shown at 4 dpf after treatment with 64 μM LWO mixture (A) and 43 μM MWO mixture (B), and at 6 dpf after 48 h of MWO mixture depuration (C). The four larvae in B are a subset of the five shown in C. At 6 dpf, cartilaginous skeletal precursors were stained with Alcian blue as described in Methods, and the head skeletons were dissected for flat mounting and microscopy. (D) Vehicle-treated control larva, (E) representative larva treated with LWO-mixture from (A), and (F–H) representative larvae treated with MWO-mixture from group in C. Arrow in C indicates larva corresponding to the head skeleton in H. Dashed lines in D–H indicate the anterior–posterior dimensions of the control skeleton.

showed severely reduced peripheral blood circulation in the head (Web Movies 9 and 10) and trunk (Web Movies 11 and 12). Moreover, a significant anemia was apparent (Web Movies 13 and 14), with markedly reduced numbers of circulating erythrocytes. Examination of affected embryos by DIC microscopy showed widespread cell death in the brain and trunk regions of the neural tube, indicated by the granular appearance of neural tissue (Fig. 7D and data not shown). We did not determine if cell death was necrotic or apoptotic, but hemorrhaging was not observed. It is unlikely that cell death in the neural tube is secondary to loss of peripheral circulation because this was not observed in embryos with complete circulatory failure at the same stage due to three-ring PAH treatment (Figs. 7B and C) or sib morpholino injection.

Discussion

In the context of aquatic toxicology, PAHs are generally thought to act as nonspecific or "baseline" toxicants through the nonpolar narcosis mode of action, or through activation of the aryl hydrocarbon receptor (AhR) pathway leading to cytochrome P450 1A (CYP1A) induction. In
addition, a wealth of studies have documented carcinogenic and immunotoxic effects of PAHs, though the former is usually associated with high molecular weight (≥5 rings) series (reviewed by Payne et al., 2003). In zebrafish embryos, we observed two distinct types of developmental toxicity for PAHs containing 2–4 rings: (1) cardiac dysfunction and its sequelae associated with some three-ring compounds, and (2) peripheral vascular defects, anemia, and neuronal cell death associated with pyrene, a four-ring compound. Edema and cardiovascular defects are common in fish embryos treated with either PAHs or AhR ligands such as dioxins, leading to a general view that these two classes of compounds act on fish early life history stages by a common pathway. Our findings and a series of studies describing the effects of dioxins on zebrafish embryos indicate that different PAH subclasses act through distinct toxic mechanisms, some novel, in embryonic and early larval stages of teleosts.

Remarkably, dibenzothiophene and phenanthrene are each individually capable of inducing a suite of defects that closely mirrors that observed in other teleost embryos exposed to complex PAH mixtures enriched in two- to four-ring compounds (e.g., Carls et al., 1999; Couillard, 2002; Heintz et al., 1999; Marty et al., 1997; Vines et al., 2000). However, it was previously unclear whether PAHs have direct and independent effects on different structures (e.g., heart, eye, jaw, body axis), or if the suite of PAH-induced developmental defects are functionally interrelated. Comparison to embryos with the silent heart phenotype indicates that most or all of the morphological defects induced by these three-ring PAHs are consequences of cardiac dysfunction. Because sin− cardiac troponin T is not expressed in tissues other than the heart, all aspects of the mutant phenotype are highly specific consequences of cardiac dysfunction.

Three-ring PAHs and cardiac function

Due to their lipophilicity and simple structure generally containing only carbon and hydrogen, low molecular weight PAHs (fewer than four rings) are widely considered to have low baseline toxicity attributed to nonpolar narcosis. The original principle underlying nonpolar narcosis (the functional equivalent of anesthesia) is nonspecific disruption of membrane integrity due to incorporation of hydrophobic organic compounds (for review, see Schultz, 1989; van Wezel and Opperhuizen, 1995). Nonpolar narcosis is thought to be achieved when membranes accumulate a certain mole fraction of contaminant, empirically determined in fish to reflect the total tissue levels or body burdens in the range of 2–8 mmol/kg wet weight (reviewed by Escher and Hermens, 2002; McCarty and Mackay, 1993). Studies in a variety of organisms have shown that PAH toxicity increases with increasing lipophilicity, as expressed by the log octanol–water partition coefficient (log Kow), up to the point where toxicity declines in association with low water solubility and bioavailability (e.g., Sverdrup et al., 2002). This relationship appears to be true for compounds with log Kow of 2–5. Basic quantitative structure–activity relationships predict that many of the PAH solutions used in our studies could theoretically produce tissue PAH levels that would be lethal by nonpolar narcosis (Verhaar et al., 1992). Thus it may appear that some of our results are consistent with the nonpolar narcosis model, with toxicity increasing from naphthalene (Kow 3.3) to phenanthrene (Kow 4.5), but decreasing for chrysene (Kow 5.8). However, several observations indicate that nonpolar narcosis is not the mode of toxic action at work for three-ring PAHs in fish embryos.

In principle, if narcotic or anesthetic compounds act by nonspecific disruption of membrane function, any excitable cell type should be affected. However, contemporary studies indicate that general anesthetics do not act nonspecifically on lipid bilayers, but selectively perturb the action of ligand-gated ion channels in the central nervous system, with minimal effects on cardiac function (reviewed by Franks and Lieb, 1994, 1998, 1999; Park, 2002; Patel, 2002). In contrast, our results indicate that three-ring PAHs are poor anesthetics that disrupt cardiac function selectively while having little effect on neuronal function. Doses of phenanthrene or dibenzothiophene that stop circulation do not immobilize or render embryos insensitive to mechanosenсор stimulation, the hallmarks of narcosis. In this light it is important to recognize that the nonpolar narcosis syndrome in fish was actually defined using the general anesthetic MS-222, a neuronal sodium channel blocker (McFarland, 1959; McKim et al., 1987). Instead, the effects of the active three-ring PAHs on cardiac function are remarkably similar to compounds that cause bradycardia due to inhibition of repolarizing potassium currents in the heart (Milan et al., 2003), suggesting the cardiac-specific KCNH2 potassium channel subunit as a possible molecular target (Keating and Sanguinetti, 2001; Mitcheson et al., 2000). Another possible target is the L-type calcium channel α1C (C-LTCC) subunit, which is required for normal atrioventricular conduction in zebrafish (Rottbauer et al., 2001).

The specific and local effects of PAHs on AV conduction also argue against the nonpolar narcosis model. In a study of the bioaccumulation and depuration of radiolabeled PAHs by zebrafish embryos and larvae, naphthalene and phenanthrene accumulated to similar levels after 24–30 h of exposure (7–8 mmol/kg dry weight for embryos, 4–5 mmol/kg for larvae; Petersen and Kristensen, 1998). This is also the time at which we observed significant effects on cardiac conduction. These authors also noted “bilateral bent chords” in phenanthrene-treated animals while naphthalene-treated embryos were unaffected. This indicates that two-ring PAHs do not affect the same target in the cardiac conduction system as do three-ring PAHs, despite reaching essentially identical tissue levels. Using the published correlation between log Kow and bioconcentration factors determined for zebrafish (Petersen and Kristensen, 1998), we estimate that fluorine should accumulate to levels 96%
of that for dibenzothiophene, a difference unlikely to account for the inability of fluorene to readily induce the AV conduction block. Whether the absence of an effect for anthracene reflects structural specificity cannot be determined because the predicted biocentration factor indicates that anthracene tissue levels would be 10- to 20-fold lower than phenanthrene. In zebrafish bioaccumulation studies, radiolabeled four-ring pyrene reached levels of approximately 3 mmol/kg dry weight in zebrafish larvae, respectively, and also resulted in dorsal curvature (Petersen and Kristensen, 1998). However, our studies indicate that this is not due to effects on cardiac conduction. Overall, our data support a direct and specific effect of a subset of three- or four-ring PAHs on cardiac conduction.

Pyrene toxicity and induction of the Ah receptor pathway

Pyrene induced a very different suite of defects at a different stage in development, including loss of peripheral circulation, anemia, delayed onset of pericardial edema, and cell death in the neural tube. These effects all manifested after 3 dpf. These distinct effects of pyrene are very similar to those described for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a potent ligand for the Ah receptor. A series of studies documenting the effects of TCDD in zebrafish described reductions in circulation in the head and trunk beginning around 3 dpf, with pericardial edema, failure of erythropoiesis, and apoptotic cell death in the neural tube (Belair et al., 2001; Dong et al., 2001, 2002; Henry et al., 1997; Teraoka et al., 2002). These effects were correlated with CYP1A induction as early as 36 hpf and were ameliorated to a similar degree by an Ah receptor antagonist or CYP1A inhibitor (Andreasen et al., 2002; Dong et al., 2001, 2002). Moreover, gene silencing with AhR or CYP1A antisense morpholinos demonstrated that TCDD-induced pericardial edema in zebrafish requires an active AhR pathway (Teraoka et al., 2003). Given the qualitative similarities between our results with pyrene and the recent work on TCDD, it is likely that pyrene acts via the AhR pathway. Irrespective of pyrene's mode of action, it appears that the three- and four-ring PAHs have fundamentally different effects on fish embryos.

Implications for the characterization of sublethal effects of PAH exposure in fish

Although the blockade of cardiac conduction by three- or four-ring PAHs is reversible, these preliminary studies suggest that transient changes in conduction may have subtle and perhaps irreversible effects during later stages of heart development. Initial stages of cardiac looping were unaffected by PAHs, but significant remodeling occurs after looping, including development of the cardiac valves, formation of the trabeculae, and thickening of the ventricular myocardium (Hu et al., 2000). Some of these processes continue through several weeks of larval development in zebrafish (Hu et al., 2000), and genetic analysis indicates that many aspects of cardiac remodeling and maturation are dependent on function (reviewed by Glickman and Yelon, 2002). Although three-ring PAH-exposed fish had a normal cardiac rhythm at 48-72 hpf after dephosphorylation, the morphology of the heart was different. Because hemodynamic forces influence multiple aspects of myocardial formation (Hove et al., 2003; Sedmera et al., 1998; 1999; Taber et al., 1993), there are likely to be significant long-term effects of three- or four-ring PAHs on this basis alone. Therefore, a more complete analysis of cardiac structure in PAH-treated fish, especially those reared for longer periods, is needed. In particular, an analysis of maturation of the trabeculae is important because these structures provide the main atrioventricular conduction pathway in the hearts of lower vertebrates (Sedmera et al., 2003). Also, defects in the cardiac trabeculae were observed in pink salmon larvae that had edema after exposure to weathered crude oil during embryogenesis (Martyn et al., 1997). It is possible that PAH-exposed fish in the natural environment may experience sublethal reductions in cardiac function that translate, in turn, to impaired performance at later life history stages. Although affected fish might appear grossly normal, their physiology and behavioral performance could be impaired. This could explain, for example, the reduced rate of marine survival among pink salmon exposed to PAHs as embryos (Heinritz et al., 2000).

Finally, our findings also raise the possibility that impacts of PAHs on kidney development could potentially contribute to sublethal effects that have not been anticipated previously. Although glomerular assembly fails in the absence of circulation, it is unknown whether there is a threshold hemodynamic force required to induce assembly, or if more moderate reductions in pressure would have consequences for kidney development that could affect individual survival. This is a particularly important question for freshwater and anadromous fishes. In addition, there are a host of other secondary effects that could conceivably contribute to reduced overall fitness. For example, reduced jaw structures could impact prey choices, growth, and overall size, factors which ultimately influence survival (Sogard, 1997). Similarly, it is unclear how transient changes in shape might affect subsequent development of the neural tube, where spatial relationships are important in the specification of spinal cord cell types (reviewed by Jessell, 2000). We anticipate that many of these questions can be answered efficiently using zebrafish as an experimental system for mechanistic studies of PAH toxicity in fish.

Note added in proof

The zebrafish breakdance mutant, which is characterized by 2:1 atrioventricular conduction block, was recently determined to disrupt the cardiac delayed rectifier K+ channel (Langheinrich et al., 2003).
Acknowledgments

The authors would like to thank Carla Stehr for her work in establishing the zebrafish breeding colony at the NWFSFSC, Jana Labenia and Tiffany Kao for excellent technical assistance and zebrafish husbandry, David Baldwin for help with video microscopy acquisition and editing software, Dave Raible for advice on the use of microinjection apparatus and discussion of data, Jim Lister for help with morpholino injections, Tor Linbo for advice on Alcian blue staining, Chuck Kimmel for advice on the head skeleton data, Frank Stockdale for generously providing the S46 antibody, and Didier Stainier, Grant Weinstein, and Calum McCrae for advice on analysis of zebrafish cardiovascular phenotypes. Monoclonal antibody MF20 was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the Department of Biological Sciences, University of Iowa, Iowa City, IA 52242. This work was supported by the National Research Council Research Associateships Program (J.P.I.) and a NWFSFSC Internal Grant (N.I.S.). Neither funding source was involved in the design or implementation of the described studies.

References


Fish embryos are damaged by dissolved PAHs, not oil particles

Mark G. Carlsa,*, Larry Hollanda, Marie Larsena, Tracy K. Collierb, Nathaniel L. Scholzb, John P. Incardona

a Alaska Fisheries Science Center, Auke Bay Laboratories, 17109 Point Lena Loop Road, Juneau, AK 99801, USA
b Ecotoxicology & Environmental Fish Health Program, Environmental Conservation Division, Northwest Fisheries Science Center,
2725 Montlake Boulevard East, Seattle, WA 98112, USA

Article Info

Article history:
Received 18 January 2008
Received in revised form 18 March 2008
Accepted 23 March 2008

Keywords:
Fish embryos
Dissolved PAH toxicity
Mechanism of oil damage
Particulate oil

Abstract

To distinguish the toxicity of whole oil droplets from compounds dissolved in water, responses of zebrafish embryos exposed to particulate-laden, mechanically dispersed Alaska North Slope crude oil (mechanically dispersed oil (MDO)) were compared to those of embryos protected from direct oil droplet contact by an agarose matrix. Most polycyclic aromatic hydrocarbons (PAHs) in MDO were contained in oil droplets; about 16% were dissolved. The agarose precluded embryo contact with particulate oil but allowed diffusive passage of dissolved PAHs. The incidence of edema, hemorrhaging, and cardiac abnormalities in embryos was dose-dependent in both MDO and agarose and the biological effects in these compartments were identical in character. Although mean total PAH (TPAH) concentrations in MDO were about 5–9 times greater than in agarose, dissolved PAH concentrations were similar in the two compartments. Furthermore, mean differences in paired embryo responses between compartments were relatively small (14–23%, grand mean 17%), typically with a larger response in embryos exposed to MDO. Therefore, the embryos reacted only to dissolved PAHs and the response difference between compartments is explained by diffusion. Averaged over 48 h, the estimated mean TPAH concentration in agarose was about 16% less than the dissolved TPAH concentration in MDO. Thus, PAHs dissolved from oil are toxic and physical contact with oil droplets is not necessary for embryotoxicity.

Published by Elsevier B.V.

1. Introduction

Oil occurs in the aquatic environment in both dissolved and particulate phases. While the toxicity of dissolved polycyclic aromatic hydrocarbons (PAHs) has been the focus of considerable research in recent decades, the relative contribution of particulate oil is still poorly understood. Although oil coating is obviously detrimental for organisms reliant on feathers or fur for insulation, oil coating of plankton and other aquatic organisms during various life history stages has received little study. In this paper we focus on the toxic implications to fish embryos of particulate oil generated mechanically without chemical dispersant.

Fish embryos (with intact chorions) are useful for toxicological studies involving oil because they are externally simple (essentially uniform spheres). They have minimum surface area in contact with surrounding water, do not ingest material and, other than the chorion, they have no external structures such as gills, setae, or feathers that can be fouled. In addition, fish embryos are highly sensitive to PAHs dissolved from oil; the biological effects of petrogenic PAHs in developing fish have been described in detail and are consistent across species (Marty et al., 1997; Carls et al., 1999, 2005; Heintz et al., 1999, 2000; Kiparissis et al., 2003; Incardona et al., 2004, 2005; Rhodes et al., 2005; Farwell et al., 2006). For example, exposure to low TPAH concentrations (1–5 μg/l) is associated with a syndrome marked by pericardial or yolk sac edema, with other morphological damage, delayed development, or death (Marty et al., 1997; Carls et al., 1999, 2005; Heintz et al., 1999, 2000). Studies in zebrafish embryos showed that the edema that forms with oil exposure is cardiogenic. Exposure to weathered crude oil or individual tricyclic PAHs (fluorene, dibenzothiophene and phenanthrene) caused specific defects in cardiac rate, rhythm, and contractility soon after the heart became functional (Incardona et al., 2004, 2005). Because cardiac function and morphogenesis are inextricably linked (Glickman and Yelon, 2002), these effects of oil exposure result in multiple secondary impacts on cardiac morphogenesis such as failure to complete looping (Incardona et al., 2005). In each of these previous studies, whole oil was never detected or in embryos. Nevertheless, a potential causative role for particulate oil in the observed developmental toxicity has been suggested (Brannon et al., 2006). This highlights the need for an experimental approach to definitively separate the contributions of dissolved and particulate oil to early life stage toxicity in fish. To accomplish this,
zebrafish embryos were embedded in an agarose matrix to preclude direct contact with particulate oil. The agarose matrix was covered with particle-laden, mechanically dispersed oil (MDO) with additional embryos added to the MDO for paired comparison (Fig. 1). By assessing the paired cardiac responses of embryos with and without physical contact to oil droplets, we had the opportunity to determine if oil droplets have detrimental physical effects (e.g., coating), or act as PAH delivery systems through direct contact with the chorion, or if embryos accumulate dissolved PAHs from water, or a combination of these possibilities.

2. Materials and methods

2.1. Embryo assays

Maintenance of adult zebrafish (*Danio rerio*), collection of fertilized eggs, and MDO exposures were all performed in water adjusted to a conductivity of ~1500 μS/cm, pH 7.5–8.0 with Instant Ocean salts (system water). Fertilized eggs were collected and washed with system water within 2 h of spawning, conventionally staged (Kimmel et al., 1995), and any inviable or abnormal early embryos (<5%) were discarded.

To preclude exposure to oil particles, blastula-stage zebrafish embryos (n = 10 per replicate) were embedded in agarose in the bottom of 60 mm glass Petri dishes. Embedding of zebrafish embryos in agar or agarose is a routine method that does not adversely affect development (Westerdijk, 2000; Distel and Koster, 2007). To avoid heat damage, low gelling temperature agarose (type VII-A, Sigma–Aldrich, St. Louis, MO) was used. Agarose was diluted in system water to 1% (w/v), solubilized by boiling, and cooled to 42°C. About 4 ml of liquid agarose were added to each dish to form a 1.4 mm thick layer; embryos were added as the agarose approached gelling at room temperature. The agarose layer was then covered by 10 ml of MDO, and 10 additional embryos (per replicate) were added to the overlying MDO fraction. The latter, particulate-exposed embryos settled on the surface of the agarose layer (Fig. 1). Embryos were incubated in the dark at 28.5°C. The agarose had no discernable effect on embryo development.

Treatment levels (n = 7) ranged from no MDO (controls) through 100% stock MDO, each replicated 3 times. Two MDO stocks were prepared in 11 separatory funnels, 0.25 ml oil in 250 ml system water (MDO<sub>100</sub>) and 2 ml in 200 ml water (MDO<sub>50</sub>). Each funnel was vigorously shaken for 2 min and allowed to separate for 4 h; stock MDO was turbid and brown. Prior to preparation, the oil was artificially weathered by heating at 70°C until approximately 20% evaporated (by mass) to mimic realistic environmental conditions (Bence and Burns, 1995; Marty et al., 1997). Composite water samples were collected from replicate treatments at the end of the experiment; hydrocarbons were extracted with dichloromethane as previously described (Short et al., 1996).

Embryos were qualitatively assessed for physiological effects of PAH exposure (Incardona et al., 2005), including pericardial edema, abnormal heart looping, and intracranial hemorrhaging after 2 d exposure. Embryos were dechorionated, allowed to straighten, then anesthetized with tricaine methane sulphonate. After live observation, all specimens were fixed in methanol + 10% DMSO and stored at -20°C. Immunofluorescence with cardiac chamber-specific antibodies MF20 and S46 and measurement of cardiac looping were carried out as described previously (Incardona et al., 2004, 2005). Observation order was random and all treatments were scored blind for both live and preserved specimens.

Alkane and PAH exposure in MDO and agarose were quantified over the exposure period with low-density polyethylene membranes [PEMs, about 2.5 cm x 2.5 cm x 98 μm (Carls et al., 2004)]. Additional MDO and agarose samples were collected after exposure, each combined across replicates in the highest dose. Agarose was rinsed with distilled water before collection to remove surface oil. Each PEM was deployed either in MDO or agarose at maximum dose levels plus controls in separate Petri dishes during embryo exposure. The PEMs were cleaned by soaking in pentane + 1 h before deployment, collected after 2 d exposure, and weighed to the nearest 0.01 mg after extraction.

2.2. Particle and chemical migration through agarose

To characterize the physical and chemical interactions between MDO and agarose, additional analytical experiments were completed under conditions that matched the embryo exposures. The oil particle size distribution in MDO<sub>100</sub> was determined by passage through, or retention on, filter paper with differing pore sizes (0.1–100 μm; Supplemental 1). Several detection methods were compared, including gravimetric, photometric, and gas chromatog-
rhapsy/mass spectroscopy (GC–MS). The effectiveness of particle exclusion by 1% agarose was tested by casting agarose membranes (0.80 ± 0.05 mm thick) and observing chemical migration through them by GC–MS (Supplemental 2). The rate of PAH diffusion from MDO into 1% agarose under assay conditions was quantified by placing MDO$_{low}$ (10 mL) in contact with agarose (4 mL) for 48 h at 27–28°C. Samples were periodically collected from multiple Petri dishes and combined for GC–MS analysis (Supplemental 3).

2.3. Hydrocarbon measurement

Water, agarose, and PEMs were extracted for hydrocarbons, the latter as previously described (Carls et al., 2004). In brief, surface contamination was removed from PEMs, the plastic was spiked with internal standards, extracted in 80:20 mL pentane/dichloromethane with sonication, concentrated, and dried with sodium sulphate. Water and agarose samples were extracted twice with dichloromethane after addition of six internal standards (Short et al., 1996). Extracts were passed through a micro silica-gel column and eluted with 20 mL of 1:1 pentane/dichloromethane to remove potentially interfering compounds such as large waxes and pigments. Alkane concentrations were measured by gas chromatography with a flame ionization detector and PAHs were measured by GC–MS using selected ion monitoring. Phenanthrene presence was confirmed by GC–MS in a limited scan mode (80–325 atomic mass units). For quality control, a method blank, spiked method blank, and two reference samples were analyzed with every 12 samples as described previously (Short et al., 1996). Method detection limits were about 1–5 ng/g in PEMs and 1–8 ng/g in water and agarose. Concentrations below the method detection limits are reported as 0. Total PAH concentrations were calculated by summing concentrations of 44 individual PAHs, ranging from 2 to 5 rings (napthalenes, biphenyl, acenaphthylenes, acenaphthene, fluorenes, dibenzothiophenes, phenanthrenes, anthracene, fluoroanthene, pyrene, fluoranthene/pyrenes, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[j]pyrene, perylene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, and benzo[g,h,i]perylene). Quantified alkane included C5 through C34, pristane, and phytane. Composition data are detailed in Supplemental 4.

2.4. Data analysis

Data were analyzed by two-factor analysis of variance [compound (MDO or agarose) × oil dose]; replicate was nested in dose for analysis of heart lesion. The Bonferroni inequality (α divided by the number of comparisons) was applied to pairwise contrasts between MDO and agarose means to ensure the probability of incorrect rejection was no more than 0.05 for all comparisons. Treatments where embryo response was 100% were excluded when describing response differences between MDO and agarose compartments because this endpoint condition obscures relationships. Conventional regression techniques were used to analyze supplementary data. Mean total PAH concentrations in MDO and agarose were estimated from ancillary chemical experiments (Supplemental 3); these estimates were used to determine lowest effective concentrations. Embryo response was related to estimated dissolved TPAH concentrations in agarose and MDO with logit analysis and curve smoothing techniques (Vellelman and Hoaglin, 1981).

3. Results

3.1. Embryo bioassays

The physical isolation of zebrafish embryos from particulate oil did not protect them against MDO-induced developmental toxicity. Dose-dependent developmental abnormalities were clearly evident in agarose-embodied animals (P < 0.001; Fig. 2). The heart was the most sensitive indicator of toxicity. The mean incidence of abnormal heart looping was 0% in controls and ranged from 60 ± 6.0% in the lowest dose (the estimated mean TPAH concentration was 15 μg/L) to 100% at higher doses (Fig. 2b and e). The mean incidence of pericardial edema increased from 0% (control) to 100% (Fig. 2c). Intracranial hemorrhaging was dose-dependent, ranging up to 44 ± 13% in the highest dose (Fig. 2a). Cardiac looping was also delayed by exposure to dissolved PAHs (P < 0.001; Fig. 2d).

The effects of direct crude oil MDO exposures on embryos were similar to those in agarose despite greater mean TPAH concentrations (see below). Direct crude oil MDO exposures produced a comparable, dose-dependent pattern of embryo toxicity (P < 0.001; Fig. 2). As with embryos embedded in agarose, the heart was the most sensitive target organ. The mean incidence of abnormal heart looping was 100% in high doses and 93 ± 7% in the lowest concentration, where the estimated mean TPAH concentration was 106 μg/L (with 17 μg/L dissolved; Fig. 2b). The mean incidence of pericardial edema increased from 0% (control) to 100% and intracranial hemorrhaging reached 57 ± 3% in the highest dose. Cardiac looping was abnormally delayed by exposure to MDO (P < 0.001; Fig. 2).

The extent of toxic injury among embryos embedded in agarose was generally lower than in embryos exposed directly to MDO. Mean paired differences were 14, 15, and 23% for intracranial hemorrhaging, abnormal cardiac looping, and pericardial edema, respectively, across responses. The mean difference between treatment pairs was 17% (95% confidence bounds 7–28%). Although differences in abnormal heart looping between corresponding agarose and MDO treatments were significant (0.01 < P ≤ 0.005), paired differences in most other responses (e.g., cardiac looping) approached statistical significance but were not significant (Fig. 2). Overall, the familiar syndrome of PAH-induced developmental defects, which occur among a wide range of teleosts (Marty et al., 1997; Carls et al., 1999; Heintze et al., 1999; Couillard, 2002; Pollino and Holdway, 2002; Rhodes et al., 2005), was less pronounced among embryos embedded in a matrix of agarose. Mortality was not observed in these 2d assays.

Particulate oil was present in MDO and absent in agarose; TPAH concentrations were greater in MDO. After 2d, TPAH concentrations were 668 μg/L in MDO$_{high}$ and 39 μg/L in the agarose from corresponding Petri dishes, thus were 17 times greater in MDO than agarose. Total PAH uptake in PEMs placed in MDO$_{high}$ (589000 ng/g) and corresponding agarose (114,000 ng/g) differed by a factor of 5, a measure of average conditions. Similarly, the estimated mean TPAH concentration in MDO was 8.6 times greater than in agarose (see next paragraph). Phytane concentrations (evidence of particulate oil) ranged from 5 μg/L in MDO$_{low}$ to 34 μg/L in MDO$_{high}$; Phytane concentrations were 1650 ng/g in the PEM in MDO$_{high}$. In contrast, phytane was not detected in corresponding agarose at the end of the assay and it was not present in the embedded PEM.
3.2. Physical chemistry

Supplemental experiments also demonstrated that PAHs diffuse into water and agarose even though particulate oil is excluded from agarose. Composition in diffusate (water where MDO was removed by passage through agarose) was characteristic of dissolved PAHs, with relatively more lower molecular weight PAHs and relatively fewer higher molecular weight PAHs; no chrysenes were detected. The median oil particle size in weathered Alaska North Slope MDO was about 8 μm, and >99% of the particles were >0.45 μm in diameter (Fig. 3 and Supplemental 1). Estimated pore sizes in 1% agarose range from 0.10 to 0.28 μm (Stellwagen, 1992; Maaloum et al., 1998; Hasse and Scholz, 2006). Consistent with these measures, the agarose matrix blocked passage of all particulate oil (Supplemental 2). The concentrations of TPAHs in agarose were limited by diffusion (Fig. 4 and Supplemental 3) and reached about 80% saturation in 4 h (normalized to dissolved TPAH concentration in MDO; r² = 0.988, Michaelis–Menten uptake model). Estimated over 48 h, the mean TPAH concentration in agarose (133 μg/l) was about 16% less than dissolved TPAH concentration in MDO (158 μg/l; Supplemental 3) and much less than mean TPAH concentrations in whole MDO (8.6 times), where 84% of PAHs were present in particulate form. The composition of PAHs in agarose and dissolved PAHs in MDO were very similar (<2% difference). Both profiles were dissimilar from whole MDO, which contained a larger proportion of higher molecular weight PAHs.

4. Discussion

Agarose proved to be an excellent size-exclusion matrix. Particulate oil was never detected internally either chemically (phytane) or spectrophotometrically. Only in agarose samples where external surface contamination was possible (by oil slicks) were traces of whole oil detected (Supplemental 3), suggesting whole oil has little affinity for this substance. Within method detection limits, separation between non-particulate and particulate treatments was complete. This performance, plus survival of embedded control embryos without damage identifies the technique as promising for continued studies.
Fig. 3. Relationship between filtrate absorbance and phytane concentration to filter paper pore diameter. Absorbance was measured at 350 nm; phytane concentrations were measured by gas chromatography/mass spectrometry. Error bars are ± standard error; n = 5; phytane measurements were not replicated.

Particulate oil is not directly toxic to fish embryos. Rather, PAHs must enter solution to be biologically available. This is demonstrated directly by a dose-dependent increase in cardiac-related developmental abnormalities in zebrafish embryos isolated from particulate oil via a size-exclusion agarose matrix. The observed effects in agarose-embedded embryos and embryos in direct contact with MDO were identical in character and were consistent with the established cardiovascular impacts of trycyclic PAHs (Incardona et al., 2004, 2005); Supplemental 5. If oil droplets had direct physical effects on fish embryos, they would not be expected to produce the same phenotype. For example, if oil droplets had an effect similar to smothering of eggs by fine sediments and blocked or reduced oxygen transport across the chorion, embryos might exhibit signs of hypoxia. However, the effects of hypoxia in zebrafish are clearly distinct from and have little overlap with the effects of petrogenic PAH exposure (Padilla and Roth, 2001; Shang and Wu, 2004; Kajimura et al., 2005). (Likewise, there was no evidence of hypoxia in agarose-embedded embryos.) Other plausible mechanisms that would link the physical interaction of oil droplets with the chorion to functional and morphological effects on the developing heart within the embryo have not been identified.

Intracranial hemorrhaging was the least responsive of the three abnormalities scored in this study. The maximum response incidence became asymptotic around 40 or 50%, evidently because as cardiac abnormalities increase, blood flow to the brain decreases, thus limiting the extent of intracranial hemorrhaging (Icardona et al., 2005).

The embryos responded to dissolved PAHs in both compartments, not whole oil particles. The ratio between mean TPAAH concentrations in whole MDO and agarose (5–9) was too large to explain the relatively small mean differences in embryo response (14–23%) between these compartments. Rather, the dissolved TPAAH concentration in MDO was similar to that in agarose, albeit 16% higher when averaged over 48 h because concentrations in agarose were limited by diffusion. The modest embryo response differences are consistent with dissolved TPAAH concentrations between compartments and inconsistent with TPAAH concentration differences when particulate oil is included in the MDO measurement. Thus, the contribution of particulate oil to the observed toxicity was negligible under these conditions. Similarly, embryo mortality and PAH accumulation in pink salmon embryos in direct contact with oiled gravel were not significantly different from mortality and accumulation in embryos exposed to dissolved PAHs in effluent water only (Heintz et al., 1999); further evidence that dissolved PAHs cause damage and direct contact with whole oil does not.

The role that particulate oil plays in embryo toxicity is apparently primarily that of a reservoir for dissolved PAHs (and other chemical constituents). Dissolved PAH concentrations in water drop more slowly when particulate oil is present than when it is not (Supplemental 3). Fish chorions or vitelline envelopes are largely an extracellular matrix material composed of secreted glycoproteins (Darie et al., 2004; Monné et al., 2006) with nanoscale pores (0.17 μm for zebrafish (Cheng et al., 2007) and apparently are not particularly hydrophobic. Consistent with this, we observed oil particles in MDO drift past the chorion without adhering to it (Supplemental 6). Therefore, the toxic reservoir is particulate oil in water, not whole oil in contact with embryos, and PAHs dissolved from that reservoir were the source of damage.

We do not expect that embryos of other teleost species with smooth chorions will react differently to particulate oil. For example, pink salmon and zebrafish both are protected by chorions with pore sizes small enough to block most if not all oil particles (Stehr and Hawkes, 1979); <0.5% of the oil particles were small enough to pass through zebrafish chorions in our experiment, and there was no evidence that oil particles were attracted in any way to the chorion. Furthermore, in other experiments where tissue was

Fig. 4. Estimated relationship among dissolved and particulate TPAAH concentrations in MDOavg and agarose. The MDO concentration is the sum of all TPAAH in water (dissolved plus particulate). The dissolved concentration was measured by passing MDO through 0.7 μm glass fiber filters; the difference is the estimated TPAAH concentration due to particulate oil (est. particle). The relationship between TPAAH dissolved concentration in water and in agarose was determined in a separate experiment to minimize contamination of the agarose surface by particulate oil; Supplemental 3 and used to model the relationship in this time series (which approximates conditions experienced by zebrafish embryos). The estimated uptake in agarose is based on Michaelis-Menten kinetics (r2 = 0.988).
analyzed for hydrocarbons, no particulate oil was detected—this despite the contention by Brannon et al. (2006) that these assays were confounded by particulate oil. Hydrocarbon uptake rates are about the same for herring eggs (similar in size to zebrafish eggs) and pink salmon eggs, 16 and 19d, respectively, to reach peak concentrations (which are dose-dependent) (Carls et al., 1999, 2005).

Thus, there is no reason to believe that the larger eggs with more lipid somehow create a gradient that would be more likely to attract oil particles. Similarly, the chorions of Pacific herring, pink salmon and zebrafish are smooth and lack any filaments that might entrap oil particles (Stehr and Hawkes, 1979; Ohta, 1984; Cheng et al., 2007). How particulate oil might influence embryos with filamentous chorions remains to be tested.

Our conclusion that dissolved PAHs caused the observed toxicity in the absence of particulate oil corroborates and extends the findings of previous studies. Our group has repeatedly demonstrated that dissolved PAHs (<18 µg/l TPAH) are toxic to fish embryos (Marty et al., 1997; Carls et al., 1999, 2005; Heintz et al., 1999, 2000). In these experiments, the amount of particulate oil in the rock column effluent was negligible, as indicated by a clear effluent with no visible oil sheens. Moreover, the effluent was enriched with PAHs and essentially devoid of phytane, a highly insoluble tracer of particulate oil (Marty et al., 1997; Carls et al., 1999, 2005; Heintz et al., 1999, 2000). Furthermore, phytane and visible oil were never detected in (or on) eggs in these experiments. In building on these earlier published results and others (Kiparissis et al., 2003; Rhodes et al., 2005; Farwell et al., 2006), our current study does not support the alternative hypothesis that particulate oil is directly involved in the toxicity of PAHs to fish embryos.

Our results point to the importance of distinguishing between oil effects on wildlife due to physical processes (coating) and oil effects due to chemical toxicity. At macroscopic scales, the adverse impacts of coating are well known in situations where oil adheres to organisms. However, for smaller species, such as larval and adult organisms (e.g., bivalves). Currently unknown is how micron-sized oil particles may affect planktonic organisms that may collect, ingest, or otherwise be fouled by them. However, physical coating effects do not appear to extend to fish embryos exposed to oil particles in the micron size range. At these smaller scales the chemical toxicity associated with dissolved-phase oil is the predominant form of injury, at least for eggs. This observation is consistent with the conclusion of others that conclusions in true solution are more bioavailable than chemicals in solid or adsorbed forms (NRC, 2002; Beckles et al., 2007; Golding et al., 2007). The importance of dissolved-phase oil is further supported by toxicity assays with pure compounds. For example, a toxicity model developed by Di Toro et al. (2000) relied only on assays using pure hydrocarbon compounds in dissolved phase. Similar to the current study, a partition-controlled assay that dissolved retene in polydimethylsiloxane film and then allowed equilibration with eggs placed in water (in contact with the film) also demonstrated that dissolved PAHs are toxic in the absence of particulate matter (Kiparissis et al., 2003). Studies that do not distinguish particulate and dissolved PAH toxicity may substantially underestimate PAH toxicity (e.g., compare the concentration scales in Fig. 2).

The sensitivity of fish embryos increases with the increasing molecular size and alkyl substitution of dissolved PAHs (Anderson et al., 1974; Moore and Dwyer, 1974; Rice et al., 1977; Hutchinson et al., 1980; Black et al., 1983; Neff, 1985, 2002). Moreover, mechanistic studies demonstrate that PAH constituents in crude oil are cardiotoxic to fish embryos whether present as pure compounds or in oil rock column effluent (Incardona et al., 2004, 2005, 2006). Proportionately fewer of the larger, more toxic molecules were biologically available in our agarose particulate exhaustion assays than in our previous oil rock column studies (Marty et al., 1997; Carls et al., 1999, 2005; Heintz et al., 1999, 2000). This is because the short duration of this study (2d) minimized weathering, thereby biasing the dissolved fraction toward the lower molecular weight naphthalenes. However, substantial quantities of PAHs larger than naphthalene diffused into the agarose (e.g., 5.6 ± 0.2 µg/l in the diffusate) and PAHs as large as C2-terphenyls were sequestered by the matrix-embedded PEM. The penetration of higher molecular weight PAHs into the agarose matrix was sufficient to produce the stereotypical syndrome of cardiovascular abnormalities in zebrafish embryos with an estimated mean lowest observed effective dose of 15 µg/l over the 2d exposure. This is within the previously reported range for embryo toxicity (i.e., <1 to 23 µg/l TPAH (Marty et al., 1997; Carls et al., 1999, 2005; Heintz et al., 1999, 2000; Brand et al., 2001; Kiparissis et al., 2003; Colavecchia et al., 2004; Rhodes et al., 2005; Farwell et al., 2006)).

The sublethal toxic responses observed in this study have previously been linked to reduced survival potential and mortality. Field and laboratory studies of embryonic fish have demonstrated a common syndrome of oil-induced toxicity in a range of teleosts, including marine, freshwater, temperate, and tropical species (Marty et al., 1997; Carls et al., 1999; Heintz et al., 1999; Couillard, 2002; Pollino and Holdway, 2002). The sublethal effects described in this experiment are correlated with impaired swimming, reduced growth, poor survival potential, and when sufficiently severe, direct mortality (Carls et al., 1999, 2005; Heintz et al., 1999, 2000; Sromberg and Fent, 2005; Heintz, 2000). Survival of oil-exposed pink salmon released into the Pacific Ocean was significantly reduced with respect to controls, even though the exposed fish had no visible damage at the time of release (Heintz et al., 2000).

Although not toxic until PAHs dissolve, particulate oil may threaten a variety of marine organisms. Whether mechanically or chemically dispersed in the water column, the large surface volume drop to water ratio promotes PAH uptake. These compounds are thus a toxic reservoir that will persist until PAHs are depleted, diluted, or advected away from sensitive species. Although direct particulate effects are not important for fish embryos, this cannot simply be generalized to organisms that might collect, ingest, or otherwise be fouled by particulate oil, including fish larvae. Continued study, including tests with chemical oil dispersants and a broader range of planktonic organisms, is recommended. Such a study is prudent because use of chemical dispersants as post-spill clean-up measures in U.S. waters remains under active debate and these dispersants promote formation of particulate oil.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2008.03.014.

References


Coastal Storms, Toxic Runoff, and the Sustainable Conservation of Fish and Fisheries

SARAH G. MCCARTHY, JOHN P. INCARDONA, AND NATHANIEL L. SCHOLZ*
NOAA Fisheries, Northwest Fisheries Science Center
Ecotoxicology and Environmental Fish Health Program
2725 Montlake Boulevard East, Seattle, Washington 98112, USA

Abstract.—Nonpoint source pollution in the form of stormwater runoff is one of the most important emerging threats to ecosystems along the coastal margins of the United States. A wide diversity of potentially toxic chemicals is commonly found in stormwater. These include the various pesticides, petroleum hydrocarbons, heavy metals, and other common contaminants that originate from commercial, industrial, residential, and agricultural land-use activities. These chemicals are mobilized from roads, lawns, crops, and other surfaces by rainfall and then transported to aquatic habitats via terrestrial runoff. The ongoing development of coastal watersheds nationwide is increasing the loading of nonpoint source pollutants to rivers, estuaries, and the nearshore marine environment. A central aim of the National Oceanic and Atmospheric Administration’s national Coastal Storms Program (CSP) is to enhance the resiliency of coastal ecosystems by improving the ability of coastal communities to anticipate and reduce the impacts of contaminated terrestrial runoff. Toxic chemicals in stormwater can adversely impact the health of fish, including threatened and endangered species. Nonpoint source pollution can also degrade the biological integrity of aquatic communities that support productive fish populations. This article examines the effects of stormwater runoff on fish and fisheries. Using case studies drawn from CSP project work in the Pacific Northwest and Southern California pilot regions, we show how degraded water quality can impact the health of fish during critical life history stages (i.e., spawning and rearing) as well as limit the overall effectiveness of fish habitat restoration. We also discuss some of the resources currently available to local communities to reduce the loading of toxins in stormwater, thereby increasing the resilience of aquatic communities. Finally, we identify priority areas for new research to help guide the future conservation and recovery of at-risk fish populations.

Coastal waters are one of the nation’s greatest assets, yet they are being bombarded with pollutants from a variety of sources. While progress has been made in reducing point sources of pollution, nonpoint source pollution has increased and is the primary cause of nutrient enrichment, hypoxia, harmful algal blooms, toxic contaminants, and other problems that plague coastal waters. Nonpoint source pollution occurs when rainfall and snowmelt wash pollutants such as fertilizers, pesticides, bacteria, viruses, pet waste, sediments, oil, chemicals, and litter into our rivers and coastal waters... Our failure to manage the human activities that affect the nation’s oceans is compromising their ecological integrity, diminishing our ability to fully realize their potential, costing us jobs and revenue, threatening human health, and putting our future at risk (U.S. Commission on Ocean Policy 2004).

* Corresponding author: nathaniel.scholz@noaa.gov
Today, nonpoint sources represent the greatest pollution threat to our oceans and coasts. Every acre of farmland and stretch of road in a watershed is a nonpoint source. Every treated lawn in America contributes toxics and nutrients to our coasts... the situation requires that we apply new thinking about the connection between the land and the sea, and the role watersheds play in providing habitat and reducing pollution (Pew Oceans Commission 2003).

**Introduction**

Human population growth and associated changes in land use are placing ever-increasing pressures on coastal ecosystems. At present, more than 50% of the U.S. population resides in coastal counties, which together account for only 17% of the total geographical area of the country (Crossett et al. 2004). While the relative proportion of people living near America's coasts is expected to remain relatively constant, growth projections forecast a nearly 50% increase in the overall size of the U.S. population by 2050 (Day 1996). These growth trends are expected to drive new development along America’s coastlines, thereby increasing the loading of nonpoint source pollutants to rivers, estuaries, and the nearshore marine environment.

Variation in land cover and land use within a coastal drainage typically determines the presence and relative amounts of different chemical contaminants in stormwater runoff (Tsihrintzis and Hamid 1997; Bay et al. 2003). For example, pesticides are typically detected in small streams and other surface waters in proportion to their rate of application within watersheds (Gilliom et al. 2006). Urban runoff generally contains heavy metals and petroleum hydrocarbons from motor vehicles and commercial land uses; pesticides from residential, park, and golf course applications; and pharmaceuticals from combined sewer overflows where these occur. In agricultural areas, nonpoint source runoff generally contains a greater diversity of pesticides, as well as pathogens and nutrients (Hunt et al. 1999). Various factors affect pollutant loading and transport to aquatic systems, such as length of the antecedent dry period, rates of atmospheric deposition, storm intensity, soil composition, and ground cover (Bertrand-Krajewski et al. 1998; Goonetilleke et al. 2005; Han et al. 2006).

An increase in impervious land cover within watersheds poses a key threat to aquatic ecosystems (Paul and Meyer 2001; Nilsson et al. 2003). Examples of impervious surfaces include paved roads, highways, parking lots, driveways, and roofs. A variety of indicators have consistently shown that the biological condition of aquatic habitats is significantly degraded when the total impervious area of a watershed exceeds 10% (reviewed by Beach 2002). This is notable because the current amount of impervious coverage in watersheds throughout the country is estimated to range from 12.5% to 30% (Beach 2002). Moreover, several studies have shown a negative correlation between urbanization and aquatic species diversity and abundance (Karr 1991; Booth et al. 2002; Wheeler et al. 2005). Although correlations between urbanization and aquatic habitat degradation are now widely established (Weaver and Garman 1994; Walsh et al. 2005a, 2005b; Urban et al. 2006; Gresens et al. 2007; Schiff and Beniot 2007), the causal contribution of chemical contaminants relative to physical processes (i.e., hydrology and geomorphology) remains poorly understood.

Despite the fact that nonpoint source pollution is an increasingly important determinant of aquatic habitat quality, the impacts of toxic runoff on the productivity of wild fish populations have not been widely investigated. This is due in part to the complex environmental chemistry of stormwater. Storms can mobilize unpredictable mixtures of contaminants over relatively short time intervals. Another challenge in recent decades has been a difficulty linking sublethal health effects in individual fish to the higher biological scales of populations and communities (Hinton et al. 2005). While nonpoint source pollution occasionally causes fish kills, most contaminant exposures are sublethal. This basic fact indicates a need to understand how toxics influence the physiology and behavior...
of fish in ways that ultimately determine their lifetime reproductive success (Scott and Sloman 2004). This in turn places a greater emphasis on the ecology of fish, for example, how toxics influence predator–prey interactions, disease susceptibility, migration, and other important life history processes (Rohr et al. 2006). Therefore, to effectively maintain (or restore) the resilience of aquatic ecosystems in the face of development pressures, coastal communities and natural resource managers need new scientific information specific to the ecotoxicological effects of stormwater.

This article addresses the impacts of toxic stormwater on fish and fisheries. To explore this issue, we draw from recent field investigations and contaminant-specific toxicological research to assess the impacts of stormwater on the health of fish in California and the Pacific Northwest. These studies were conducted as part of the the National Oceanic and Atmospheric Administration’s (NOAA’s national Coastal Storms Program [http://coastalstorms.noaa.gov/stormwater]) with the aim of more clearly defining the current and future threats posed by toxic runoff to fish habitats. This article is not intended as a review of the toxicological literature. Also, we do not discuss the extent to which pollution may limit specific fisheries. Our goal instead is to highlight different approaches that have been used to link real-world habitat conditions to the health and survival of fish. We also give examples of how emerging biotechnologies are making it easier to resolve subtle but important health effects in fish and to unravel some of the complexities associated with chemical mixtures.

Three specific case studies are highlighted in the following sections. The first describes unexpectedly high rates of acute mortality among adult Pacific salmon returning to spawn in restored urban streams in the greater Seattle metropolitan area. The findings are a cautionary tale for urban stream restoration. They reinforce the importance of in situ biological monitoring in aquatic systems impacted by urban runoff. They also foreshadow potential future threats to wild salmon populations in developing watersheds in northern California and the Pacific Northwest. The second case study shows how one of the most common contaminants in stormwater (dissolved-phase copper) can isolate juvenile salmon from important sensory cues in their surrounding environment, thereby increasing their vulnerability to predation. It serves as an example of how short-term, environmentally realistic pulses of pollution in fish habitats can disrupt the physiology and behavior of fish and how sublethal effects can be extrapolated to higher biological scales. The third case study gives an overview of new exploratory research to discover novel pathways of toxicity in fish during sensitive life stages. The focus is on petroleum hydrocarbons, which originate from motor vehicles (and other sources) and are ubiquitous in urban runoff. We show how an expanding toolbox of techniques in molecular biology and genetics can be used to (1) address the complicated problem of chemical mixtures, and (2) identify previously unknown biological response pathways. Among other applications, these new technologies may lead to new and sensitive indicators of health and performance in wild fish exposed to hydrocarbons in urbanizing waterways. Following the case studies, we identify some tools available to local communities for reducing the loading of toxics in stormwater, thereby increasing the resilience of aquatic communities. We close with a discussion of research priorities that will help guide the future conservation and recovery of fish and fisheries that are impacted by stormwater runoff.

**Recurrent Die-Offs of Coho Salmon Returning to Spawn in Restored Urban Streams**

As in other regions of the United States, current growth in the Pacific Northwest is driving the conversion of forested and agricultural lands to commercial and residential uses. These changes in land cover and land use are posing increasingly important threats to anadromous Pacific salmon (genus *Oncorhynchus*) species that are significant throughout the region for commercial, recreational, cultural, and ecological reasons (National Research Council 1996). Among these are several stocks of Chinook salmon *O. *
yitscha, sockeye salmon O. nerka, coho salmon O. kisutch, chum salmon O. keta, pink salmon O. gorbuscha, and steelhead O. mykiss. Due in part to the degradation and loss of habitat, many salmonid stocks have been declining in recent decades (Nehlsen et al. 1991). As a consequence, several salmonid population segments, or evolutionarily significant units (ESUs), are now listed as either threatened or endangered under the U.S. Endangered Species Act (for current species listings, see www.nmfs.noaa.gov/pr/). For example, the lower Columbia River coho and Puget Sound steelhead ESUs were listed as threatened in 2006 and 2007, respectively. In response to these numerous and geographically widespread listings of salmon populations, a major societal effort is now underway to conserve and restore freshwater and estuarine habitats.

On a more local scale, urban and suburban streams in and around cities such as Seattle, Washington have been the focus of intensive restoration activities for more than a decade. The efforts to date have been largely focused on replanting native riparian vegetation, increasing habitat complexity using weirs and large woody debris, removing culverts and other barriers to fish passage, and increasing stormwater detention in urban areas. As with many high-density regions (reviewed by Paul and Meyer 2001), lowland streams in the Seattle metropolitan area receive significant amounts of nonpoint source pollution that increase along a gradient of urbanization. Outfalls from storm drains make up a significant fraction of surface flows in these streams, particularly during the fall and winter months. Therefore, stream restoration projects have had to contend with the familiar hydrologic challenges associated with stormwater management (Bernhardt and Palmer 2007).

A key benchmark for the success of urban stream restoration projects throughout lowland Puget Sound has been the extent to which adult salmonids (primarily coho and chum) return to spawn in the improved and, in some cases, newly available habitats. Beginning in the late 1990s, field biologists with the city of Seattle, Washington Trout (since renamed the Wild Fish Conservancy), and other organizations made a surprising discovery. They found that while salmon were successfully returning to many restored streams, a high proportion of sexually mature female coho carcasses, when examined, showed large numbers of retained eggs (Figure 1). They went on to document highly erratic swimming behavior and prespawn mortality among both male and female coho. Affected fish from different urban streams displayed a common suite of symptoms, including surface swimming and gaping, fin spaying, spasming, disorientation, and loss of equilibrium. The coho usually died within a few minutes to a few hours after becoming overtly symptomatic. Visual inspections generally indicated that the affected coho spawners were in good condition, with the silver coloration typical of salmonids that have recently transitioned to freshwater from the ocean.

The ad hoc observations of coho prespawn mortality (PSM) during fall spawner surveys prompted a focused monitoring and forensic research study. The investigation began in 2002 and has continued in the years since as part of the NOAA’s Coastal Storms Program project work in the Pacific Northwest. To date, 5 years of daily stream surveys have been conducted during coho spawner migrations in the fall on Longfellow Creek in west Seattle (Figure 2A). The Longfellow drainage, a typical urban stream system, has been a focus for intensive habitat restoration in recent years. Daily surveys have also been conducted on other Seattle-area urban streams. In 2002, adult coho mortality was monitored on Fortson Creek, a nonurban forested tributary of the north fork of the Stillaguamish River as a reference location (Figure 2B). Coho spawning habitat was surveyed for live, symptomatic, and dead animals. For all dead or symptomatic female coho, the location, species, gender, length, weight, and spawning condition (percentage egg retention in females) were recorded. Spawning condition was only assessed for female coho because of the difficulty determining whether field-collected males had spawned. Rainfall and instream flow data were also collected for the different streams.
After a protracted dry period in the early fall of 2002, adult coho salmon began entering Longfellow Creek with the first major rains and ensuing freshets in early November. All of the females returning to the stream in the first several days died before spawning, and successful spawners were only observed after several significant rain events. The overall rate of female PSM for Longfellow Creek in 2002 was 86.0% (n = 57 animals) across the entire fall run. At the nonurban location (Fortson Creek), nearly all of the returning female coho survived to spawn, with an overall female PSM rate of 0.9% (n = 114). Surveys in

Figure 2. Study sites for coho salmon prespawn mortality investigations. (A) Longfellow Creek is a restored stream located in a highly residential and commercial area in west Seattle, Washington and was sampled daily during the fall coho spawning seasons, 2002–2006. (B) Fortson Creek is a non-urban forested tributary of the North Fork Stillaguamish River, near Darrington, Washington and was sampled daily during the coho run in late fall and winter, 2002. (Photographs by Carla Stehr, NOAA Fisheries)
subsequent years have revealed similarly high rates of PSM in Longfellow Creek (Table 1; N. Scholz, NOAA Fisheries, Northwest Fisheries Science Center, unpublished results) as well as other urban streams in the lowland Puget Sound geographic area. Within Longfellow Creek, the severity of coho die-offs from year to year appears to be influenced by rainfall. Preliminary results from survey data gathered thus far indicate a significant inverse relationship between total rainfall during the fall spawning season and coho PSM (Scholz, unpublished results). The highest rates of spawner mortality were observed in fall seasons that were relatively dry but punctuated by episodic storm events. Monitoring of rainfall and PSM rates will continue in Longfellow Creek over the next several years to better define the relationship between fall weather patterns, transport of contaminants in stormwater, and coho PSM.

Coho PSM in urban streams is unlikely to be causally related to other types of pre-spawn mortality in salmonids that have previously been associated with high temperature, low dissolved oxygen, overcrowding, disease, parasites, predation, or an accidental chemical spill (e.g., Gilhousen 1990; Heard 1991; California Department of Fish and Game 2004; Quinn 2005). In recent years, surface water quality monitoring and a variety of forensic analyses of affected coho from Longfellow Creek have systematically ruled out each of these hypotheses (Scholz, unpublished results). Instead, the weight of evidence suggests that adult coho are acutely sensitive to nonpoint source stormwater runoff from urban landscapes. Whether salmon are dying from exposure to a single contaminant or a mixture of contaminants is not yet known. On the one hand, most pollutants are present in urban surface waters at concentrations below those that will typically cause fish kills. On the other hand, coho spawners undergo important physiological changes as they transition from saltwater to freshwater. These changes may render them more vulnerable to toxic chemicals, alone or in combination with other environmental stressors. Research to identify the precise cause of coho PSM is ongoing.

The “urban stream syndrome” comprises a suite of common characteristics that include flashy flow regimes during storms, increased sedimentation, higher levels of contaminants, and low abundance and survival of sensitive aquatic species. This creates a tendency for systems that drain highly urbanized areas to be degraded despite localized restoration efforts (Walsh et al. 2005b). The restoration activities on Longfellow Creek were successful in terms of attracting spawning coho back to the watershed. However, postconstruction monitoring has revealed that many spawners are unable to withstand pollutants in urban stormwater runoff. These findings reinforce the current view that urban stream restoration projects need to address the physical, chemical, and biological aspects of habitat quality at appropriate scales (Booth et al. 2004; Walsh et al. 2005a; Bernhardt and Palmer 2007). It is particularly important that water quality be considered at the catchment scale.

In closing, there are several important lessons to be learned from research to date on coho PSM. First, biological monitoring is an essential component of aquatic habitat restoration. We are now aware of the threats that urban runoff poses for coho populations be-

Table 1. Annual rates of female coho pre-spawn mortality (PSM) in an urban stream (Longfellow Creek, west Seattle, Washington) and a forested reference stream (Fortson Creek, north fork Stillaguamish River, Washington). Sample size (N) indicates number of dead females of known spawning condition, not including fish that were predated upon and scavenged prior to sample date. Percentage calculated as number of PSM females divided by the overall number of females (pre- and postspawn mortality) for that spawning season.

<table>
<thead>
<tr>
<th>Site</th>
<th>Year</th>
<th>N</th>
<th>%PSM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2002</td>
<td>57</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>18</td>
<td>66.7</td>
</tr>
<tr>
<td>Longfellow Creek</td>
<td>2004</td>
<td>9</td>
<td>88.9</td>
</tr>
<tr>
<td></td>
<td>2005</td>
<td>75</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Fortson Creek</td>
<td>2007</td>
<td>114</td>
<td>0.9</td>
</tr>
</tbody>
</table>
cause postconstruction field surveys revealed the anomalous behavior and condition of spawners attracted to restored streams. Second, degraded water quality in the form of nonpoint source pollution has the potential to undermine restoration activities in urban areas that focus exclusively on physical and biological habitat processes. Third, conventional laboratory toxicity studies may not adequately capture real-world threats to fish in highly complex chemical environments such as urban streams. Finally, nonpoint source pollution is likely to become an increasingly important issue for the sustainable conservation of wild coho populations in urbanizing watersheds throughout the western United States. Models are currently being developed to identify at-risk river systems in the Puget Sound region and to forecast the potential for local coho extinctions over the next several decades of human population growth and development.

**Dissolved Copper and the Salmon Nose**

Dissolved copper is one of the most ubiquitous contaminants in stormwater runoff to aquatic systems. This reflects the many societal uses of copper in coastal watersheds. These include, for example, the incorporation of copper into roofing materials, pesticide formulations, antifoulant paint for boats, and treated wood. In urbanizing areas, one of the most important sources of copper is automobiles. Vehicle exhaust contains copper, and the action of braking releases trace amounts of copper and other heavy metals from brake pads. Copper accumulates on highways, roads, parking lots, and similar surfaces until storm events mobilize the metal in runoff. Since conventional detention and treatment systems for runoff are designed to reduce the impacts of sedimentation and altered flow, they typically do not remove dissolved-phase copper from surface waters. Therefore, because of its close association with cars and roads, copper is in many ways a signature contaminant for urban and suburban development.

Research sponsored by NOAA's Coastal Storms Program has recently focused on the impacts of dissolved copper in stormwater on salmon and steelhead in the Pacific Northwest pilot region. Copper is classically known to be highly toxic to many aquatic organisms. At high concentrations, the metal is acutely lethal to fish via a mechanism that involves the binding of copper to the gill (Niyogi and Wood 2004). Much less is known about the sublethal effects of copper, particularly following short-term exposures (i.e., on the order of hours) that are more typical of episodic stormwater runoff events. A key management concern related to runoff from impervious surfaces is whether transient, environmentally realistic exposures to copper interfere with the life history requirements of threatened or endangered salmonids in the Pacific Northwest and, more recently, in California. A related challenge has been to link sublethal effects to the survival and reproductive success of individual animals, as these processes determine the productivity and recovery potential of Endangered Species Act (ESA)-listed populations.

Recent NOAA research has focused on the salmon olfactory nervous system as an important target for dissolved-phase copper. It has been known for more than 30 years that the chemosensory system of fish is particularly vulnerable to the neurotoxic effects of copper (Hara et al. 1976; Hansen et al. 1999a). This is due, in part, to the direct contact between sensory neurons in the olfactory epithelium (the olfactory rosette; Figure 3) and pollutants in surface waters. The potential for olfactory neurotoxicity raises several important concerns for anadromous salmonids, as these species rely on chemical signals in the aquatic environment to imprint on their natal streams, detect and avoid predators, navigate during adult migrations, and synchronize their spawning. There are also several logistical advantages to focusing on the salmon nose for toxicity studies. For example, the biology of olfaction in fish has been actively studied for many years, and the basic architecture and function of the peripheral olfactory epithelium is well understood (Hara 1992) relative to many other areas of the fish nervous systems.
system. Receptor neurons are very sensitive to chemical cues, and their responsiveness to a variety of natural odorants is well documented. Finally, straightforward electrophysiological methods have been established for monitoring the effects of pollutants on the active properties of sensory neurons (Baldwin and Scholz 2005). The salmon nose is therefore an experimentally tractable system with a high degree of biological relevance for individual survival, migration, and reproduction.

Much of the recent work to date has focused on freshwater-phase juvenile salmon, as juveniles spend months to years (depending on the species) in small stream catchments that are most likely to be affected by stormwater runoff. Salmonids are known to avoid gradients of copper in aquatic habitats, such as those that might be produced by municipal point-source discharges, irrigation return flows, or direct discharges from mining activities (Hansen et al. 1999b). However, the diffuse loading of copper to streams via runoff is a form of nonpoint source pollution. In the absence of a distinct gradient, it is unlikely that salmon will be able to avoid copper from nonpoint sources.

As expected from previous work with other fish species (e.g., Harada et al. 1976), dissolved copper is a potent inhibitor of olfactory function in juvenile coho salmon (Baldwin et al. 2003). When exposed to environmentally relevant copper concentrations (0–20 parts per billion), salmon olfactory neurons become increasingly unresponsive to natural odorants (e.g., the amino acid L-serine) in a dose-dependent manner. The onset of functional anosmia occurs within the first few minutes of exposure, a window that is well within the typical duration of a stormwater pulse. Another significant finding from initial research is that the olfactory toxicity of copper is similar across receptor neurons that respond to different classes of odorants (Baldwin et al. 2003). Not surprisingly, reduced electrical activity in the peripheral sensory epithelium translates to a reduction in the response of neural networks in the olfactory forebrain (Sandahl et al. 2004). Because copper appears to be a general-purpose inhibitor of chemoreception in salmon, it has the potential to interfere with many if not all behaviors that depend on a normally functioning olfactory system.

Many studies have now shown that copper disrupts olfactory function in fish. However, the extent to which laboratory observations of physiological impairment are predictive of effects on critical behaviors has only recently been investigated. To explore the relative effects of copper on the neurobiology and behavior of juvenile coho, Sandahl et al. (2007) devised a computer-assisted imaging system to monitor the predator avoidance behavior of individual animals in response to a chemical alarm signal. Like many other species of fish,
juvenile salmon have within their skin specialized cells that contain a chemical alarm substance. When a predator attacks a fish, the mechanical tearing of the skin releases the alarm cue into the surrounding water. The cue signals a predation risk to nearby conspecifics, and neighboring fish respond with a stereotypical suite of avoidance behaviors, the most prominent of which for salmon is a tendency to become motionless (Brown and Smith 1997; Scholz et al. 2000). The alarm response of juvenile salmon is both robust and ecologically relevant and is therefore particularly well suited for investigating the olfactory toxicity of copper.

Using a combination of neurophysiological and behavioral recording methods, Sandahl et al. (2007) compared the effects of copper on olfaction and olfactory-mediated predator avoidance behaviors in juvenile coho salmon. A short-term (3 h) exposure was chosen to emulate environmental exposures to copper in stormwater runoff. The inhibitory effects of copper (at or above 2 \( \mu \)g/L) on olfactory receptor neurons correlated very closely with a loss of responsiveness to the predation cue (Sandahl et al. 2007; Figure 4). Therefore, short-term exposures to copper are sufficient to isolate juvenile salmon from important features of their sensory landscape. While copper exposures may not kill fish outright, sensory deprivation has the potential to increase mortality rates due to predation during freshwater rearing stages. This is important because even modest changes in overall rates of juvenile mortality can significantly influence the productivity of wild salmon populations (Kareiva et al. 2000).

In summary, Coastal Storms Program research on copper was designed to provide resource managers with targeted new information about one of the most common contaminants, urban stormwater runoff. The findings should have applications in geographical areas outside of the Pacific Northwest. For example, the results in coho salmon have recently been extended to steelhead (D. Baldwin and N. Scholz, NOAA Fisheries, Northwest Fisheries Science Center, unpublished results), which are presently endangered in Southern California. Future research chal-

![Figure 4. Exposure to copper diminished both olfactory sensitivity and alarm behavior in juvenile coho (figure from Sandahl et al. 2007). Electro-olfactogram (EOG) responses to skin extract (10 \( \mu \)g protein/L) were inhibited at increasing copper concentrations. Copper exposure also reduced the behavioral alarm response elicited by 0.1 \( \mu \)g protein/L of skin extract in a dose-dependent manner. Paired physiological and behavioral response means were highly correlated (i.e., fish with reduced olfactory sensitivity showed reduced alarm behavior). Error bars represent one standard error bar.](image-url)
Challenges include linking the behavioral effects of copper to more refined estimates of individual survival and reproductive success (e.g., field studies of homing and straying behavior in migratory adults [Scholz et al. 2000]). These challenges notwithstanding, it is now reasonably well established that copper in stormwater runoff has the potential to limit the recovery of ESA-listed salmon populations. This is likely to place an increasingly important emphasis on stormwater management strategies that reduce the loading of copper and other dissolved toxicants.

**Polycyclic Aromatic Hydrocarbons and the Developing Fish Heart**

Fossil fuel use contributes a large suite of potentially toxic compounds to runoff from impervious surfaces. Although polycyclic aromatic hydrocarbons (PAHs; Figure 5A) represent a small fraction of the total hydrocarbon mass of fossil fuel products, some PAHs are known to be highly toxic to fish. The increase in the number of motor vehicles over the last decade has resulted in a corresponding increase in the loading of PAHs to aquatic habitats (Van Metre et al. 2000; Lima et al. 2002; National Research Council 2003; Partridge et al. 2005). Airborne PAH levels are generally proportional to human population densities (Hafner et al. 2005), and deposition of airborne PAHs is now the largest source of aquatic contamination (Van Metre et al. 2000; Lima et al. 2002; Van Metre and Mahler 2003; Li and Daler 2004). In addition, runoff from impervious surfaces can also contain PAHs from other sources, such as gasoline, lubricating oils, and coal tar- or asphalt-based parking lot sealants (Mahler et al. 2005). Although stormwater contributes a smaller fraction of the total aquatic PAH loadings relative to other routes of transport (e.g., direct absorption of gas-phase PAHs [Gigliotti et al. 2002, 2005]), storm events can raise PAH levels in streams dramatically, thereby contributing significantly to the levels of PAHs in estuaries and other nearshore areas, particularly in sediments (Hoffman et al. 1984; Crunkilton and DeVita 1997; Menzie et al. 2002; Hwang and Foster 2006; Kimbrough and Dickhut 2006; Stein et al. 2006).

Although the sources, transport and accumulation of PAHs in coastal habitats have been extensively monitored, the biological impacts of PAHs on fish have received comparatively less attention. Moreover, the potential role for PAHs in stormwater as a limiting factor for the productivity of fisheries resources is not well understood. However, two intensive lines of investigation in previous years have shown that PAHs are a potential threat to the health of fish. The first is the well-documented effects of carcinogenic PAHs on benthic fish living in close association with historically contaminated sediments in the nearshore marine environment. The second is the impact of crude oil-derived PAHs on fish at early life history stages, an understanding gained to a large degree from research conducted on Pacific herring Clupea pallasi and pink salmon after the Exxon Valdez oil spill in Prince William Sound, Alaska. Oil spill research on herring and salmon led to the discovery of previously unrecognized pathways of toxicity involving the fish heart. More generally, these studies have underscored how little is known about the biological effects of PAHs. A much more sophisticated view of PAH toxicity has been emerging in recent years, and, with this, a recognition that predicting the effects of this complex family of compounds will require an expanded research effort. As discussed below, these data gaps have been a key focus for new research sponsored by the NOAA’s Coastal Storms Program.

PAHs occur in stormwater in highly complex mixtures that vary considerably in composition depending on the relative contributions from petrogenic (e.g., oil) or pyrogenic (i.e., combustion) sources (Figure 5B). In general, petroleum products are enriched with low molecular weight PAHs containing two or three rings, while fuel combustion products contain a higher percentage and variety of high molecular weight compounds with four rings or more (Wang et al. 2003; Lima et al. 2005). Because most low molecular weight PAHs are found as multiple alkylated
isomers, PAH mixtures can contain hundreds of individual compounds. This high degree of complexity raises at least three major research challenges that are highly relevant to the conservation of fisheries. The first is delineating the relationship between the toxicity of indi-
vidual PAH compounds and the toxicity of complex mixtures. It is now becoming clear that different compounds affect the health of fish via different biological pathways. Are there toxicological effects that stem uniquely from PAH mixtures, and do these vary with the overall composition of mixtures? The second is to link low-level PAH inputs in stormwater to the health and survival of individual fish and, by extension, to the productivity of vulnerable fish populations. This challenge is identical in most respects to the research objectives identified for dissolved copper in the preceding section. The third is to develop new diagnostic tools that can be used to assess the health of PAH-exposed fish under natural conditions. These new biological indicators, which are increasingly needed for ecosystem-based monitoring systems throughout coastal regions of the United States, should ideally be both sensitive and specific for PAH toxicity.

Studies on the toxicology of PAHs began early in the 20th century with the identification of benzo[a]pyrene as the primary causative agent of cancers associated with coal tar exposure (Phillips 1983). High molecular weight (five- and six-ring) PAHs such as benzo[a]pyrene are potent procarcinogens activated by metabolism through the aryl hydrocarbon receptor (AHR)–cytochrome P4501A (CYP1A) pathway. The AHR is a ligand-activated transcription factor that controls the expression of a battery of genes encoding enzymes that convert PAHs to watersoluble derivatives that are excreted, including mixed function oxygenases such as CYP1A family members (Schmidt and Bradfield 1996; Nebert et al. 2004). CYP1A oxidizes PAHs, converting some to reactive intermediates that can alkylate DNA (and other macromolecules), leading to somatic mutations and neoplasia. One of the most clearly delineated cause-effect relationships for PAH impacts on fish is the association of exposure to PAH-contaminated sediment with neoplastic liver lesions in benthic species such as English sole Pleuronectes vetulus (Myers et al. 2003). PAH toxicity is generally considered in these terms, but as described below, this traditional view belies the true complexity of this large family of compounds.

The toxicity of low molecular weight PAHs, which are weak AHR ligands, was largely unappreciated until the 1989 Exxon Valdez oil spill in Prince William Sound, Alaska. Similarly, this event shifted the research focus from adult fish to early life history stages. The Exxon Valdez oil spill coincided with the seasonal spawning of Pacific herring and was followed shortly after by the spawning of pink salmon. By nature of their preferred spawning habitat, these two species deposited eggs in areas that were most heavily contaminated by the spill (Peterson et al. 2003; Short et al. 2003). Alaska North Slope crude oil (and most other crude oils) contain a PAH fraction that is dominated by two- and three-ring compounds, but in which the carcinogenic high molecular weight compounds are absent or present in only trace amounts (Wang et al. 2003). Over the last decade, many studies examining the effects of dissolved petroleum-derived PAHs on development of herring, pink salmon, and other fish species documented a common malformation syndrome as well as significant sublethal effects in the absence of malformations, including reduced growth and survival to adulthood (Marty et al. 1997; Carls et al. 1999; Heintz et al. 1999, 2000; Couillard 2002; Peterson et al. 2003). Other studies in recent years have also shown that fish embryos and larvae are highly sensitive to PAH mixtures from a variety of other sources, including creosote wood preservatives, oil sands, and sediments impacted by urbanization (Vines et al. 2000; Ownby et al. 2002; Colavecchia et al. 2004; Wassenberg and Di Giulio 2004; Sundberg et al. 2005).

Recent research supported by NOAA’s Coastal Storms Program has focused on using the zebrafish Danio rerio model system to identify the specific contributions of different PAHs to early life stage toxicity in fish. Zebrafish have become a major experimental model in environmental health research. A systematic comparison of the effects of individual PAHs to weathered crude oil led to the identification of at least three distinct modes of action for PAH developmental toxicity in
fish (Incardona et al. 2004, 2005, 2006). This was largely possible due to the rapid development and accessibility of the zebrafish embryo allowing continuous monitoring of organogenesis and to the suite of genetic and molecular tools associated with the zebrafish system (Shin and Fishman 2002). Notably, research using zebrafish has produced a sophisticated understanding of the links between form and function in the developing fish heart (Glickman and Yelon 2002), which was found to be the primary target of low molecular weight PAH toxicity (Incardona et al. 2004).

Weathered crude oil and tricyclic PAHs (fluorene, dibenzothiophene, and phenanthrene) were found to cause cardiac dysfunction soon after the heart becomes active, which has multiple secondary consequences for cardiac morphogenesis. The heart is one of the first organs to be functional in the embryos of fish and other vertebrates. Because cardiac function and morphogenesis are inextricably linked, any disruption of cardiac physiology during early development ultimately impacts the subsequent shape of the heart. Consequently, developing embryos exposed continuously to cardiac toxins have hearts that fail to develop and become atretic (string-like), ultimately leading to the structural defects previously associated with exposure to petroleum hydrocarbons. In this case, genetic analysis using zebrafish embryos indicated that the AHR-CYP1A pathway played a protective rather than causal role in toxicity and that these tricyclic PAHs are directly cardiotoxic (Incardona et al. 2004, 2005). Potential targets for petrogenic tricyclic PAHs include ion channels, structural proteins, or regulatory enzymes involved in the cardiac contraction cycle.

In distinct contrast to individual tricyclic PAHs or weathered crude oil, the four-ring pyrogenic PAH ben[a]anthracene acts through the AHR pathway to cause a different type of heart malformation, in the same manner as more potent AHR ligands such as dioxin (Prasch et al. 2003; Teraoka et al. 2003; Carney et al. 2004; Antkiewicz et al. 2005). A third type of toxicity was observed with exposure to another pyrogenic four-ring compound, pyrene, which resulted in systemic toxicity that was dependent on metabolism by CYP1A in the liver (Incardona et al. 2006). Other high molecular weight PAHs that are abundant in stormwater have not yet been characterized at this level of detail, although their potency as AHR ligands suggests that they would act similarly to ben[a]anthracene.

In summary, deciphering the toxicology of PAH mixtures in stormwater has proven to be a complex research challenge. Investigations to date have shown that the developing fish heart is vulnerable to a variety of impacts from multiple members of the PAH family, each acting through distinct cellular pathways. These findings have been a major step forward in terms of understanding how PAHs affect the health of fish, alone and in mixtures. The discovery that the heart is a primary target organ also represents a significant advance towards the eventual goal of developing new diagnostic tools for assessing the health of fish throughout coastal areas of the United States. However, there are still a large number of PAH compounds whose individual toxicity is unknown, and PAH mixtures in stormwater are more complicated than purely petrogenic mixtures represented by oil spill models. The immediate embryonic effects of exposure to a handful of individual PAHs and petrogenic PAH mixtures are now understood in fair detail, but more information is needed on the long-term impacts of transient sublethal exposure and effects of exposure in later larval and juvenile stages. The effects of more complex mixtures representative of urbanized sediments are also unknown, and it is unclear how pathways of petrogenic and pyrogenic PAH toxicity might interact. Research findings in zebrafish need to be validated in native species. To this end, studies on early life stage Pacific herring and California halibut Paralichthys californicus are now underway as part of the NOAA’s Coastal Storms Program. Ultimately, a full appreciation of the impacts of urbanization and stormwater runoff on key marine and aquatic resources will require a more detailed un-
derstanding of mechanisms of PAH toxicity. Because survival of early life history stages is crucial for recruitment to adult populations, particularly at low population sizes (Myers et al. 1999), there is considerable potential for population-level impacts stemming from the myriad sublethal physiological effects of these compounds.

**Achieving Resilience in Coastal Ecosystems**

Resilience, a central theme in ecology, refers to the capacity of ecosystems to maintain structure and function in the face of a disturbance or a sustained forcing pressure such as development (Holling 1973; Walker et al. 2004). Human activities such as overfishing can decrease resilience, thereby increasing the potential for an ecosystem to undergo undesired regime shifts (Folke et al. 2004). For example, a coral reef in a highly impacted area may become more susceptible to disease and bleaching, causing a shift to a reef dominated by algae (Folke et al. 2004). As is evident from recent research on coho salmon in Pacific Northwest urban streams, nonpoint source pollution poses some important and difficult challenges to local efforts aimed at improving the resilience of aquatic habitats.

Effective mitigation strategies are essential in vulnerable areas that are impacted by degraded runoff. Coastal areas are not only important in terms of natural ecosystem function, they are also highly coveted by humans for economic and esthetic reasons and thus can be referred to as social-ecological systems. Therefore, as a human element is introduced, the ability of the resource managers to mitigate for resilience and prevent the ecosystem from reaching a critical threshold becomes integral (Walker et al. 2004). Stormwater management techniques may need to vary according to the vulnerability or current state of the target ecosystem. For some specific land uses, it may be less important to document the nature and extent of the toxicological injury to fish than to conduct research to help understand which source control measures are most effective. This includes, for example, using biologically based methods to monitor the effectiveness of stormwater filtration, riparian buffers, low impact development, and other mitigation options. In most cases, source control for contaminants is very expensive, and the benefits that these resource management options provide to aquatic species are not well understood.

As an example of NOAA's sponsorship of new technologies to promote resilience, the Cooperative Institute for Coastal and Estuarine Environmental Technology has funded targeted research on the development and efficiency of stormwater management techniques that help protect nearshore aquatic environments from degraded runoff (http://ciceet.unh.edu/). Among their funding recipients is the University of New Hampshire Stormwater Center (www.unh.edu/erg/cstev/). They have installed 11 different stormwater devices at their research facility and provide fact sheets on the results of their research for planners, engineers, researchers, and restoration practitioners who are deciding among technology choices. Information on manufactured devices (such as infiltration devices and manhole retrofits), filters, bioretention systems, gravel wetlands, and porous pavement also give practitioners the ability to compare technologies and choose the one that best fits their land use and site in terms of which contaminants need to be removed, project budget, and available physical space.

**Future Research Priorities**

Society currently lacks much of the scientific information needed to manage nonpoint source pollution and reduce the impacts of toxic stormwater runoff on coastal fisheries. The rapid rate of current development along the coastlines of the United States (Paul and Meyer 2001; Beach 2002; Nilsson et al. 2003; Crosetti et al. 2004) represents a persistent forcing pressure that will continue to degrade the quality of spawning, rearing, and migratory habitats for fish. In addition, the suc-
cess or failure of habitat restoration activities throughout the country will hinge, in part, on an understanding of the limitations imposed by nonpoint source pollution. This is true for restoration in urban areas (Simenstad et al. 2005) as well as in geographical regions that will become urban or suburban in the next few decades (Bernhardt and Palmer 2007). The trend towards more impervious surfaces in coastal watersheds is going to make it increasingly difficult to maintain resilient fish populations. Unfortunately, climate change is likely to complicate this task even further. Climate change and pollution are expected to combine in terms of their threats to fisheries (Schindler 2001). This is particularly true for terrestrial runoff, as this is largely driven by the frequency and intensity of storms. Below, we identify priority areas for new research, modeling, and risk assessment. Advances in each of these areas will substantially improve the scientific foundation for sustaining fish populations and the ecosystems that support them.

A Need for More “Eco” in Ecotoxicology

Assessing and predicting the community-level effects of toxics is a challenge that is becoming progressively more important in community ecology (Rohr et al. 2006). Contaminants can have a wide variety of indirect effects on fish and fisheries in coastal areas. Trophic cascades are a common source of indirect effects (Fleeger et al. 2003), and these can lead in turn to decreased prey availability and starvation (Bennet et al. 1995) among other outcomes. Other examples of indirect effects include increased vulnerability to predation (Labenia et al. 2006), disease susceptibility and pathogen-induced mortality (Arkoosh et al. 2001), and competition with nonnative, contaminant-tolerant species. Understanding the impacts of terrestrial runoff on these and other ecological processes will invariably require more “eco” in the field of ecotoxicology, that is, combining a multispecies approach to research with new advances in community-based modeling.

Studying Individual Animals while Managing Populations

Conservation and recovery planning for vulnerable coastal fisheries typically occurs at the scale of natural populations and, increasingly, at the level of communities and ecosystems (Arkema et al. 2006). An enduring challenge in ecotoxicology is to link the health of individual animals to these higher scales. To this end, population models are playing an increasingly important role (Sromberg and Meador 2006). To align empirical studies with quantitative models, future research should provide the biological and demographic information necessary to model how real-world exposures to toxics in terrestrial runoff might alter the lifetime reproductive success of individuals within a population. These population models can then be used to forecast future extinction risks associated with nonpoint source pollution and also to estimate the relative importance of surface runoff as an obstacle to habitat recovery in coastal watersheds and estuaries.

Evaluating Single Chemicals in a World of Multiple Stressors

For decades, conventional toxicological investigations have typically focused on the adverse health effects of individual contaminants. As shown by the earlier example of copper and salmon olfaction, this approach can yield useful information on priority contaminants in stormwater. In the real world, however, nonpoint source pollution almost always involves the delivery of complex mixtures of chemicals to aquatic habitats. These mixtures, combined with other (nonchemical) stressors, can impact the health of fish in ways that are highly unpredictable and still poorly understood. For example, an interactive effect of multiple stressors may explain the unexpected mortality of adult salmon returning to spawn in Pacific Northwest urban streams. Moreover, since current-use pesticides frequently occur as mixtures in streams (Hoffman et al. 2000), Coastal Storms Program research has focused on the interactive toxicity of pesticides in mixtures (Scholz et al. 2006). This has led recently
to the unexpected finding that some mixtures of common insecticides produce synergistic (i.e., greater than additive) neurotoxicity and mortality in juvenile salmon (C. Laetz and N. Scholz, NOAA Fisheries, Northwest Fisheries Science Center, unpublished results). Also, as noted earlier, the impacts of contaminants on fish survival can be proportionately greater when fish are coexposed to toxics and another environmental stressor such as a pathogen (Arkoosh et al. 2001; Clifford et al. 2005). Understanding and minimizing the effects of multiple stressors is one of the major research challenges in modern ecotoxicology (Eggen et al. 2004). The associated complexity is one of the primary reasons why resource management agencies have had such difficulty confronting nonpoint source pollution (U.S. Commission on Ocean Policy 2004).

A Need for Species-Centric Versus Chemical-Centric Risk Assessment

Ecological risk assessment is a widely used tool in ecotoxicology. Although risk assessment is a relatively young discipline with many different applications to date, most risk assessments involving toxic substances are chemical-centric. This usually involves assessing the potential toxicity of a single chemical to a wide diversity of aquatic species, often using a classical comparative metric such as the median lethal concentration (LC50). Chemical-centric risk assessments are typically developed to inform specific types of resource management activities. These include, for example, the U.S. Environmental Protection Agency's registration or re-registration of a pesticide, or the development of aquatic life criteria under the Clean Water Act. While chemical-centric risk assessments may be useful for these specific purposes, they are very poorly suited for evaluating the impact of nonpoint source pollution on at-risk fish and fisheries. LC50-based risk assessments may not adequately capture important ecological considerations, including delayed effects, indirect effects, and impacts on sensitive species or life history stages. In the case of threatened or endangered fish populations (i.e., Pacific salmon), the biological requirements of the species are a more appropriate conceptual basis for risk assessments involving polluted stormwater. Species-specific risk assessments are desirable because they incorporate a higher degree of ecological realism. They emphasize the survival potential of a fish in the real world, where a particular chemical is only one of many environmental stressors.

Identifying Cost-Effective Pollution Control Measures and Mitigation Strategies that Work

With a few exceptions, the sources of many nonpoint source pollutants in aquatic habitats are reasonably well known. These include, for example, the heavy metals and petroleum hydrocarbons that originate from motor vehicles. Engineered solutions to surface runoff typically focus on reducing toxic loads. Loading reductions will invariably improve overall water quality, but they may not go far enough to ensure resilience if there is still a sufficient amount of toxic runoff to impact fish and their habitats. Therefore, it will be important to combine emerging technologies for stormwater management with targeted toxicological research, ideally involving the in situ monitoring of fish health as well as other indicators of ecological response.

Acknowledgments

We thank NOAA's Coastal Services Center and Coastal Storms Program, the U.S. Fish and Wildlife Service's Environmental Contaminants Program, the California Department of Fish and Game Oil Spill Response Trust Fund/Oiled Wildlife Care Network, and the City of Seattle—Seattle Public Utilities for funding the work described in the three case studies. We would also like to thank our collaborators with the City of Seattle (Laura Reed, Katherine Lynch, and Keith Kurko), the U.S. Fish and Wildlife Service (Jay Davis), King County (Dean Wilson, Fritz Grothkopp, and Lorin Reinelt), and NOAA Fisheries (Jana Labenia, Jennifer McIntyre, Mark Myers, Linda Rhodes, Gina Ylitalo, Julann Sprom-
berg, Blake Feist, Heather Day, Tiffany Linbo, David Baldwin, Cathy Laetz, Carla Stehr, Mark Carls, and Tracy Collier), as well as countless volunteers who assisted with field surveys for the prespawn mortality investigation.

References


Oceanic and Atmospheric Administration, National Ocean Service, Management and Budget Office, Special Projects, Silver Spring, Maryland.


(Oncomelania gubhuscha) embryos incubating downstream from weathered Exxon Valdez crude oil. Environmental Toxicology and Chemistry 18(3):494–503.


Chemosensory Deprivation in Juvenile Coho Salmon Exposed to Dissolved Copper under Varying Water Chemistry Conditions

JENIFER K. MCINTYRE,†
DAVID H. BALDWIN,‡
JAMES P. MEADOR,∥ AND
NATHANIEL L. SCHOLZ*†

School of Aquatic and Fishery Sciences, University of Washington, Box 355020, 1122 NE Boat Street, Seattle, Washington 98105, and NOAA Fisheries, Northwest Fisheries Science Center, Ecotoxicology and Environmental Fish Health Program, 3723 Montlake Boulevard E., Seattle, Washington 98112.

Received June 29, 2007. Revised manuscript received November 14, 2007. Accepted November 26, 2007.

Dissolved copper is an important nonpoint source pollutant in aquatic ecosystems worldwide. Copper is neurotoxic to fish and is specifically known to interfere with the normal function of the peripheral olfactory nervous system. However, the influence of water chemistry on the bioavailability and toxicity of copper to olfactory sensory neurons is not well understood. Here we used electrophysiological recordings from the olfactory epithelium of juvenile coho salmon (Oncorhynchus kisutch) to investigate the impacts of copper in freshwaters with different chemical properties. In low ion strength artificial freshwater, a short-term (30 min) exposure to 20 μg/L dissolved copper reduced the olfactory response to a natural odorant (10−3 M L-serine) by 82%. Increasing water hardness (0.2–1.6 mM Ca) or alkalinity (0.2–3.2 mM HCO3−) only slightly diminished the inhibitory effects of copper. Moreover, the loss of olfactory function was not affected by a change in pH from 8.6 to 7.6. By contrast, olfactory capacity was partially restored by increasing dissolved organic carbon (DOC; 0.1–0.6 mg/L). Given the range of natural water quality conditions in the western United States, water hardness and alkalinity are unlikely to protect threatened or endangered salmon from the sensory neurotoxicity of copper. However, the olfactory toxicity of copper may be partially reduced in surface waters that have a high DOC content.

Introduction

The neurotoxicity of dissolved copper to the sensory systems of fish has been the focus of considerable research over the past few decades. The olfactory epithelium of fish, which contains ciliated olfactory sensory neurons (OSNs) embedded in a layer of mucus, is in direct contact with surface waters and is therefore particularly vulnerable to the toxic effects of copper and other pollutants. Numerous studies have shown that copper diminishes the sensitivity and responsiveness of OSNs to chemical cues (1). Moreover, low-level copper exposures interfere with predator avoidance behaviors that are important for survival (2–5). In salmon, the relationship between a loss of sensory capacity (as measured using electrophysiological recordings) and behavioral impairment is highly correlated (0–20 μg/L Cu; 3). At higher exposure concentrations (≥20 μg/L) copper causes the degeneration of the sensory epithelium (5, 7). These effects manifest on a time scale of hours (3, 6) or even minutes (8). This makes the fish olfactory system a particularly suitable end point for evaluating the toxicological effects of copper in the aquatic environment.

Copper is classically known to disrupt osmoregulation in fishes by interfering with sodium uptake in the gill (9, 10). This mechanism causes acute lethality at high exposure concentrations. The toxicity of copper to the fish gill epithelium is influenced by the hardness, alkalinity, pH, and dissolved organic matter (DOM) content of water. These constituents can protect against the acute toxicity of free ionic copper by either competing for binding sites at the sodium transporter (cations) or by reducing the bioavailability of copper via complexation (anions and DOM) (Figure S1 in the Supporting Information). The biotic ligand model (BLM) is widely used to predict the degree to which these water chemistry parameters will protect against acute lethality caused by copper binding to the fish gill (11–15). The BLM also provides a conceptual basis for the U.S. Environmental Protection Agency’s (EPA) approach to risk assessment for metals (14), and is being incorporated directly into the national copper aquatic life criterion (15).

The extent to which the BLM can be used to estimate the sublethal neurobehavioral toxicity of copper in fish is less clear. The gill epithelium and olfactory epithelium are very different in terms of both architecture and physiological function (Figure S1). In contrast to the cellular processes underlying osmoregulation and gas exchange, the primary function of OSNs is to transduce odorant binding and receptor activation to electrical signals (action potentials) that are ultimately propagated to the olfactory forebrain. Olfactory signal transduction involves a complicated and finely tuned biochemical cascade (Figure S1) (16). The "ligand" for copper in the fish nose has not been identified, but may involve one or more proteins that are unrelated to sodium exchange (17). Accordingly, the predictive capacity of the BLM, as originally developed for gill-mediated lethality, may or may not extend to sublethal effects on the fish olfactory system.

To explore the effect of water chemistry on olfactory neurotoxicity, we used a well-established neurophysiological approach (18, 19) to monitor the olfactory neurotoxicity of copper in juvenile coho salmon (Oncorhynchus kisutch), while varying water hardness, alkalinity, pH, and dissolved organic carbon (DOC). We exposed fish to copper for 30 min at 20 μg/L (0.315 μM), a concentration that is (1) environmentally relevant for salmon exposed to copper in urban runoff (20) and (2) likely to produce a robust olfactory inhibition as a baseline for assessing the potential protective effects of different water chemistries (18, 19). To emulate the natural diversity of water chemistry conditions in Pacific salmon habitats, we water each approach to encompass the range of values reported by the U.S. Geological Survey’s National Water Quality Assessment Program for freshwater streams and rivers in the Puget Sound region as well as the Willamette, Yakima, and Sacramento River drainage basins. These surface waters provide spawning and rearing habitat for salmon populations that are listed as either threatened or endangered under the U.S. Endangered Species Act (ESA; see NOAA Office of
TABLE 1. Water Quality Parameters for Artificial Freshwaters, Deionized Water (DW), Dechlorinated City Water (DCW), and Hatchery Water (HW), including pH and Concentrations of Major Inorganic ions, Alkalinity, Hardness, Dissolved Organic Carbon (DOC), Dissolved Copper (DCu), and Ionic Copper (ICu) *

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ca (mM)</th>
<th>Mg (mM)</th>
<th>Na (mM)</th>
<th>K (mM)</th>
<th>Cl (mM)</th>
<th>SO₄²⁻ (mM)</th>
<th>HCO₃⁻ (mg/L as CaCO₃)</th>
<th>DOC (mg/L)</th>
<th>DCu (µg/L)</th>
<th>ICu (µg/L)</th>
<th>ICu % of DCu</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>0.001d</td>
<td>0.002</td>
<td>0.003</td>
<td>0.028</td>
<td>0.017</td>
<td>0.001</td>
<td>1</td>
<td>1</td>
<td>0.25</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>low-ion</td>
<td>0.2</td>
<td>0.2</td>
<td>7.5</td>
<td>0.203</td>
<td>0.097</td>
<td>0.188</td>
<td>0.021</td>
<td>13</td>
<td>0.1</td>
<td>14.05</td>
<td>5.08</td>
</tr>
<tr>
<td>Caₜ</td>
<td>0.4</td>
<td>0.2</td>
<td>7.5</td>
<td>0.432</td>
<td>0.099</td>
<td>0.199</td>
<td>0.021</td>
<td>15</td>
<td>0.3</td>
<td>13.20</td>
<td>4.88</td>
</tr>
<tr>
<td>Ca₂</td>
<td>0.8</td>
<td>0.2</td>
<td>7.5</td>
<td>0.898</td>
<td>0.081</td>
<td>0.183</td>
<td>0.019</td>
<td>11</td>
<td>0.9</td>
<td>13.03</td>
<td>4.99</td>
</tr>
<tr>
<td>Ca₃</td>
<td>1.6</td>
<td>0.2</td>
<td>7.8</td>
<td>1.820</td>
<td>0.093</td>
<td>0.183</td>
<td>0.021</td>
<td>13</td>
<td>1.0</td>
<td>14.97</td>
<td>5.22</td>
</tr>
<tr>
<td>Alk₁</td>
<td>0.2</td>
<td>0.8</td>
<td>8.2</td>
<td>0.211</td>
<td>0.090</td>
<td>0.178</td>
<td>0.013</td>
<td>40</td>
<td>3.0</td>
<td>16.15</td>
<td>0.64</td>
</tr>
<tr>
<td>Alk₂</td>
<td>0.2</td>
<td>1.6</td>
<td>8.5</td>
<td>0.197</td>
<td>0.091</td>
<td>0.151</td>
<td>0.014</td>
<td>29</td>
<td>0.8</td>
<td>14.80</td>
<td>0.17</td>
</tr>
<tr>
<td>Alk₃</td>
<td>0.2</td>
<td>3.2</td>
<td>8.6</td>
<td>0.200</td>
<td>0.084</td>
<td>0.308</td>
<td>0.015</td>
<td>60</td>
<td>0.9</td>
<td>16.40</td>
<td>0.07</td>
</tr>
<tr>
<td>Alk₄</td>
<td>0.2</td>
<td>3.2</td>
<td>7.6</td>
<td>0.196</td>
<td>0.088</td>
<td>0.308</td>
<td>0.014</td>
<td>137</td>
<td>0.9</td>
<td>16.33</td>
<td>0.82</td>
</tr>
<tr>
<td>FA₁</td>
<td>0.2</td>
<td>2.5</td>
<td>7.4</td>
<td>0.183</td>
<td>0.086</td>
<td>0.178</td>
<td>0.022</td>
<td>27</td>
<td>0.9</td>
<td>13.07</td>
<td>0.45</td>
</tr>
<tr>
<td>FA₂</td>
<td>0.2</td>
<td>2.5</td>
<td>7.2</td>
<td>0.181</td>
<td>0.086</td>
<td>0.181</td>
<td>0.022</td>
<td>27</td>
<td>0.9</td>
<td>13.07</td>
<td>0.45</td>
</tr>
<tr>
<td>FA₃</td>
<td>0.2</td>
<td>2.5</td>
<td>7.1</td>
<td>0.184</td>
<td>0.089</td>
<td>0.187</td>
<td>0.021</td>
<td>27</td>
<td>0.9</td>
<td>13.07</td>
<td>0.45</td>
</tr>
<tr>
<td>NGC</td>
<td>0.2</td>
<td>2.5</td>
<td>7.1</td>
<td>0.181</td>
<td>0.086</td>
<td>0.181</td>
<td>0.021</td>
<td>27</td>
<td>0.9</td>
<td>13.07</td>
<td>0.45</td>
</tr>
<tr>
<td>DP</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.m.</td>
<td>n.m.</td>
<td>n.m.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>DCW</td>
<td>0.400</td>
<td>0.078</td>
<td>0.13</td>
<td>0.01</td>
<td>0.194</td>
<td>0.03</td>
<td>0.84</td>
<td>42</td>
<td>0.9</td>
<td>0.20</td>
<td>n.m.</td>
</tr>
<tr>
<td>HW</td>
<td>1.022</td>
<td>0.11</td>
<td>6.957</td>
<td>0.11</td>
<td>1.746</td>
<td>0.33</td>
<td>1.00</td>
<td>150</td>
<td>1.1</td>
<td>4.27</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* For measured parameters, values are the mean of three replicate samples. The measured concentrations of conventional cations were 93 ± 10% (mean ± SD) of nominal values. Dissolved organic carbon (DOC) concentrations were 0.11 ± 0.01 mg/L in AFW in the absence of added DOM and 6.03 ± 0.13 mg/L in AFW containing FA. The DOC content of AFW containing 10 mg/L NOM was lower than that of AFW containing FA (4.11 ± 0.23 mg/L DOC). Measured concentrations of dissolved copper were 80 ± 9% (mean ± SD) of nominal values. All alkalinity present as bicarbonate. ionic copper concentrations from MINTEQ modeling at test temperature (13 °C). Reporting limit. Calculated from alkalinity. pH lowered with HCl. All water quality parameters in DI were below reporting limits. Not measured.

of Protected Resources, http://www.nmfs.noaa.gov/pr/species/fish/ for current species listings). This approach allowed us to assess the extent to which the neurobehavioral toxicity of dissolved copper to ESA-listed salmonids may be altered in river systems with different water chemistries.

Materials and Methods

Animals: Juvenile coho salmon (n = 80; 188 ± 2 mm, 71 ± 2 g [mean ± SEM]) were reared at the Northwest Fisheries Science Center (Seattle, WA) and maintained under ambient lighting in dechlorinated municipal city water adjusted for hardness and pH (DCW; Table 1). Prior to copper exposures, fish were acclimated overnight (14–18 h) in 30 l glass aquaria containing 30 l of the appropriate artificial fresh water (AFW; see below). Acclimation tanks were aerated and maintained under static conditions at 13 °C with ambient light.

Artificial Fresh Water: We used data from the U.S. Geological Survey’s National Water Information System Web Site (NWISWeb; http://nwis.waterdata.usgs.gov/nwis) to identify a natural range of water chemisties in freshwater salmon habitats throughout the western United States. This included more than a decade of surface water quality monitoring (1990–2003) for streams in the Puget Sound region as well as the Willamette, Yakima, and Sacramento River basins. Monitoring data were drawn from 7–24 sites (5–21 streams) for each of the four basins. The nominal compositions of the different artificial fresh waters (AFW) are shown in Table 1.

To establish a baseline toxicological response, juvenile coho were exposed to copper in a low ionic strength AFW (0.2 mM Ca, 0.2 mM Na, 0.1 mM Mg, 0.01 mM K; Table 1). Hardness was increased by adding calcium as CaCl₂ (to 0.4, 0.8, 1.6 mM Ca). Although in natural waters hardness is influenced by cations other than calcium (e.g., Mg²⁺), the term “hardness” is used interchangeably with calcium concentration here. Alkalinity was increased by adding bicarbonate as NaHCO₃ (to 0.8, 1.6, 3.2 mM HCO₃⁻). In the highest alkalinity water, pH was reduced from 8.6 to 7.6 with 0.37 M HCl. Reference fulvic acid (FA) and natural organic matter (NOM) isolated from the Suwannee River, Georgia were obtained from the International Humic Substances Society (IHSS; St. Paul, MN, http://www.ihss.gatech.edu) and were added to AFW to increase DOM (to 2.5, 5, 10 mg/L FA, or 10 mg/L NOM).

For dissolved copper exposures, AFW was made daily from ion stock solutions using salts (CaCl₂·2H₂O, NaHCO₃, MgSO₄·7 H₂O, KCl) from Sigma-Aldrich (www.sigma-aldrich.com). The exception was AFW containing DOM, which was prepared 24 h prior to use to allow the amended copper and DOM to equilibrate (24). Copper was added to AFW from a stock solution of 1 g/L Cu (2.68 g/L CuCl₂·2H₂O) to reach a nominal concentration of 25 µg/L (0.315 µM). All stock solutions were prepared weekly and stored at 5°C. Deionized water (DI) was used in all preparations. Measured values for each ionic constituent, alkalinity, pH, hardness, DOC, dissolved copper, and ionic copper are shown in Table 1.

Electrophysiological Recordings: Odor-evoked field potential recordings were used to measure the relative impacts of dissolved copper in different AFWs. The experimental procedures for recording electro-olfactograms (EOGs) have been published previously (19); see also (3, 8, 22). Trials began by perfusing the olfactory chamber with AFW for 15 min. Over the subsequent 15 min, preexposure EOGs were obtained by delivering a natural odorant (10⁻³ M L-serine) to the sensory epithelium for 10 s every 5 min. Following 30 min of continuous AFW perfusion, the olfactory chamber was perfused with AFW containing 20 µg/L Cu for 30 min. The periodic presentation of odor pulses continued during the exposure interval. The maximum negative phasic displacement from the electrical baseline (the odor-evoked EOG) was used to quantify the olfactory response (in mV). For each individual fish, the amplitude of the postexposure EOG was divided by the pre-exposure amplitude to calculate a relative olfactory response (Figure S2). EOG recordings were collected from n = 5–10 fish for each AFW except for AFW containing NOM (n = 4).

Chemical Analyses: Measurements of conventional ions and dissolved copper were conducted on triplicate samples.
of AFW collected from the experimental exposure system (the tube delivering solutions to the salmon olfactory chamber). The DI water used to make AFW was also analyzed as were the waters used to rear and maintain the fish (hatchery water) and the supply of dechlorinated city water (DCW) to the hatchery. Analyses for conventional ion composition, hardness, and alkalinity were conducted by an EPA-certified laboratory (AmTest Inc., Redmond, WA) using standard methods. Samples containing dissolved copper were analyzed by an EPA-certified laboratory (Frontier Geosciences Inc., Seattle, WA) using inductively coupled plasma mass spectrometry (ICP-MS) with a detection limit of 0.04 μg/L (see ref 22).

For a subset of AFWs (low-ion control, 0 FA, 2.5 FA, 5 FA, 10 FA, 10 NOM, HW), dissolved organic carbon samples were analyzed by Shimadzu TOC-Vcsh (University of Washington Oceanography Technical Services; Seattle, WA). A Fisher Scientific Accumet AB15 (calibrated daily) was used to measure the pH of AFW samples.

**Copper Ion Measurement.** Free ionic copper concentration [Cu²⁺] was measured at room temperature (22 °C) on AFW collected from the olfactory perfusion system with a cupric ion selective electrode (Cu ISE) (Orion 94-29; Thermo Orion Inc., Beverly, MA) paired with a double junction reference electrode (Orion 90-02) and an ISE meter (Orion 720A). The copper ISE electrode was maintained and calibrated daily for low level (Cu²⁺) measurements according to manufacturer's specifications (23). For measurements that fell within the calibration range (low-ion and Ca manipulations), [Cu²⁺] was determined by linear interpolation between the adjacent calibration points. For the low-copper samples that fell below the calibration range, [Cu²⁺] was expressed in mV from the mV reading for the lowest calibration standard (0.6 μg/L) in the associated calibration curve (0.6–30.5 μg/L Cu).

**Modeling Copper Speciation and Median Effect Concentrations.** Methods for modeling copper speciation, the use of the Biotic Ligand Model to predict median lethal concentrations, and the use of regressions to estimate the median inhibitory concentration for olfactory inhibition are presented in the Supporting Information.

**Statistical Analyses.** Statistical comparisons included one-way analysis of variance (ANOVA), simple linear regression, and t tests. Linear regressions were used to test the effect of hardness, alkalinity, and DOC on the precopper EOG amplitude and the relative EOG amplitude at the end of the copper exposure. An ANOVA was used to identify differences in the average postexposure EOG amplitude among fish in each of the AFWs. Differences from unexposed controls were subsequently tested with Dunnett's post hoc test. t-Tests were used to test for differences in relative EOG amplitude for (1) 10 mg/L NOM vs FA and (2) low vs high pH for 3.2 mM HC0₃⁻. The relative importance of each water parameter (hardness, alkalinity, DOC) for predicting copper toxicity at the gill vs the nose was assessed by examining the slopes for the LC50 (via gill toxicity) vs the IC50 (for olfactory toxicity). Slopes were considered not statistically different if the LC50 slope coefficient fell within the 95% confidence interval for the IC50 slope coefficient. All statistics were performed using SPSS 11.5 for Windows (www.spss.com) with a significance level of α = 0.05.

**Results**

**Measured and Modeled Composition of Artificial Fresh Water.** The measured concentrations of conventional cations and dissolved copper were 93 ± 10% (mean ± SD) and 80 ± 9% of nominal values, respectively (Table 1). Measured DOC concentration and nominal FA concentration were highly correlated (Pearson correlation coefficient; r² = 0.990, df = 14, p < 0.001; DOC = 0.38 × FA + 0.15). Measured and modeled concentrations of free copper were in close agreement and ranged from 38% of dissolved copper concentration in AFW with added calcium to <1% of dissolved copper concentration at the highest concentrations of bicarbonate or DOM (Table 1).

**Influence of Water Chemistry on the Olfactory Neurotoxicity of Copper.** Consistent with previous studies (8, 22), a short-term (30 min) exposure to dissolved copper significantly reduced the amplitude of the juvenile coho olfactory response to the natural odorant 10⁻⁷ M L-serine. In the control (low-ion) AFW, 20 μg/L copper reduced the size of the odor-evoked EOGs by 82% relative to pre-exposure responses (Figure 1). The relative olfactory response of noncopper exposed controls in low-ion AFW did not decline appreciably over the duration of the recording interval. The size of the olfactory response at the end of the experimental perfusion was 102 ± 6% (mean ± SE) of the initial response (Figure 1). The responsiveness of the olfactory system during the pre-exposure perfusion interval was unaffected by changes in the concentration of bicarbonate (simple linear regression; p = 0.824) or DOC (p = 0.460). The amplitude of the odor-evoked EOG showed a slight but significant negative relationship to concentration of calcium (r = -0.317, slope = -0.268, p = 0.003).

Copper exposures in each of the different AFWs significantly reduced the coho olfactory response relative to unexposed controls (F1,12 = 31.658, p < 0.001; Dunnett's post hoc: all p < 0.001). The only exception was AFW containing the highest concentration of FA (10 mg/L), in which the olfactory responses of copper-exposed fish were indistinguishable from controls (Dunnett's post hoc: p = 0.077). None of the other AFW formulations conferred complete protection against the neurotoxic effects of copper (Figure 1). Artificial water containing 10 mg/L FA was more protective than AFW containing 10 mg/L NOM (t test: p = 0.003), which likely reflects the higher organic carbon content of the former. The inhibitory effect of copper on odor-evoked EOGs was not influenced by pH (Figure 1; t test: p = 0.039).

Increasing water hardness, alkalinity, or DOM reduced copper toxicity (simple linear regression: p < 0.001), albeit by different degrees. As shown in Figure 1A and B, the influence of water hardness and alkalinity was relatively minor. On a unit-equivalent (mg/L) basis, the slope of the relationship between relative EOG amplitude and the concentration of each water chemistry constituent was more than an order of magnitude lower for calcium or bicarbonate than for DOM (Figure 1C). On a molar-equivalent basis, this relationship differed by more than 2 orders of magnitude (Table S1). Therefore, the protective effects of DOM are much greater than the protection afforded by either water hardness or alkalinity.

**Water Chemistry in Western River Systems Is Unlikely to Protect Salmon from the Olfactory Toxicity of Copper.** Based on previous monitoring investigations in west coast streams (U.S. Geological Survey WNWISWeb; http://nwis.waterdata.usgs.gov/nwis, calcium and alkalinity are unlikely to substantially reduce the impacts of dissolved copper on salmon olfaction in natural habitats. In the surveyed streams of the Puget Sound, Willamette River, Yakima River, and Sacramento River basins, environmental calcium did not reach concentrations sufficient to reduce copper toxicity by half (2.8 mM; 113 mg/L, Figure 2, top panel). Similarly, less than 1% of surveyed surface water samples had bicarbonate concentrations that would reduce copper toxicity by half (6.5 mM: 398 mg/L). By contrast, many western river systems are likely to contain sufficient DOC to at least partially reduce the olfactory neurotoxicity of copper (Figure 2, bottom panel). For example, 15% of all surface water samples collected in the above basins contained enough DOC to reduce sublethal copper toxicity by approximately half. Only a small minority...
BLM extends to the salmon olfactory system. Specifically, for each water quality parameter (hardness, alkalinity, and DOC), we compared the slopes of the relationships of the median lethal concentration (50% mortality, or LC50, as predicted by the BLM) and the median inhibitory concentration (copper concentration causing a 50% loss of chemosensory capacity, or the IC50, as determined empirically from results presented here and in a previous study [ref (19)] (see Supplemental Methods in the Supporting Information). As shown in Figure 3, water chemistry has a markedly different influence on these two toxicological end points, with the classical end point (acute lethality) having much greater sensitivity to variation in water hardness, alkalinity, and DOC. In comparing the two tissues, the influence of calcium was 3-fold greater for the gill relative to the nose. The effects of bicarbonate and DOC were 80-fold and 20-fold higher for the gill, respectively. Slopes were significantly divergent for all three parameters (LC50 slopes well above the 95% CI for IC50 slopes). The BLM also predicted a substantial decrease in the LC50 (from 312 to 111 μg/L) following a 1-unit drop in pH (from 8.6 to 7.6). However, no change in olfactory toxicity was observed in association with this change in pH in the present study (i.e., in the AFN with the highest alkalinity). These results collectively indicate that the ligands for copper in the gill and the nose are likely to be distinct.

**Discussion**

It is now well established that the fish olfactory system is particularly vulnerable to the neurotoxic effects of dissolved copper in the aquatic environment. Relative to classical toxicological measures (i.e., acute lethality), olfaction has several significant advantages as an end point for assessing the risks that copper poses to fish, including threatened and endangered species. First, copper toxicity in the olfactory epithelium manifests on a time scale of minutes (vs a 96-h LC50). Second, copper causes a loss of sensory function at very low, environmentally realistic exposure concentrations (at or below 5 μg/L). Third, olfaction underlies an important suite of life history traits in fish, and copper-induced anosmia is a good predictor of impairment to critical behaviors (3). Finally, as we have shown in this study, the toxicity of copper to the fish nose is only marginally influenced by water chemistry over the ranges that commonly occur in river drainages of the west coast of the United States. It should therefore be considerably easier to predict the site-specific toxicity of copper to the olfactory system as compared to the gill.

River systems in the western United States that provide habitat for ESA-listed Pacific salmon and steelhead are typically soft with a low alkalinity and a low DOC content. With a few exceptions (i.e., when DOC levels exceed 6 mg/L), the site-specific chemistries of these surface waters are unlikely to completely protect the salmon olfactory system from the neurotoxic effects of dissolved copper. However, it should be noted that LC50s for copper vary by as much as 4-fold in natural waters having different sources of organic matter (24, 25). We used a FA derived from Suwanee River NOM, and it remains to be determined whether copper’s chemosensory toxicity will vary with NOM from different river basins in the western United States. Nevertheless, our findings suggest that copper originating from motor vehicles, pesticide formulations, building materials, boat yards, and other sources has the potential to interfere with the chemosensory-mediated behavior of Pacific salmon under the majority of exposure conditions in western U.S. streams.

Copper has been shown to disrupt olfaction in a diversity of fishes (discussed in ref (3)) as well as other aquatic species (5). Accordingly, our current findings are likely to extend to

---

**FIGURE 1.** The olfactory response of juvenile coho salmon is reduced in the presence of copper. Relative olfactory response to 10⁻³ M L-serine after 30-min exposure to 20 μg/L (0.315 μM) copper in artificial fresh water of varying calcium (A), bicarbonate (B), or dissolved organic carbon (DOC) concentration (C). NOM = natural organic matter. Dashed and dotted lines across the top of each panel are, respectively, the mean and lower 95% confidence limit for the mean olfactory response of negative control fish. Each data point represents the amplitude of the olfactory response of one individual relative to its preexposure response amplitude (Figure S2). Regression lines and 95% confidence intervals of the regressions are also included. Dark bars along the x-axes show the range of average concentrations of each water chemistry parameter surveyed in streams in the western U.S. subregions of Puget Sound, Willamette River, Yakima River, and Sacramento River (U.S. Geological Survey, NWISWeb).
other fish species in polluted freshwater habitats worldwide. The extent to which our results can be extended to fishes in estuaries or saltwater is less clear. This is because the cation and DOC contents of these habitats are considerably higher than the range of values examined here. Also, the salmon olfactory system undergoes physiological changes when these anadromous animals migrate from freshwater to the ocean, and this may alter the expression or function of the as-yet unidentified ligand(s) for copper. Additional studies using seawater-acclimated fish are therefore recommended.

Copper has a higher affinity for bicarbonate (log \( K = 14.3 \)) than for the biotic ligand in the fish gill (log \( K = 7.4 \)) (13). At the gill, the free ionic form of copper is the most important causal agent in determining acute lethality (11, 13). Complexation with bicarbonate reduces the bioavailability of copper to the gill, thereby reducing toxicity. By contrast, the inhibitory effects of copper on salmon olfactory neurons were only modestly affected by increasing bicarbonate and pH, even though free copper was dramatically reduced in these AFW formulations (Figure S3). This suggests that the ligand(s) in the salmon olfactory epithelium may have a relatively higher affinity for copper, and that bicarbonate-complexed copper is bioavailable to OSNs.

It is unlikely that copper inhibits olfaction by targeting the receptor proteins in the apical cilium or microvilli of OSNs that bind amino acids and other odors. Previous studies in coho salmon (26), rainbow trout (27), and Atlantic salmon (28) have shown that amino acid–receptor binding is inhibited only at copper concentrations exceeding 100 \( \mu \)M, which is well above the concentration used in this study (20 \( \mu \)g/L, or 0.315 \( \mu \)M). Copper also does not appear to form nonstimulatory complexes with L-serine (26), an observation supported...
by the gradual decrease in EOG amplitude with copper exposure (not shown, but see Figure 4 in ref (8)), rather than an instantaneous drop in EOG amplitude. Signal transduction in OSNs involves a complex biochemical cascade (16). Mammalian studies have shown that micromolar concentrations of copper can block ion channels in neurons (29–31), and the metal may also interfere with transmembrane conductances in fish sensory neurons. In addition, if copper is translocated to the cytosol of salmon OSNs, it could potentially block the function of many key proteins, including, for example, voltage- and ligand-gated channels, G proteins, cyclases, kinases, phosphatases, and other proteins that control the excitability properties of sensory neurons (Figure S1; see also ref (16)). More work is needed to identify the biotic ligand(s) in the fish OSN.

Our present results contribute to a growing awareness that short-term exposures to environmental pollutants such as copper can interfere with the sensory biology of aquatic species (32). In addition to metals, recent studies have also shown that several current-use pesticides (e.g., refs 22, 33, 34) as well as stream acidification (35) can cause chemosensory deprivation and altered olfactory-mediated behaviors in fish. The toxic effects of copper also extend to the lateral line (36, 37), another important sensory system that underlies schooling and predator evasion behaviors in fish. In the case of Pacific salmon, a key priority for future research is to determine how a copper-induced loss of olfactory capacity affects life history traits that determine individual survival and lifetime reproductive success. These include, for example, olfactory imprinting, predation mortality, homing behavior, and spawning success. Information at these higher scales is needed to more effectively manage the conservation and recovery of ESA-listed salmon populations in the many western watersheds that are currently undergoing high rates of urban and suburban development.

Acknowledgments
This research was funded by the NOAA Coastal Storms Program, supported in part by a grant from the NIEHS Superfund Basic Sciences Program P42ES-004696, and an EPA STAR graduate fellowship awarded to J.K.M. We thank Tiffany Linbo, Frank Sommers, Scott Hecht, Chris Mebane, Tony Hawks, and Tracy Collier and three anonymous reviewers for helpful comments on the manuscript.

Supporting Information Available
Supplementary Methods are presented to give a more detailed description of copper speciation modeling and modeling of LC50s and IC50s. Supplementary Results and Discussion are included to describe copper speciation in artificial fresh water (AFW) samples and to compare our current findings on the influence of hardness and alkalinity on copper olfactory toxicity to previously published results. Table S1 shows regression statistics for the influence of calcium, bicarbonate, and fulvic acid on copper olfactory toxicity. Figure S1 provides a schematic illustration contrasting the potential actions of copper at the gill epithelium and the olfactory epithelium. Figure S2 shows how copper-induced olfactory neurotoxicity (percent relative EOG amplitude) was measured. Figure S3 indicates how free ion copper concentrations varied in AFW formulations based on direct measurements using the copper electrode and modeled estimates using VMINTSQ. Figure S4 compares our findings in this study for the influence of calcium and bicarbonate to previous studies of copper-induced olfactory toxicity in fish (8, 38, 39). This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited


(37) Hernandez, P. P.; Moreno, V.; Olvarri, F. A.; Allende, M. L. Sublethal concentrations of waterborne copper are toxic to lateral line neuronomasts in zebrafish (Danio rerio). Hearing Res. 2006, 218, 1-10.


ES071603E
Additions and Corrections

2008. Volume 42. Pages 1352–1358

Jennifer K. McIntyre, David H. Baldwin, James P. Meador, and Nathaniel L. Scholz*: Chemosensory Deprivation in Juvenile Coho Salmon Exposed to Dissolved Copper under Varying Water Chemistry Conditions

It has come to our attention that a computational error when modeling IC50s (equation presented correctly in Supplemental Methods, line 26) has resulted in erroneous data in Figure 3 and the associated results in the text of the manuscript. The error occurred when manipulating the equation to solve for IC50 (from its previously published form which solved for relEOG) and resulted in an under-prediction of the IC50s for Figure 3. The affected results text appears at the end of the section “Comparison of Copper Toxicity to the Salmon Gill and Nose under Different Water Chemistries”. In this section we compared the slope of the IC50s with the slope of the LC50s for each water quality parameter (Ca, HCO3, DOC). We reported that the slopes for IC50s were significantly less than those for LC50s and further reported by what magnitude the slopes differed. The recalculated slopes for each IC50 are still significantly different from those for its respective LC50 but the magnitude of the difference has been reduced. These changes in IC50 values do not affect our discussion. The affected text (with corrections) and the affected figure (with a corrected figure) follow.

Corrections To Results Text (p 1355; Changes Highlighted). As shown in Figure 3, water chemistry has a markedly different influence on these two toxicological end points, with the classical end point (acute lethality) having much greater sensitivity to variation in water hardness, alkalinity, and DOC. In comparing the two tissues, the influence of calcium was 2-fold greater for the gill relative to the nose. The effects of bicarbonate and DOC were 41-fold and 4-fold higher for the gill, respectively. Slopes were significantly divergent for all three parameters (LC50 slopes well above the 95% CL for IC50 slopes).

Affected Figure 3 (Incorrect and Corrected Figures Appear Side by Side for Comparison).

ES800790V
10.1021/es800790v
Published on Web 07/31/2008
FIGURE 3. Water chemistry parameters are less protective at the fish nose than at the fish gill against toxicity from dissolved copper. Exposure concentrations of dissolved copper predicted to result in 50% toxic effect at the gill (open squares = LC50) or the nose (closed circles = IC50) for the various concentrations of calcium, bicarbonate, or DOC in artificial fresh waters tested. LC50s were generated by the Biotic Ligand Model (BLM) for each artificial fresh water treatment, as described in the text. IC50s were calculated from published dose–response relationships for copper in juvenile coho salmon, as described in the text. The open circle is a point estimate of the IC50 from the regression parameters (Table S1). The dark bars along the x-axes show the range of average measured concentrations of each parameter in western U.S. streams. The corrected figure appears on the right.
Environmental Toxicology

EFFECTS OF WATER HARDNESS, ALKALINITY, AND DISSOLVED ORGANIC CARBON ON THE TOXICITY OF COPPER TO THE LATERAL LINE OF DEVELOPING FISH

Tiffany L. Linbo,† David H. Baldwin,‡ Jenifer K. McIntyre,† and Nathaniel L. Scholz‡
†Northwest Fisheries Science Center, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, Washington 98112, USA
‡School of Aquatic and Fishery Sciences, University of Washington, 1122 Northeast Boat Street, Box 355020, Seattle, Washington 98105, USA

(Received 19 June 2008: Accepted 7 January 2009)

Abstract—Conventional water chemistry parameters such as hardness, alkalinity, and organic carbon are known to affect the acute lethality of copper to fish and other aquatic organisms. In the present study, we investigate the influence of these water chemistry parameters on short-term (3 h), sublethal (0–40 μg/L) copper toxicity to the peripheral mechanosensory system of larval zebrafish (Danio rerio) using an in vivo fluorescent marker of lateral line sensory neuron (hair cell) integrity. We studied the influence of hardness (via CaCl₂, MgSO₄, or both at a 2:1 molar ratio), sodium (via NaHCO₃ or NaCl), and organic carbon on copper-induced neurotoxicity to zebrafish lateral line neurons over a range of environmentally relevant water chemistries. For all water parameters but organic carbon, the reductions in copper toxicity, although statistically significant, were small. Increasing organic carbon across a range of environmentally relevant concentrations (0.1–4.3 mg/L) increased the EC₅₀ for copper toxicity (the effective concentration resulting in a 50% loss of hair cells) from approximately 12 μg/L to approximately 30 μg/L. Finally, we used an ionoregulatory-based biotic ligand model to compare copper toxicity mediated by targets in the fish gill and lateral line. Relative to copper toxicity via the gill, we find that individual water chemistry parameters are less influential in terms of reducing cytotoxic impacts to the mechanosensory system.

Keywords—Zebrafish Hair cells Mechanosensory Bioavailability Biotic ligand model

INTRODUCTION

Metals such as copper are toxic to the peripheral sensory systems of fish and other aquatic organisms. Dissolved copper specifically impairs the normal function of olfactory and mechanosensory neurons by reducing their physiological responsiveness to environmental cues [1] and, at higher exposure concentrations, via cell death [2]. Sensory isolation in turn interferes with behaviors mediated by these senses, including predator detection and avoidance [3–7], social interaction [8], prey detection [9], and rheotaxis (orienting toward flow [10]). Copper and other so-called information disruptors [11] can therefore have important impacts on the survival, distribution, and reproductive success of fish.

In fish and other aquatic organisms, the acute lethality of copper is known to be mediated by the gill epithelium. The relative concentrations at which copper kills fish are influenced by various water chemistry parameters such as hardness, alkalinity, pH, and dissolved organic carbon [12]. Cations (e.g., Ca²⁺ and Na⁺) reduce the bioavailability of metal ions to the binding site (biotic ligand) on the gill by competition, whereas anions (e.g., HCO₃⁻, CO₃²⁻, Cl⁻, and SO₄²⁻) and dissolved organic carbon (DOC) bind to the free metal ions to form inorganic and organic complexes, respectively. Because complicated reduction in the availability of copper to the biotic ligand, the lethal effect of copper is reduced in waters enriched in anions and DOC [12]. The influence of water chemistry is commonly estimated using the biotic ligand model (BLM).

The BLM was developed to predict the acute toxicity of copper to freshwater aquatic organisms in waters with varying chemical compositions [13]. The BLM has been used more recently to derive site-specific water quality criteria that are intended to be protective of the most sensitive freshwater taxa (e.g., Ceriodaphnia dubia [14]).

Despite several important assumptions and limitations [15], the BLM has served as a useful predictor of copper toxicity mediated via the fish gill in waters of different chemistries [12]. However, for biotic ligands in fish tissues other than the gill, water chemistry may have less of an influence on copper toxicity. For example, copper-induced neurotoxicity to the peripheral olfactory epithelium of juvenile coho salmon (Oncorhynchus kisutch) was less influenced by changes in water hardness, alkalinity, and DOC than was predicted from an ionoregulatory-based rainbow trout (Oncorhynchus mykiss) BLM [16,17].

In the present study, we used larval zebrafish to investigate the effects of water chemistry on copper toxicity to the mechanosensory lateral line. In teleosts, the peripheral mechanosensory system is comprised of assemblages of receptor neurons (neuromasts) along the surface of the fish. Each neuromast contains a rosette of ciliated hair cells. These receptor cells are responsive to water displacement around the body of the animal. Mechanosensory information is transduced by hair cells and then propagated to the central nervous system. Zebrafish are a convenient experimental model with a diversity of established molecular markers that allow the direct visualization of the mechanosensory system in vivo. Also, the ontogeny and anatomy of the zebrafish lateral line have been described in considerable detail [18,19],
and the neurotoxic effect of copper on zebrafish mechanosensory receptors has been previously described [2,20,21]. In the present study, using conventional in vivo methods for measuring hair cell death [2], we determined how environmentally relevant variations in water hardness, alkalinity, sodium, and DOC influence copper-induced neurotoxicity in the lateral line of larval zebrafish. These empirical results were then combined with empirical data from the olfactory system [16,17] and modeled data from the fathead minnow (Pimephales promelas) BLM to compare the relative influence of different water chemistry parameters on acute toxicity to the fish lateral line system, olfactory system, and gill.

MATERIALS AND METHODS

Animals

Zebrafish were obtained from our breeding colony maintained at the Northwest Fisheries Science Center (Seattle, WA, USA). Adult wild-type (AB strain) zebrafish were allowed to spawn, and the embryos were collected and sorted into 100-mm plastic petri dishes (Falcon®, VWR) containing embryo medium or modified embryo medium (artificially constituted test waters with different chemical properties). Embryo medium consisted of distilled water amended with CaCl2·2H2O, MgSO4·7H2O, NaCl, NaHCO3, and KCl at the concentrations in Table 1 (all chemicals minimum 99.0% purity; Sigma). Embryo medium or test water was renewed every 24 h. Embryos and larvae were incubated at 28.5°C in the appropriate test water (except for DOC manipulations, wherein all fish were in embryo medium) and raised until 4 d postfertilization (dpf).

Test waters

To determine the effects of water chemistry parameters on copper-induced hair cell death, different salts or organic matter were added to the embryo medium to alter hardness, alkalinity, and DOC across a range of environmentally relevant surface water concentrations as determined from the U.S. Geological Survey’s National Water Information System Web Site (NWISWeb; http://nwis.waterdata.usgs.gov/nwis). The six different water chemistry formulations (Table 1) varied hardness (via CaCl2 alone, MgSO4 alone, or both at a 2:1 molar ratio), alkalinity (via NaHCO3), sodium (via NaCl), and DOC (via natural organic matter [NOM]). Test waters were prepared by adding stock solutions made from CaCl2·2H2O, MgSO4·7H2O, NaCl, NaHCO3 (Sigma), and NOM (52.5% carbon, Suwannee River, International Humic Substances Society; http://www. ihss.gatech.edu/) to embryo medium. The test media were stored at 28.5°C.

For the DOC test, a NOM stock solution was prepared 24 h before the copper exposure and allowed to dissolve at room temperature. The day of the copper exposure, the NOM test waters were then diluted to the final concentrations (0, 2.5, 5, and 10 mg/L NOM) to which the animals were exposed. Each test was then spiked with the copper stock solution to achieve the appropriate metal concentration (see below). In a separate experiment to test whether toxicity is reduced by copper complexation to NOM (a process which requires time [22]), a NOM stock solution was prepared 48 h before exposure. Copper was then added to the diluted NOM test water (~1.1 mg carbon/L) 24 h prior to exposure and allowed to equilibrate overnight at room temperature.

Copper exposures

For each test water formulation, larvae were exposed to five copper concentrations (0, 5, 10, 20, and 40 μg/L) diluted from a copper stock solution using CaCl2·2H2O (Sigma; min-
Effect of water chemistry on copper neurotoxicity in fish

Immuno 99.0% purity). Copper stock solutions were prepared 24 h before the exposure and stored at 4°C.

Larvae at 4 dpf were separated into plastic six-well plates (Costar®, Fisher Scientific). Fifteen larvae were added to each well containing 6 ml of the water treatment. Triplicates (3 wells of 15 larvae each) were performed for each water treatment and copper concentration. For experiments involving the different water formulations, the 6 ml of water treatment was renewed for each well before the copper exposure. Copper (from stock) was then added to each well to achieve the appropriate concentration. In the equilibrated NOM and copper experiment, the embryo medium was exchanged with 6 ml of the appropriate premixed solution. The six-well plates containing zebrafish larvae in test water amended with dissolved copper were incubated at 28.5°C for 3 h. Hair cell death within the zebrafish neuromast has previously been shown to be complete by 3 h [2].

Determination of hair cell neurotoxicity

To visualize the individual mechanosensory neurons in vivo, larvae were immersed in 0.05% 4-(4-diethylaminostyryl)-N-methylpyridinium iodide (DASPEI; Sigma) for 8 min, rinsed in embryo medium, and anesthetized with tricaine methane sulfonate (ethyl 3-aminobenzoate methane sulfonate [MS-222]; 250 μg/ml: Sigma) for 2 min. The DASPEI staining was the basis for quantifying the number of hair cells per neuromast. Larvae were then mounted on depression slides in embryo medium, and hair cells were counted using a Nikon Eclipse E600 compound microscope (Meridian Instruments) fitted with a mercury lamp and a fluorescence filter (excitation 460–500 nm). Because a previous study [2] found that the toxicity of dissolved copper is similar across different zebrafish neuromasts, the number of hair cells in a single representative neuromast (O2, located on the otic vesicle [19]) was quantified. Hair cell counts were obtained from one of the bilaterally symmetrical O2 neuromasts for each of 10 randomly selected larvae per well to give a total of three replicates of 10 larvae per water treatment and copper concentration.

Water chemistry analysis

Dissolved copper concentrations were measured for all stock solutions for each water parameter test. Also, for one exposure concentration (40 μg/L), water samples were collected both before and after the exposure (a composite from 18 wells containing 15 larvae per well after 3 h at 28.5°C). Copper solutions were analyzed by inductively coupled plasma mass spectrometry by a U.S. Environmental Protection Agency (EPA)-certified laboratory (Frontier GeoSciences). The pH values of embryo media and test waters were measured using a pH meter (Accumet basic AB15; Fisher Scientific). The pH glass electrode was calibrated daily with pH 4, pH 7, and pH 10 buffers (VWR). Analysis of ion concentrations, hardness, and alkalinity was performed for each artificially formulated test water with the exception of embryo medium containing DOC. Samples were analyzed by a U.S. EPA-certified analytical laboratory (AmTest Laboratories) using standard methods. Total organic carbon was measured in triplicate for each of the DOC test waters using a Shimadzu TOC VCSH analyzer (University of Washington Oceanography Technical Services).

Data analyses

For each combination of water test medium and copper concentration, the means of the three replicates were used for analysis. With these means, the dose–response relationship between copper and DASPEI hair cell staining was calculated by the same nonlinear regression as previously described [2]:

\[ y = m / (1 + (x/k)^n) \]

where y is the number of hair cells per neuromast of a copper-exposed fish, m is the mean number of hair cells per neuromast of an unexposed fish. x is the copper concentration, k is the EC50 (effective concentration resulting in 50% loss of hair cells), and n is the slope. The dose–response relationships were calculated using Kaleidograph (Synergy Software). For comparison of the EC50 of dose–response relationships, an F test was performed using Prism (GraphPad Software).

The relationship between each water chemistry parameter (e.g., hardness as CaCl2) and copper toxicity was analyzed by linear regression for the lateral line EC50s and the BLM-predicted LC50s (concentration resulting in 50% mortality of the test animals; see below) using Prism. Prism was used to run F tests to compare the slopes and intercepts of the linear regressions.

Biotic ligand modeling

The copper BLM (version 2.2.3, HydroQual; http://www.hydroqual.com/wr.blm.html) was used to compare the relative influence of water chemistry on copper toxicity predicted by the BLM (LC50s) and the observed toxicity to the zebrafish lateral line sensory neuron loss (EC50). The water quality parameters used in the present study (Table 1) and 10% humic acid and 0.01 μM sulfide served as default input values. The fathead minnow BLM was selected for estimating LC50s because it is a warm-water-tolerant, freshwater species for which the BLM has been extensively refined and validated in previous studies (e.g., [12,23–25]).

RESULTS

The measured compositions of the different artificial freshwaters were within 20% of the nominal or target values (Table 1). Similarly, all measured copper concentrations were within 20% of the nominal values (data not shown). The measured copper concentration of the 40 μg/L copper solution in embryo medium was reduced 44% after the 3-h exposure (from 33.2 to 14.7 μg/L). This loss during the exposure interval was likely due to uptake by the zebrafish larvae, adherence to the plastic wells, or both. Thus, the calculated copper toxicities (EC50s), which in the present study were based on initial nominal copper concentrations, may underestimate actual copper toxicity. Because hair cell death happens within 30 min of exposure [2] and the exposure time frame in the present study was 3 h, cytotoxicity likely occurred at a copper concentration between nominal and the 44% copper loss. The measured values for DOC equaled approximately 40% of the nominal concentrations for NOM. This reflects the fact that NOM is not entirely composed of DOC.

Influence of major ions on copper neurotoxicity

The effect of hardness on copper toxicity to the zebrafish peripheral mechanosensory system (EC50) was determined by altering the concentrations of CaCl2, MgSO4, and both salts at a ratio of 2:1 (molar). When hardness was increased from soft water (45 mg/L as CaCO3) to hard water (320 mg/L as CaCO3), EC50s increased approximately 50% across the hardness range tested, regardless of which cation was varied (Table 1). The relationship between water hardness (expressed as total Ca2+ and Mg2+ in
millimolar in Fig. 1) and copper toxicity (Table 2) was not significantly different among cations or ratios ($F_{2,6} = 2.92, p = 0.13$; pooled slope = 2.48, pooled intercept = 10.1).

To assess the effects of increased alkalinity and Na$^+$ ions, copper toxicity in test waters containing four different concentrations of NaHCO$_3$ and NaCl was compared. Toxicity decreased when either sodium salt was added, with EC50s increasing approximately 59% across the range of sodium concentrations tested (Table 1). The effects of NaHCO$_3$ and NaCl were similar, and relationships between salt concentration and copper toxicity for the two salts (Table 2) were not significantly different ($F_{1,4} = 0.028, p = 0.88$; pooled slope = 1.09, pooled intercept = 10.9). This indicates that carbonate complexation and the subsequent change in pH caused by the addition of NaHCO$_3$ had no significant effect on copper toxicity to the lateral line.

**Organic carbon reduces copper toxicity to the lateral line**

Varying concentrations of NOM were used to evaluate the relationship between organic carbon and copper toxicity to the mechanosensory system. Addition of DOC to test waters significantly decreased copper toxicity to the mechanosensory system. Specifically, the EC50s for hair cell death increased by an average of 437% across the DOC range tested (Table 1). Notably, the two highest concentrations of NOM fell within the lower half of the dose–response curve for the range of copper concentrations tested in the present study. Although the values for these higher NOM concentrations were sufficient for the purposes of calculating an EC50, the smaller data sets produced standard errors that were correspondingly higher (Table 1). The reduction in copper neurotoxicity by DOC was greater than that for hardness, sodium, or alkalinity across the ranges tested (Fig. 1). Although the molecular weight of the NOM used in the present study is unknown, on a unit-equivalent basis (mM), the effect (i.e., slope) of DOC on copper toxicity would be two or more orders of magnitude greater than those for the cations (Table 2) due to the typically large molecular weights of DOC compounds [26].

**Premixing copper and organic matter does not reduce toxicity**

To determine the effect of allowing copper and organic matter to complex for 24 h prior to exposure, DASPEI labeling of hair cells was compared between animals exposed to copper mixed with NOM solutions for 24 h (equilibrated) and copper added to NOM solutions at the start of the exposure (spiked). Although allowing copper to equilibrate with NOM resulted in a slight shift of the dose–response curve to higher copper concentrations (Fig. 2), the resulting change in the EC50 (from 21.7 ± 0.8 μg/L for spiked to 26.6 ± 1.0 μg/L for equilibrated) was not significant ($F_{1,4} = 5.21, p = 0.085$).

**A gill-based BLM does not predict lateral line toxicity**

The BLM is conventionally used to predict the acute, gill-mediated lethality of copper as a function of water chemistry [12]. To assess whether these predictions also extend to the fish lateral line, we compared the slopes of the effective concentration (hair cell loss; EC50) to the BLM-predicted lethal concentration (LC50) for each water chemistry parameter. In all cases, the BLM predicted that changes in water chemistry would have greater influence at the fish gill than was experimentally determined for the lateral line system in the present study (Fig. 1). Slopes for the relationships between water quality parameter concentration and copper toxicity were signifi-

![Fig. 1](image-url)

Fig. 1. Water quality parameters have less influence on copper toxicity to mechanosensory receptors (EC50, effective concentration that causes 50% loss of hair cells; open symbols and solid lines) than on the biotic ligand model (BLM)-predicted lethality (LC50, lethal concentration of 50% of test animals; solid symbols and dashed lines) for the water quality conditions of (A) hardness (as combined Ca$^{2+}$ and Mg$^{2+}$), (B) sodium, and (C) dissolved organic carbon in Table 1. For hardness and for sodium, EC50s are pooled because there is no difference in the slope of their relationships (see Results section). Open symbols are estimates of EC50s ± standard error based on nonlinear regressions. Note: the y axis of panel B is on a logarithmic scale.
Effect of water chemistry on copper neurotoxicity in fish

Table 2. Linear regression relationships between concentrations of various water parameters and measures of copper toxicity for mechanosenasation (EC50 = copper concentration resulting in a 50% loss of hair cells of the zebrafish lateral line), olfaction (IC50 = copper concentration resulting in a 50% inhibition of electrophysiological olfactory response in the coho salmon [16,17]), and mortality (LC50 = copper concentration resulting in 50% mortality of fathead minnow using the biotic ligand model [BLM]).

<table>
<thead>
<tr>
<th>Water parameter (units)</th>
<th>Lateral line EC50 (µg Cu/L)</th>
<th>Intercept</th>
<th>$r^2$</th>
<th>Slope (95% CI)*</th>
<th>Offactory IC50 (µg Cu/L)</th>
<th>BLM LC50 (µg Cu/L)</th>
<th>Slope (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl2 (mM)</td>
<td>2.31 (1.41-3.21)</td>
<td>9.72</td>
<td>0.984</td>
<td>5.52 (2.74-8.31)</td>
<td>9.7 (8.4-11.0)</td>
<td>8.1 (6.7-9.5)</td>
<td>9.9 (8.6-11.3)</td>
</tr>
<tr>
<td>MgSO4 (mM)</td>
<td>3.12 (0.99-5.26)</td>
<td>10.0</td>
<td>0.952</td>
<td></td>
<td>8.1 (6.7-9.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCl2-MgSO4 (mM)</td>
<td>2.02 (1.07-2.96)</td>
<td>10.6</td>
<td>0.977</td>
<td></td>
<td>8.1 (6.7-9.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaHCO3 (mM)</td>
<td>1.06 (~0.039-2.17)</td>
<td>10.6</td>
<td>0.896</td>
<td>2.54 (1.53-3.55)</td>
<td>318 (123-515)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl (mM)</td>
<td>1.11 (0.54-1.68)</td>
<td>11.1</td>
<td>0.972</td>
<td></td>
<td>4.8 (3.7-6.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>9.12 (4.71-13.5)</td>
<td>12.9</td>
<td>0.975</td>
<td>9.9 (6.48-13.35)</td>
<td>48.2 (38.9-58.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 95% confidence interval of the slope.
* All slopes were significantly different from zero ($p < 0.05$), except for NaHCO3, where $p = 0.053$.

* Dissolved organic carbon.

...greater for LC50s than those for EC50s (above the 95% confidence interval for EC50s). Specifically, they were greater by more than a factor of three for CaCl2, MgSO4, and NaCl, more than 300 times for NaHCO3, and five times higher for DOC (Table 2).

The relative differences between the predicted and observed slopes demonstrate that the influences of water quality parameters on copper toxicity via the gill and the lateral line were dissimilar. The most notable difference between the BLM-predicted toxicity to the fish gill and the observed toxicity to the fish mechanosenasory system was for the effect of the two sodium salts. In addition to the change in pH, NaHCO3 was predicted to reduce copper toxicity more than 10-fold over the effect of NaCl at the fish gill. By contrast, NaHCO3 and NaCl had the same influence on the toxicity of copper to the mechanosenasory system (Fig. 1B), irrespective of the increase in pH (Table 1).

DISCUSSION

Our findings indicate that water chemistry parameters can influence copper toxicity to the peripheral mechanosenasory system of larval fish. Increasing hardness ions, sodium ions, and DOC all resulted in some reduction of copper neurotoxicity to individual sensory neurons in zebrafish lateral line neuromasts. Changes in the divalent cations Ca$^{2+}$ and Mg$^{2+}$ over an environmentally relevant hardness range slightly decreased copper toxicity. For example, an increase in hardness of 1 mM or 100 mg/L (as CaCO3) decreased toxicity (as indicated by an increase in the EC50) by approximately 2.5 µg/L (Fig. 1A). The contributions of each ion were similar for the different Ca$^{2+}$/Mg$^{2+}$ ratios examined. Alkaline waters also slightly reduced copper neurotoxicity. For example, an increase in NaHCO3 of 5 mM (alkalinity change of ~250 mg/L as CaCO3) decreased toxicity by approximately 5.5 µg/L. However, because the influences of NaCl and NaHCO3 were approximately equal, the effect of alkalinity can be attributed to the sodium content of the artificial freshwaters and not to an increase in bicarbonate ions or to a shift in pH. An example of how Na$^+$ influences copper toxicity in the zebrafish lateral line is evident when comparing the present results with a previous study [2]. Specifically, the higher EC50 for copper toxicity to lateral line neurons that was reported earlier [2] is attributable to the relatively high concentration of Na$^+$ in the water used in that study (Table 1; see also the formula used in Table 2). Overall, our present findings indicate that the most effective parameter for reducing copper toxicity to lateral line neurons was DOC. This effect of DOC presumably reflects complexation and reduced copper bioavailability.

Although most of the water chemistry parameters had a limited effect on the copper-induced degeneration of mechanosenasory neurons, hair cells have the ability to regenerate when zebrafish larvae are restored to clean water [2]. Zebrafish sensory neurons regenerate over the course of approximately 48 h. The extent to which different water chemistries influence the time course and extent of hair cell regeneration remain to be investigated.

The findings in the present study for the peripheral mechanosenasory system are similar to the results of previous studies focused on the peripheral olfactory system of fish. Investigations involving Atlantic salmon (Salmo salar) and coho salmon (O. kisutch) have found only a limited influence of hardness [16,17,27,28] or alkalinity [16,17,29] on copper-induced neurotoxicity to olfactory receptor neurons. In studies involving the salmon olfactory system, copper neurotoxicity to sensory neurons has generally been measured as a reduction in physiological responsiveness to olfactory stimuli (odorants). The influence of water chemistry on copper toxicity by changes in calcium hardness, bicarbonate alkalinity, and DOC was similar for both the coho olfactory system [16,17] and the zebrafish lateral line system (the present study). This is indicated by overlapping confidence
intervals (95%) for the slope coefficients (Table 2). Therefore, in terms of water chemistry and copper toxicity, the two fish sensory systems appear to be similar to each other and distinct from the gill as a separate target organ.

Given the similar form (ciliated sensory neurons), function (signal transduction), and locations of mechanosensory and olfactory receptors (in direct contact with copper dissolved in water), it is perhaps not surprising that the different chemical properties of water produced similar outcomes for the two sensory systems. The sensory receptors of lateral line neuromasts and the olfactory epithelium are comprised of ciliated neurons embedded in a mucus membrane directly exposed to ambient water. These receptors transduce mechanical and chemical stimuli, respectively, into electrical information relayed to sensory processing centers in the fish brain. Similarities in receptor form and function could also explain why both sensory systems responded to the diverse suite of toxicant physicochemical properties based on gill epithelial cells that are very different from sensory receptors in terms of both architecture and physiology [30,31].

Although an ionoregulatory BLM may be useful for predicting the influence of site-specific water quality conditions on the acutely lethal effects of copper to fish, the accuracies of these predictions are diminished when this form of the BLM is applied to the fish peripheral mechanosensory system. The cations Ca²⁺, Mg²⁺, and Na⁺, in conjunction with the anions Cl⁻ or SO₄²⁻, do not appear to compete with copper as effectively for the as-yet-unidentified biotic ligand(s) in the lateral line compared to the ligands of the fish gill. Also, the BLM assumption that inorganic or organic constituents of surface waters render dissolved copper unavailable to the biotic ligand did not hold for the mechanosensory system. Results from the present study also show that the inorganic ligand HCO₃⁻ along with an increase in pH, did not reduce the bioavailability of copper to the lateral line beyond that of its associated cation (Na⁺), in contrast to the predicted effect of HCO₃⁻ and pH at the gill. This suggests that copper may be available and toxic to zebrafish lateral line neurons in inorganic complexes (e.g., Cu–carbonate) that are biologically unavailable to the gill. Also, although DOC did reduce the toxicity of copper to mechanoreceptors, this was less of a reduction than that predicted by an ionoregulatory BLM. Interestingly, the BLM assumes that copper has reached equilibrium in the exposure water. Although toxicity via the fish gill is reduced when copper has reached equilibrium with DOC [22], this was not the case for the lateral line. Copper toxicity to the zebrafish peripheral mechanosensory system was not significantly decreased when copper was allowed to equilibrate with DOC for 24 h. Hence, the lateral line may be vulnerable to some Cu–DOC complexes, as has been shown in some studies for the fish gill [32,33]. Alternatively, the binding affinity of the biotic ligand for copper in the mechanosensory system may be greater than that of the biotic ligand for copper at the fish gill. For example, the biotic ligand(s) associated with mechanosensory receptor neurons may be binding dissolved copper that remains bound to carbonates and DOC at the gill.

There is an important distinction between a BLM that is predictive of sublethal, sensory-dependent behavioral effects in fish and BLM-derived water quality criteria for copper that may be protective for neurobehavioral toxicity. As we have shown here, the gill-based BLM does not accurately predict copper toxicity to fish sensory systems, particularly under conditions of varying alkalinity. It may be possible to improve predictive accuracy with a modified BLM specifically parameterized for sensory toxicity (see below). This will require an improved biological understanding of copper binding affinities in sensory tissues, among other parameters. It should be noted, however, that the intended use of the BLM as a regulatory tool [14] may result in site-specific criteria that generally protect against toxicity to the olfactory and lateral line systems of fish. This is because the criteria mode of the BLM considers toxicity data for the most sensitive aquatic species (e.g., *C. dubia*). Relative to fish (e.g., fathead minnows), the parameter for critical copper accumulation (LA50) is much lower for *C. dubia*. The net effect is that the BLM-derived criteria are below our calculated EC50s for copper toxicity to the lateral line in waters with different chemical compositions. A corresponding criterion maximum concentration (CMC) can be calculated for each of the different water chemistries shown in Table 1 (data not shown). In all cases, the resulting CMC is below the respective EC50 for copper-induced toxicity to the zebrafish lateral line. For example, the exposure water with a DOC of 2.4 mg/L (NOM-2 in Table 1) has a BLM-derived CMC of 6.05 μg/L copper, which is below the EC50 of 38.8 μg/L for hair cell death. This comparison is simplistic because an EC50 for sensory neuron cell death is likely well above the threshold for adverse impacts on fish behavior. Nevertheless, the example illustrates how the inclusion of sensitive taxa in the BLM-derived CMC produces criteria that are below the EC50s calculated in the present study.

The basic structure and function of the lateral line are highly conserved across teleosts [34]. Therefore, the zebrafish can serve as a useful model to investigate the effects of physicochemical water properties on dissolved copper toxicity to the lateral line of native fish species. Additionally, by providing important sensory input regarding environmental cues, such as the presence of predators, the mechanosensory system underlies behavioral responses, such as predator avoidance, that are critical to the survival of fish [35]. Not surprisingly, studies have found that impairing the mechanosensory system of fish can lead to reductions in important behaviors, such as startle and orienting responses [6,9,21]. Therefore, the mechanosensory system is an important endpoint to consider when assessing the potential impacts of copper on fishes. Moreover, simple fluorescent imaging procedures used in the present study should be useful in terms of screening a wide range of dissolved toxicants for lateral line neurotoxicity in fish. Extending our current findings to the mechanosensory-mediated behaviors of wild fish species remains an important area of future research.

In summary, we have shown that the fish mechanosensory system is sensitive to dissolved copper at low exposure concentrations (e.g., EC50s of 11–20 μg/L) that last for only a short duration (3 h). Copper toxicity to the fish lateral line is a particularly important consideration in urban and urbanizing areas, where storm events (<12 h) transport pulses of dissolved copper into aquatic habitats at concentrations that span or exceed the copper levels used in the present study (e.g., 3.4–64.5 μg/L in northern California, USA [36]). Overall, our results indicate that environmentally relevant levels of dissolved copper from stormwater discharges and other inputs to fish habitats have the potential to impair critical behaviors that depend on a properly functioning lateral line. Although copper toxicity to the lateral line may be ameliorated by DOC (and to a much lesser extent hardness and alkalinity), this reduction will be less than that predicted from an ionoregulatory BLM parameterized for ligands in the fish gill. In the future, it should
be possible to use the empirical data generated in the present study, as well as recent data for the salmon olfactory system [16,17], as a basis to develop a set of BLM parameters that are more representative of sublethal sensory endpoints in fish. This holds promise for a model with greater predictive accuracy than the current gill-based BLM.

Acknowledgements—We thank Jamie Colman and the Neurobiology and Development Lab at the Northwest Fisheries Science Center for assistance and Scott Hecht, Frank Sommers, and an anonymous reviewer for comments on the manuscript. The present study was supported by the National Oceanic and Atmospheric Administration Oceans and Human Health Initiative, the National Oceanic and Atmospheric Administration Coastal Storms Program, and a U.S. Environmental Protection Agency Science to Achieve Results predoc toral fellowship to J.K. McIntyre.

REFERENCES