Final Report:

Environmental Stresses and Skeletal Deformities in Fish from the Willamette River, Oregon, USA

By

Daniel L. Villeneuve[†¥], Lawrence R. Curtis[†], Jeffrey J. Jenkins[†], Kara E. Warner[†], Fred A. Tilton[†], Michael L. Kent[‡], Virginia G. Watral[‡], Michael E. Cunningham[§], Douglas F. Markle[§], Doolalai Sethajintanin[†], Oraphin Krissanakriangkrai[†], Eugene R. Johnson[†], Robert Grove[†], Kim A. Anderson[*†].

Oregon State University

Department of Environmental and Molecular Toxicology, [‡] Department of Microbiology, and [§] Department of Fisheries and Wildlife

Corvallis, OR USA 97331

June 15, 2004

To: Oregon Watershed Enhancement Board

ABSTRACT

The Willamette River, one of only 14 American Heritage Rivers, flows through the most densely populated and agriculturally productive region of Oregon. Previous biological monitoring of Willamette River fish detected elevated frequencies of skeletal deformities in fish from certain areas of the lower (NP [NP], rivermile [RM] 26-55) and middle (near Wheatland Ferry [WF], RM 72-74) Willamette River, relative to those in the upper Willamette (i.e. near Corvallis [CV], RM 125-138). The objective of this study was to determine the likely cause of skeletal deformities in populations of Willamette River fish. Characterization of deformity loads in Willamette River fish collected in 2002 and 2003 demonstrated that deformity loads remained 2-3 times greater at the NPPool (NP) and WF locations than those observed at the CV location. There were some differences in water quality parameters between the NP and CV sites, but they did not readily explain the difference in deformity loads. Concentrations of bioavailable metals were below detection limits (≈1-5 μg/L). Concentrations of bioavailable polychlorinated biphenyls (PCBs) and chlorinated pesticides were generally below 0.25 ng/L. Concentrations of bioavailable polycyclic aromatic hydrocarbons were generally less than 5 ng/L. Chlorpyrifos (averaged less than 1.5 ng/L) was the only organophosphate pesticide detected as bioavailable in water. Concentrations of most persistent organic pollutants were below detection limits in ovary/oocyte tissue samples and sediments and those that were detected were not significantly different among sites. Bioassay of Willamette River water extracts provided no evidence that unidentified compounds or the complex mixture of compounds present in the extracts induced skeletal deformities in cyprinid fish. However, metacercariae of a digenean trematode were directly associated with a large portion of the lesions detected in fish collected from the Willamette River and the lesions were reproduced in fathead minnows exposed to cercariae extracted from field collected snails. As a whole, there was very little evidence to suggest that chemical contaminants were responsible for the greater deformity loads observed at NP and WF. Instead, the weight of evidence suggests that parasitic infection was the primary cause of skeletal deformities observed in Willamette River fish.

INTRODUCTION

The Willamette River in western Oregon is one of only 14 American Heritage Rivers (Uhrich and Wentz 1999). The river is the 13th largest river in the United States in terms of stream flow and yields more runoff per square mile than any other river in the U.S (Uhrich and Wentz 1999). It flows north from Eugene for approximately 187 miles through mixed agricultural and urban areas to Portland, Oregon's largest metropolitan area, before joining the Columbia River just 10 feet above sea level (Figure 1). The Willamette basin is home to 70% of Oregonians and the Willamette Valley is renowned as one of the most highly productive agricultural regions in the Pacific Northwest (Wentz et al. 1998, Altman et al. 1997). The Willamette River provides a significant migratory corridor, nursery habitat and adult forage for runs of salmon, and nearly 50 species of fish have been identified in the river (Altman et al. 1997). Recreational or sport fishing is extremely popular, and resident species are fished throughout the year. Numerous animal species utilize the Willamette River during various seasons.

As part of on-going efforts to manage and protect aquatic life in the Willamette River basin, the Oregon Department of Environmental Quality initiated investigations of skeletal deformities in fish from the Willamette River in the early 1990s. Biological monitoring was widely used as a means to evaluate the health of aquatic ecosystems and the potential impacts of antropogenic activities on those systems. It was suggested that skeletal deformities in fish served as useful bioindicators of pollution (Bengtsson 1979; Valentine et al. 1972; Lemly 1997), and evaluation of skeletal deformities in juvenile fish was used extensively to monitor the health of fish populations (Sloof 1982, Bauman and Hamilton 1984, Moore and Hixson 1977, Bengtsson 1991, Lindesjöö and Thulin 1992). In 1992-1994 the incidence of skeletal deformities in northern pikeminnow (NPM) (*Ptychocheilus oregonensis*;) collected from the NP (NP) region, extending from river mile (RM) 55 to 26.5 (Figure 1), ranged from 22-74% (Ellis 2000, Ellis et al. 1997). Skeletal deformity rates in NPM were also elevated (21.7%) in the middle Willamette River (around RM 72, Wheatland Ferry; Figure 1). In contrast, the skeletal deformity rates in juvenile NPM collected from the upper Willamette River (RM 185-125) ranged from

1.6-5.3% (Ellis 2000, Ellis et al. 1997). The NPM was not the only species impacted. Of 15 species collected from the NP region, and associated tributaries, in 2000, skeletal deformity rates exceeded 25% in 10 species (Markle et al. 2002). As a whole, biomonitoring of skeletal deformities in Willamette river fish suggested that fish from the NP region and middle Willamette River, had significantly greater deformity rates than fish from the upper Willamette River.

In the mid-late 1990s, proposals to tap the NP region of the Willamette River as a source of drinking water for urban expansion heightened public concern related to the reports of deformed fish (http://www.hevanet.com/safewater/recentnewshome.htm). In 1998, for example, 85% of people surveyed expressed "extreme" concern about the level of toxic chemicals in the river (Oregon Daily Emerald, Feb. 26, 1998). In response to these concerns the 71st Oregon Legislative Assembly Enacted Senate Bill 234 in 2001. The bill "placed a high priority on gathering specific data about and improving the scientific understanding of the extent and probable cause of fish deformities in the Willamette River" (Oregon Senate Bill 234). This study was undertaken in direct response to that legislation.

A wide variety of chemical, physical, and biological stressors have been associated with the occurrence of skeletal deformities in fish. A variety of chemicals are known to induce neuromuscular damage that can result in skeletal deformities. These include heavy metals such as lead (Holcombe et al. 1976; Hodson et al. 1980; Bengtsson and Larsson 1986) and numerous organophosphate pesticides (McCann and Jasper 1972). Chemicals can also cause skeletal deformities by impairing developmental processes and bone formation. Compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), toxaphene, and cadmium have been reported to cause skeletal deformities through such mechanisms (Henry et al. 1997, Mehrle et al. 1982, Mayer et al. 1978, Olsson et al. 1999, Teraoka et al. 2002, Cheng et al. 2000). In addition to chemicals, skeletal deformities have been linked to water quality problems including low pH (Frojnar 1977, Beamish 1977), low dissolved oxygen (Garside 1959, Alderdice et al. 1957), and elevated temperatures (Gabriel 1944, Kwain 1974). Nutritional deficits, particularly ascorbic acid and tryptophan deficiencies, have been linked to skeletal deformities in fish (Halver et al. 1969, Kloppel and Post 1975). Inbreeding has

been shown to cause skeletal malformations including scoliosis, lordosis, curved neural spines, fused vertebrae, and compressed vertebrae (McCay and Gjerde 1986, Ponyton 1987, Prion 1978). Finally, numerous infectious biological agents including viruses, bacteria, and parasites have been reported to cause skeletal deformities (Kent et al. 1989, La Patra et al. 2001, Hedrick et al. 1998, Matthews et al. 2001). Although the association of fish skeletal deformities with a wide variety of stressors makes it a useful endpoint for biological monitoring, the observation of a high incidence of skeletal deformities, alone, has little diagnostic value.

This study attempted to identify or diagnose the cause(s) of skeletal deformities associated with fish collected from the middle and lower Willamette River, with particular emphasis on the NP region. Skeletal deformities associated with fish collected from the upper, middle, and lower Willamette River in 2002 and 2003 were characterized to determine whether recent conditions were similar to those reported previously and to further describe spatial and temporal patterns of deformity loads. *In situ* monitoring of river water quality coupled with *in situ* sampling and analysis of bioavailable organic contaminants and metals compared water quality and potential for direct exposure to known chemical contaminants at the NP and CV study sites and determine whether these factors likely caused of the deformities. Analysis and comparison of sediment samples and fish tissue from NP versus CV, evaluated potential trophic or maternal transfer of known persistent organic pollutants (POPs) as a potential cause. Bioassay of river water potential to produce skeletal deformities in embryo-larval fathead minnows (FHM) (*Pimephales promelas*) under controlled laboratory conditions evaluated the potential role of unknown chemicals or complex mixtures in causing the skeletal deformities observed in Willamette River fish. Finally, field collected fish were examined for parasites, and the association of parasitic infection with skeletal lesions was quantified. Together, these components provided a weight-of-evidence-based, empirical, approach that identified the likely cause of skeletal deformities observed in fish collected from the NP region of the Willamette River.

METHODS

Fish Collection and Deformity Characterization

Larval and juvenile fish were sampled from May-October 2002 and May-August 2003. Fish were collected by beach seine, cast net, and dip net. The three primary sampling areas were NP (RM 47.5-53; lower Willamette), Wheatland Ferry (WF; RM 72-74; middle Willamette) and CV (RM 125-138; upper Willamette). Specimens were fixed immediately in the field by immersion in 10% buffered formalin. Seventeen different species were collected, with NPM, *Richardsonius balteatus* (redside shiner), *Catostomus macrocheilus* (largescale sucker), *Mylocheilus caurinus* (peamouth), and *Acrocheilus alutaceus* (chiselmouth), representing the most commonly collected species (total sample sizes > 1000; Cunningham et al. 2004-submitted).

Specimens fixed for a minimum of two weeks were X-rayed in a Faxitron MX-20 cabinet X-ray machine using AGFA Structurix D4 DW ETE industrial radiography film. Film was developed using a Kodak X-OMAT Model M6B developer. Radiographs were inspected for deformities using a 10-15X ocular over a light table. Radiographs of approximately 15,700 fish were examined. Presence or absence of twelve different categories of skeletal deformities were scored (Cunningham et al. 2004-submitted). Analyses were based on the number of deformity categories present per individual (deformity load) (Markle et al. 2002). Based on random reevaluation of 550 fish, reader error was not significant (Cunningham et al. 2004-submitted). Additional details regarding fish collection and deformity characterization are reported elsewhere (Cunningham et al. 2004-submitted).

In situ water quality and bioavailable contaminants

Site Description and Sample Collection: The sampling sites facilitated investigation of seasonal and spatial bioavailable contaminant concentrations in the Willamette River at NP and at upriver sites (CV) (Figure 1). Two stations were designated at NP, one on the south side of the river (RM 47; N 45° 16.02', W 122° 54.59') and one a few miles downriver on the north side of the river (RM 44; N 45° 15.27', W

122° 53.58'). In addition, two stations were designated at CV (RM 135; [1] N 45° 29.13', W 122 39.06'; [2] N 45° 27.37', W 122° 39.47'). The NP 1 (RM 47) sampling station was about 25-30 feet from the shoreline and the water depth was 27 feet. NP 2 sample station (RM44) was about 30 feet from the shore the water depth was 20 feet. The CV sites were about 15 feet from the shore and the water depth was 7-11 feet. Flow near the CV sites in May 2002 was *ca.* 9,000-10,000 ft^{3}/sec. By late July the flows had decreased by a factor of 2 to *ca.* 4,500 ft^{3}/sec. Willamette River flow was greater at the NP sites. Flow in May 2002 was about 17,000 to 20,000 ft^{3}/sec. By late July the flows decreased by a factor of 3 to about 7,000 ft^{3}/sec. The CV sites were shallow (2-10ft) and characterized by shallow gravel and sediment riprap. In the NP area the Willamette was much deeper (20-60 feet) and there were no shallow gravel beds near the study sites.

Field sampling was conducted from May to July in 2002 and 2003. Three sampling campaigns were completed per year, one each in approximately *ca*. May, June and July. Each sampling event was 21 days. All samples (*in situ* sampling devices and grab samples) were collected 1 ft from the river bottom. The sampling campaigns were designed to capture the river conditions during fish egg laying and early fry development. Nutrient and water quality parameters including: dissolved oxygen, conductivity, specific conductance, salinity, total dissolved solids, resistivity, temperature, pH, ORP (oxidation reduction potential), depth, ammonium/ammonia, nitrate, and turbidity; were collected on an hourly basis with a YSI 6920 Sonde (YSI, Yellow Springs, OH).

Dissolved, bioavailable organic contaminants and metals were collected by deploying passive sampling devices (PSD) and diffusion gradient thin films (DGT) in protective mesh cages. PSDs consisted of neutral lipid (i.e. triolein) enclosed in layflat polymeric tubing (Environmental Sampling Technologies, St. Joseph, MO; Huckins et al. 1990). Five individual PSD and DGTs were included in each cage. Each cage was suspended with "float-cable-cage-cable-anchor" arrangement that ensured that the cage would stay at the station and would stay suspended one foot from the river bottom. The five PSD were later composited for analysis. PSD were kept on ice in sealed airtight containers during transport to and from field sites. Complete PSD descriptions have been previously published (Huckins et al.

1993). The PSD were were gently cleaned of any sediment or algae after deployment at the site utilizing a tub filled with site water to minimize air exposure. No fouling impedance was employed in the calculations of estimated water concentration since algae growth on the devices was nil to minimal.

Analytical Procedure: PSD were extracted by hexane dialyses, in amber jars. Sample volumes were reduced using a TurboVap. The samples were then run through gel permeation chromatograph (GPC) and fractions containing organochlorine and PAH contaminants were collected. Appropriate fractions were determined by analyzing standards and fortified samples (Sethajintanin et al. 2004-in press). Appropriate fractions were analyzed using GC-ECD (organochlorine contaminants) and HPLC-DAD and fluorescence (PAH contaminants). All sample manipulations were either performed in brown amber or foil wrapped containers to minimize UV/Vis exposure.

The organochlorine contaminants fraction was separately concentrated to 0.5 ml and analyzed by gas chromatography with dual capillary columns (DB-XLB and DB-17ms, J&W Scientific Inc.) and dual ECD detectors (Ni^{63}) with an injection volume of 2 μL. DB-XLB and DB-17ms (each was 30 m x 0.23 mm ID x 0.25 μm film thickness) were used for quantification and confirmation. The GC-ECD was a Varian Star ® Model 3600 operated with the 8200 autosampler with the splitless mode. Helium and nitrogen were the carrier gas and makeup gas, respectively. Both columns were temperature programmed as follows: initial column temperature was 100 °C with a 1- min hold, then increased from 100 to 130 °C at 10 °C/min, and from 130 to 285 °C at 3 °C/min. The final temperature was held for 4 min. Injector and detector were set at 250 °C and 350 °C, respectively. Chromatographic data were integrated and calculated using Varian Star 4.0-® software. Quantification was accomplished using five to eight standards. Correlation coefficients for the standard calibration curves were >0.998. Analytes were reported when detected on both columns and only samples containing residues exceeding the blanks were considered positive.

Organophosphate insecticides were extracted as above and also analyzed by gas chromatography with a DB-17 column with electron capture detection and a DB-XLB column with a thermionic specific detector for nitrogen and phosphorus.

The PAH contaminants fraction was separately concentrated to ca. 1.0 mL. PAH detection and quantitation was performed on a Hewlett Packard 1100 HPLC with dual detection by flourescence or diode array, both with multiple wavelengths. The fluorescence detector had an excitation wavelength at 230 and emission wavelengths at 360, 410, and 460 nm; diode array had detection signals at 254, 242, and 230 nm. Only three compounds, fluorene, acenapthylene, and indeno(123cd)pyrene, were detected by diode array, the rest were detected with the fluorescence detector. The column used was a Phenomenex Luna C18, with 3*u* particle size. The instrument was run with a constant flow rate of 0.75 mL/min and a timed gradient for the acetonitrile / water eluent system. The time program ran at 40% acetonitrile for 10 min., was gradually ramped up to 70% acetonitrile for 15 min., and then ramped up to 90% acetonitrile for 10 min.. The program was held at 90% acetonitrile for 3 min. and then returned to 40% and analyzed by HPLC with diode array detection and fluorescence detection.

After the DGT were retrieved and in the laboratory, the resin-gel was removed and immersed for 24 h in 0.6 mL of 1M trace metal grade nitric acid. An aliquot was removed and diluted to 40 mL with 18MΩ*cm water. The pH was adjusted to ca. 4.5 to 5.5 prior to analysis by anodic stripping voltammetry (ASV). All grab water samples were filtered thru a 0.45 µm membrane filter prior to metal analyses by ASV. ASV (TraceDetect, Seattle, WA) quantified the metals reported. Reduction potentials were verified with standards for each metal tested.

Quality control (QC): Field, trip, and extraction blanks were used with each sampling campaign. The field blank PSD sample were opened and exposed to the atmosphere during deployment or recovery. These field blanks were processed and analyzed exactly as deployed PSD samplers. Field extraction blanks, were opened in the field and washed simulating the process of removing the light sediment or algae on the passive sampling devices. Samples containing residues exceeding the blanks were considered positive for residues. Transport blank values were multiplied by the water volume they would have been exposed to if left with the other PSDs. The CV site was designated as field duplicate. Field duplicates represented 30% of all samples collected. All QC sample types were included in each analytical batch of analysis. Laboratory QC samples included reagent blanks, fortified samples and

laboratory duplicates, each QC type represented 5-10 % of the total number of samples analyzed in any given batch. They were prepared and analyzed in the same fashion as the field samples. Standard curves were typically composed of ≥ 4 standard concentrations for all organic analyses and ≥ 3 for all inorganic analyses.

Data Analysis: The theory and mathematical models required for estimation of analyte water concentrations from the concentration in the PSD lipid have been previously described (Huckins et al. 1993). The following equation was used to calculate the dissolved (bioavailable) water concentration:

$$C_w = C_{spmd}V_{spmd}/R_st$$

Where C_w is the concentration of analyte in water, C_{spmd} is the concentration in lipid (spmd), *t* is the exposure time in days, V_{spmd} is the volume of lipid/membrane, and R_s is the PSD sampling rate. Sampling rates (R_s) for a large series of organochlorine and PAH contaminants have been previously established.

The mass of the metal in the DGT resin gel (M) was determined from the ASV quantitation. The theory and mathematical models required for estimation of the analyte water concentrations form the concentration in the DGT have been previously described. The following equation was used to calculate the labile (bioavailable) water concentration:

$$M = Ce\ (V_{HNO3} + Vgel)/fe$$

where Ce was the concentration of metals in the 1M HNO_3 elution solution, V_{HNO3} was the volume of HNO_3 added to the resin gel, Vgel was the volume of the resin gel, and *fe* was the elution factor for each metal. The concentration of the metal measured by DGT (C_{DGT} ="bioavailable" water concentration) was determined from the following equation:

$$C_{DGT} = M\Delta g/(DtA)$$

where Δg was the thickness of the diffusive gel (0.8mm) plus the thickness of the filter membrane (0.13 mm), D was the diffusion coefficient of metal in the gel, t was deployment time and A was the exposure area ($A=3.14\ cm^2$).

The Mann-Whitney test was applied for statistical comparisons of site differences in bioavailable contaminants.

Analysis of POPs in Northern pikeminnow ovary tissue

The NPM (*Ptychocheilus oregonensis*) was the species chosen for analysis of maternal transfer of POPs. They are abundant in both regions of the Willamette, are relatively easy to collect, reach moderately large sizes, and have been heavily impacted in the NP region (Markle et al. 2002; Ellis 2000). Adult NPM were collected from NP (N 45° 16.007, W 122° 55.031) and CV (N 44° 28.250, W 123° 14.300) study areas (Figure 1) in May-June 2002 using a combination of hook and line and electrofishing. Wet weight of the fish collected for analysis of maternally transferred POPs ranged from 375-975 g. There was no significant difference in the mean wet weights of the fish collected from the two study sites. Field collected animals were transported to the laboratory on ice. Ovary tissue and associated oocytes were removed from gravid females, using clean, solvent rinsed, dissection tools, and placed into certified I-Chem® jars. Tissue samples were stored at -20°C until extracted.

Samples were shipped to GLP- (Good Laboratory Practices) certified analytical laboratories for quantification of a range of POPs. Twenty-one chlorinated pesticides were quantified by gas chromatography with electron capture detection (GC/ECD) according to EPA method 8081A (ODEQ Laboratory, Portland OR). Twenty-eight polychlorinated biphenyl (PCB) congeners were quantified by GC/ECD according to EPA method 8082 (ODEQ Laboratory, Portland, OR). Additionally, concentrations of seven polychlorinated dibenzo-*p*-dioxin (PCDD) congeners and 10 polychlorinated dibenzofurans (PCDFs) were quantified by high resolution GC/MS (Axys Analytical, British Columbia, Canada). Method detection limits (MDLs) for chlorinated pesticides and PCBs ranged from 2.5-3.3 μg/Kg wet wt. MDLs for PCDDs and PCDFs ranged from 0.10-0.13 ng/Kg wet wt.

A total of 5 ovarian tissue/oocyte samples (each from a separate fish) per study area were analyzed. For the purposes of statistical analysis and plotting of figures, concentrations below the method reporting limit (MRL) or detection limit were assumed to be equal to one half of this limit. When the assumptions of parametric statistics were met, t-tests were used to test for differences among study sites. Kolmogorov-Smirnov's test was used in cases where parametric assumptions were not met.

Analysis of POPs in sediment

Grab samples of surficial sediment were collected from the NP and CV region of the Willamette River. In 2002, three samples were collected at NP location N 45°16.308, W 122°59.460, and three samples were collected at CV location N 44°31.567, W 127°15.384. In 2003, three samples were collected at NP location N 45°15.567, W 122°54.231 and three samples were collected at CV location N 44°32.887, W 123°15.432. Sediment samples were scooped directly into certified I-Chem® jars and transported to the laboratory on ice. Samples were stored at –20°C until shipped for analysis. Sediment samples were extracted and analyzed at the ODEQ laboratory, Portland, OR. Sediment extracts were analyzed for 22 chlorinated pesticides by GC/ECD (EPA method 8081A), 8 nitrogen/phosphorous pesticides by GC/NPD, and 29 PCB congeners by GC/ECD (EPA method 8082 A). MDLs for chlorinated pesticides and PCBs were around 0.33 μg/Kg wet wt. The MDL for nitrogen/phosphorous pesticides was 10 μg/kg wet wt.

Preparation of River Water Extracts for Bioassay

Water samples were collected from four study locations during the summer of 2003. The sampling sites included two NP locations (NP: N 45°15.567, W 122°59.142 and AI N 45°16.145, W 122°59.142), Wheatland Ferry (WF: N 45°05.447, W 123°02.655), and CV (N 44°32.887, W 123°15.432). On each sampling day, samples were collected from CV and one of the other three sampling locations. Grab samples were collected in 20 L stainless steel containers and three 20 L samples were collected at each site. Samples were typically collected at a depth of approximately 1 m

and containers were opened and sealed (all air removed) underwater. In all cases, collections were made at least 30 cm below the surface and at least 30 cm above the sediment. Sample extraction was completed within 96 h of sample collection.

Samples were extracted by drawing them with vacuum through through DVB-phobic and DVB-phillic solid phase extraction disks (Bakerbond Speedisk® 8072-06, 8068-06, J.T. Baker, Phillipsburg, NJ) arranged in series (5 independent stations of phobic and philic disks in series). Flow rates were 15-30 ml/min. Each of the five Speedisk® stations was used to extract 12 L. After extraction was completed, the disks were dried under vacuum, and stored in airtight containers at –20°C overnight. Each disk was eluted three times with 5 ml of methanol. Eluents were passed through a column of Na_2SO_4 to remove any water present. Eluents from both the phobic and phillic disks were pooled together for each sample site (~150 ml) then evaporated to 3 ml under a steady stream of N_2 gas using a Zymark Turbovap II. Resulting extracts were transferred to amber glass vials and stored at –80°C until used for bioassay.

Since the extraction procedure was designed to capture a broad spectrum of known and unknown contaminants (wide range of K_{ows}), it was not feasible to determine extraction efficiencies for all possible components. The basic solid phase extraction approach described above has been characterized for over 100 current use pesticides and persistent organic pollutants (Usenko and Simonich 2004, personal communication). As a quality assurance test for this study, chlorpyrifos was spiked into water samples at multiple stages of the extraction process including. The greatest losses occurred during the solid phase extraction step. However, greater than 70% of the chlorpyrifos added to either river water or deionized water was recovered in hexane extracts of the final MeOH eluent. This was consistent with recoveries demonstrated by other researchers (Usenko and Simonich, 2003 personal communication). Overall, the extracts prepared were considered representative of a broad mixture of chemical contaminants present in the river, but quantitatively accurate proportions of each component was not assured.

Fathead minnow skeletal deformity assay

Larval FHM less than 24 h post-hatch were obtained from Chesapeake Cultures (Hayes, VA, USA). Larval FHM (24-48 h post-hatch) were randomly assigned to 400 ml beakers containing 100 ml of dechlorinated tapwater (dtw). Each beaker was stocked with n=30 larval FHM. Beakers were then randomly assigned to one of eight treatment groups. The treatment groups for the study were as follows: control (CON; 200 ml of dtw); solvent control (SC; 0.05% MeOH in dtw); 8X-, 4X-, and 1X-CV; 8X-, 4X-, and 1X-NP, AI, or WF. 8X, 4X, 1X represents a volume of the appropriate extract dissolved in 200 ml dtw to provide a concentration equivalent to 800%, 400%, 100%, respectively, of the river water concentration of the extract's constituents assuming 100% recoveries. Methanol was added to each of the 4X and 1X treatments such that the total MeOH concentration was equivalent to that of the 8X treatments and SC (0.05%). Fifty percent of the test solution was renewed daily by drawing the solution down to 100 ml and adding 100 ml of fresh test solution containing nominal concentrations of extract and/or solvent. The location of each beaker on the exposure bench was assigned randomly.

After 5 days of exposure, surviving fish were counted and transferred to 1 L plastic containers for grow out to ~ d. 25-30 post-hatch. During the grow-out period fish were maintained in dtw supplied from a flow-through system. Throughout both the exposure and grow-out periods, water temperatures were maintained at 24-26°C, photoperiod was 16 h light, 8 h dark and FHM were fed *Spirulina* (Algae Feast, Earthrise, Petaluma, CA, USA) twice daily and brine shrimp nauplii (GSL Brine Shrimp, Ogden, UT, USA) once daily. Water quality (dissolved oxygen, pH, ammonia, and nitrite) was monitored daily.

At the end of the grow-out period, fish from each container were transferred, live, to a 5 cm diameter plastic tube with fine mesh at one end (PVC insert). The entire batch of fish was immersed in a 0.2% calcein (Sigma C-0875; St. Louis, MO, USA) solution (pH 7.0). Fish were live calcein stained for 10 min, transferred to clean water for 10 min to destain, and then euthanized by immersion in a 200 mg/L solution of MS-222 (Finquel®, Argent, Redmond, WA, USA). Euthanized specimens were immediately examined by fluorescence microscopy using a Leica MZFL111 dissecting microscope

(Bartles and Stout, Bellevue, WA, USA) equipped with a mercury lamp and fluorescein/green fluorescence protein filter. Calcein staining allows for direct visualization of calcified skeletal structures (Du et al. 2001). Each specimen was examined for skeletal deformities including scoliosis, lordosis, fused vertebrae, compressed centra, extra or missing spines, etc. Screening of several hundred fish as part of assay development confirmed that all these types of deformities were detectable by this method. Vertebral development was also scored on a scale of 1-5 using a criteria defined for this study. Digital images of each fish and close-ups of deformities, if detected, were captured and archived using ImagePro Plus 4.5.1 (Media Cybernetics, Silver Springs, MD, USA). In some cases, examinations were spread over 2-3 d. Replicates examined each day were selected randomly.

Survival to the end of the exposure period (6 d post-hatch), survival to the examination (28-30 d post-hatch), and percent of surviving fish with a skeletal deformity, and was determined for each replicate. Developmental score distributions were determined for each treatment. One-way analysis of variance was used to test for differences in survival or incidence of deformities, among treatments. A non-parametric Kruskall-Wallis test on ranks was used to test treatment-related differences in developmental score distributions.

Parasite Characterization and Laboratory Infection Assays

Characterization of parasite association with vertebral lesions in fish collected from the Willamette River was based on examination of histological sections of formalin preserved fish, as well as whole mounts of trypsin-cleared, alcian blue and alizarin red S-stained fish (Kent et al. 2004). The methods and statistical analysis used for the parasite characterization were reported elsewhere (Kent et al. 2004).

For laboratory transmission studies, laboratory-reared FHM were obtained from Cheasapeake Cultures, Hayes, VA. Fish were held in dechlorinated tap water (23-26°C) to ensure unexposed fish did not become infected. Fish were mainatained in static water aquaria with biological filters. Fish were delivered at 3 – 7 day old, and were initially feed paramecium cultures until about 10-14 days old, then

switched to a mixutre of brine shrimp naupallii (GSL Brine Shrimp) and freeze-dried Spirulina algae (Algae Feast). After about 3- 4 wk fish were then feed TetraMin flake food (Tetra Sales, Blacksburg, VA).

Fluminicola virens snails were collected from the NP area of the Willamette River (Figure 1) from June – August 2003. Cercariae consistent with those described by Niemi and Macy (1974) were harvested by holding individual snails in isolation in 24 well tissue culture plates in 2 ml water. For transmission studies, larval FHM of varying age (Table 4) were exposed to known concentrations of cercariae or control water. Several exposures were conducted as initial trials with very young fish resulted in high mortality in exposed fish (Table 4).

Incidence of infection, vertebral deformities, and association of worms with deformities was determined by examination of whole, preserved fish that were cleared with trypsin and stained with with alcian blue and alizarin-red S (Dingerkus and Uhler 1977; Potthoff 1984). Fish were collected at either 55 or 70 days post exposure. Cleared fish were placed in a Petri dish, covered with glycerin, and the entire fish was examined at 25 or 50 X. Fish were also evaluated by radiography as described by Markle et al. (2002).

RESULTS & DISCUSSION

Deformity Loads in Willamette River Fish

Lack of reliable information on normal background deformity rates in unstressed fish populations limits value of skeletal deformities in fish as a biomonitoring tool. One study in salmonids suggests 2-5% may be a normal background rate of skeletal deformities in wild populations (Gill and Fisk 1966). However, it is unclear how much this background rate may to vary among different species, and among geographically distinct populations. Since, in most cases, background deformity rates in unstressed populations are unknown, biomonitoring approaches based on skeletal deformities rely on the detection of changes in deformity loads from year to year, or on comparisons between locations with

similar habitat, climate, etc. In the case of Willamette River fish marked geographic disparity in the frequency of skeletal deformities in fish that suggest stress on fish populations in the NP and Wheatland Ferry regions of the river.

Deformity characterization for 2002-2003 were consistent with studies from previous years that suggested that the incidence of skeletal deformities in Willamette River fish were elevated in the lower (NP) and middle (Wheatland Ferry) Willamette River relative to up-stream areas (CV) (Ellis 2000, Markle et al. 2002). Among the five species most commonly sampled, the percent frequency of deformities was generally 2-3 times greater at the NP and Wheatland Ferry locations than at CV (Table 1). The only exception to this was the large-scale sucker (*Catostomus macrocheilus*), which was notable as the only catostomid species of the five most commonly collected. The other four species were cyprinids. Among the cyprinid species, mean deformity loads for fish from Wheatland Ferry were significantly greater than those for fish from CV (Table 1). The same was true for fish from NP, although for peamouth the difference was not statistically significant (Table 1). There were no obvious geographic or habitat differences that explained the differences observed (Cunningham et al. 2004). Overall, biomonitoring of skeletal deformities in fish collected at different locations along the Willamette River in 2002-2003 suggested fish populations near NP and Wheatland Ferry were stressed relative to those near CV.

Water Quality Characterization

Ammonium, nitrate, pH, temperature, dissolved oxygen, oxidative reduction potential, and specific conductance data were collected hourly during all 21 d sampling events. At each individual station, some parameters showed strong temporal and spatial variation while others did not. As expected, the water temperature varied during each 24 h cycle. The diurnal temperature variation was larger at the CV sites (1-2° C), whereas the NP sites varied 1°C or less over a 24 hr cycle. Temperature increased throughout the sampling season. In May, temperatures were generally 12 ± 1C in May and by the end of the sampling period were ca. 22C ± 2C. The temperature patterns were the same for both

seasons (2002 and 2003). The pH varied from 7.2 to 7.8 at the NP sites for both 2002 and 2003. The CV sites had larger diurnal changes in pH from 7.2 to 8.8 during some 24 hr cycles, usually in late May and early June. These large diurnal pH changes were seen in both 2002 and 2003. At all sites there were small decreases in pH from the 2002 season to the 2003 season. The nighttime pHs were similar at all locations; however, there was a strong geographic difference in daytime pH. The CV sites were often 1+ pH units higher than the NP sites. A few studies have linked low pH conditions (less than 5.5) to the development of skeletal deformities in suckers (Frojnar 1977; Beamish 1972), but we are not aware of any reports that link pH 7.2-8.8 conditions to skeletal deformities in fish.

The oxidation reduction potential (ORP) probe tended to drift after about 7-10 days of deployment. Since all deployments were 21 days, data after 7 days was excluded from the analysis. The ORP was consistently lower at the NP sites as compared to the CV sites. During some sampling events the difference was small, <10% lower, while for other events the difference was as great as a factor of 2. There was no apparent difference during the season at the CV sites, and little difference between 2002 and 2003. There was some evidence that the ORP increased during the season at the NP sites and there was some difference between 2002 and 2003.

The ORP value is a direct reading of the activity of the oxidizing and reducing agents in the water as they correspond to oxidation-reduction reactions. In general, the CV waters were more oxidizing than the NP waters, (or the NP waters are more reducing than the CV site water). Thus, based solely on ORP, microbial growth may have been favored at the NP site.

Specific conductivity (SC) was very similar for all sites (CV and NP). There was a slight increase at all sites as the seasons progressed (May-July). The SC pattern was similar for both 2002 and 2003. The dissolved oxygen (DO) pattern was the same for all sites. A diurnal pattern was apparent, and a slight decrease in DO was seen from May through July in both 2002 and 2003. A seasonal increase in ammonia was measured at all sites. The concentration increased about a factor of 5, from 0.05 to 0.25mg/L. In general, the CV sites had higher ammonia than the NP sites. The nitrate probe was not robust, in 2002 the drift generally occurred within 24 hr of deployment, in 2003 the probe failed

within a few hours after deployment. The limited data indicated that nitrate was low early in the year (May) and increased through July. Although the data was limited, it appeared that the nitrate concentrations were higher at the NP sites compared to the CV sites. As a whole, water quality monitoring provided no compelling evidence to suggest that differences in nutrient concentrations, pH, temperature, DO, or specific conductivity were likely causes of the different deformity loads observed at NP versus CV, but further investigation of a possible link between ORP differences and susceptibility to infection may be warranted.

Bioavailable Contaminants

Polycyclic aromatic hydrocarbons (PAHs): Total bioavailable PAHs were low at all locations, for all sampling events, (generally <5 ng/L). Of the 16 PAHs measured, 13 PAHs were below detection limits (<0.1 ng/L) in 2002. In 2003, 10 PAHs were typically below detection limits. Three PAHs, phenanthrene, fluoranthene, and anthracene, were detected at sites in 2002 and 2003. At the CV sites phenanthrene and anthracene were equally abundant in 2002, while fluoranthene was somewhat less abundant (Table 2). Of the three PAH detected at the NP sites in 2002, phenanthrene was the most abundant (Table 2). In 2003, anthracene, and phenanthrene were the most abundant of the 16 individual bioavailable PAHs. In 2002, the CV sites consistently had higher total PAH (ΣPAH) concentrations compared to the NP study sites (Table 2). The average ΣPAH in 2002 was *ca*. 3.2 ng/L at CV, while the other sites had ΣPAH concentrations in 2002 of *ca*. ≤ 2 ng/L. In 2003 this pattern was consistent (Table 3). The average CV sites (1&2) had ΣPAH of 3.9 and 2.7 ng/L respectively, while the 2003 average NP sites (1&2) had ΣPAH of ≤ 2 ng/L. At the low concentrations detected at the two sites, PAHs were not considered to be likely contributors to the difference in deformity loads associated with the two areas.

Polychlorinated biphenyls (PCBs) PCBs analysis for this study was based on a congener-specific approach. However, concentrations of all congeners were quite low, so interpretation of results focused on total PCB concentrations. The total bioavailable PCB concentrations were generally very

low, < 0.15 ng/L at all sites and many sites were below detection limits (0.03 ng/L) (Table 2). During 2002 and 2003, the total bioavailable PCBs were generally higher at the NP sites (Table 2). However, since most of the data are below detection limits or near detection limits, it is difficult to draw any firm conclusions. As a whole, the PCB concentrations detected did not readily explain the difference in deformity loads or raise alarm.

Pesticides: The bioavailable ΣDDT concentrations were low (generally < 0.3 ng/L) at all sites in 2002 and 2003 (Table 2). On average the NP sites had the same or higher concentrations of ΣDDT than the CV sites (Table 2). The difference between sites was significant in 2003 (p=0.041) but not in 2002 (p=0.18). There was no significant difference between ΣDDT in 2002 and 2003 (p=0.589 NP; p=0.310 CV). In 2002, the DDT profile was dominated by p,p-DDD followed by DDE and DDT (Table 2). This profile would be expected from older deposits. However, in 2003, DDE dominated, followed by DDD and DDT (Table 2). DDD and DDE concentrations detected in 2002 and 2003 were not significantly different (p=0.065-1.0). Mean DDT concentrations at NP were not significantly different between 2002 and 2003 (p=0.699; Table 2), but mean bioavailable DDT detected at CV increased 3.5-fold (p=0.004; Table 2). The cause of the increased p,p'-DDT concentration was not clear, but the results suggest input from a newer source.

Dieldrin concentrations were slightly higher at the NP sites compared to the CV sites (Table 2; 2002 p=0.015; 2003 p=0.004). However, the bioavailable concentrations of dieldrin were very low, <0.15 ng/L and there was no significant difference between years (NP p=0.699; CV p=0.589; Table 2). The dieldrin concentrations detected are well below those generally thought to be toxic to fish (Mayer and Ellersiek 1986; Georgacakis and Khan 1971) and we are not aware of any reports linking ng/L concentrations of dieldrin to skeletal deformities.

Chlorpyrifos was the only organophosphate pesticide detected. Dimethoate, diazinon, and azinophos-methyl were below detection limits at all sites for all sampling campaigns (detection limits were estimated at 2, 3 and 2 ng/L, respectively). In 2003, the estimated, bioavailable, water concentration for chlorpyrifos averaged 0.74 ± 0.51 at CV, and 1.38 ± 0.33 ng/L at NP (Table 2). There

was a trend for higher chlorpyrifos concentrations in NP but this difference was not statistically significant (P=0.065). The bioavailable chlorpyrifos concentrations observed in this study were well below those reported to be toxic to fish (Giesy et al. 1999) and there were no peer-reviewed reports linking lesser concentrations of chlorpyrifos to skeletal deformities in fish.

Metals: Bioavailable heavy metals samples were collected using diffusion gel thin-films (DGT) and samples were deployed the same as the PSD. Bioavailable zinc, cadmium, lead and copper were determined for all 6 sampling campaigns. Zinc, cadmium, lead, copper and arsenic (III) were determined in filtered grab water samples pulled during the 2003 sampling deployments. The bioavailable metal concentrations were, for the most part, below detection limits. The only exception was detection of approx. 100 µg/L Zn in a single sample from NP. However, as this detection was never replicated and all other samples from the site were below detection limits, this was considered an artifact. The results provided no evidence that bioavailable heavy metals were a likely cause for the difference in deformity loads at the two sites.

Maternal Transfer of POPs

Concentrations of POPs detected in Willamette River NPM ovary/oocyte tissue were relatively low. Chlorinated pesticide concentrations were generally less than 3.3 ng/g wet wt and only three of the 21 different chlorinated pesticide residues analyzed in Willamette River NPM ovary/oocyte tissue were detected (Table 3). Of the three, 4,4'-DDE was detected with the greatest frequency and at the greatest concentrations (Table 3). Endrin and 4,4'-DDD were detected in two of the ten samples analyzed, both from fish collected from the NP region. Exposure to ppb concentrations of toxic chlorinated pesticides similar to those detected in the ovary/oocyte tissue from some of the fish analyzed, have caused adverse effects in early life stage fish (Harris et al. 1994; Villalobos et al. 2003; PAN Pesticide Database). Thus, some potential for toxic effects of maternally transferred chlorinated pesticides was possible, although more extensive study would be needed to determine how probable such effects are.

Concentrations of maternally transferred PCBs detected in NPM ovary/oocyte samples were not alarming. A total of seven different PCB congeners were detected in one or more of the NPM ovary/oocyte samples (Table 3). PCB 153 was detected the most frequently (6/10 samples) and at the greatest concentration, (up to 11.2 ng/g; Table 3). PCBs 110, 118, and 138 were each detected in a single ovary/oocyte sample from a fish collected from NP. All the PCB congeners detected were mono- or di-ortho substituted. These congeners tend to be much less toxic than the non-ortho planar PCBs (Harris et al. 1994; Walker and Peterson 1991; Zabel et al. 1995). Fish, in particular, were less sensitive to the mono-ortho PCBs than mammals or birds (van den Berg et al. 1998; Zabel et al. 1995). It was suggested that co-exposure to the relatively nontoxic mono and di-ortho PCB congeners reduced the overall uptake of the more toxic non-ortho planar PCBs (Harris et al. 1994). Mean concentrations of PCBs 8 and 18 were greater in fish collected from CV than those in fish from NP (Table 3). Conversely, the mean concentration of PCB 153 in ovary/oocyte tissue was greater in NP fish (Table 3). However, neither of these differences between sites were significant. Based on the samples analyzed, maternal transfer of PCBs was an unlikely risk for overt early life stage toxicity to Willamette River NPM.

PCDDs and PCDFs were detectable in all the ovary/oocyte samples analyzed. The specific congeners varied considerably among samples, therefore a toxic equivalents approach (van den Berg et al. 1998) facilitated analysis of the results and comparison among sites. Total 2,3,7,8-TCDD equivalents in oocyte/ovary tissue of Willamette River NPM ranged from 0.18 to 2.06 pg/g wet wt. The greatest TEQ concentration (2.06 pg/g wet wt) was detected in a fish from CV. However, the mean TEQ concentrations were nearly identical for fish collected from the two study sites (0.84 ± 0.20 versus 0.85 ± 0.31 pg/g wet wt for NP and CV, respectively; Table 3). Total concentrations of TEQs detected in NPM ovary/oocyte tissues were less than those expected to cause toxicity during early life stage development. Based on measured TCDD concentrations in fish eggs, the lowest observed effect concentration for 7 different fish species ranged from 270-2000 pg/g wet wt (Elonen et al. 1998). NOECs were greater than 175 pg/g wet wt (Elonen et al. 1998). The TEQ concentrations detected in

this study were at least 135 times lower than the LOEC for the most sensitive of the seven species tested (Elonen et al. 1998). Furthermore, the TEQ concentrations detected were at least 2.5 times less than the probable no observable adverse effect level (NOAEL) of TEQs for lake trout, which is widely regarded as the fish species most sensitive to dioxin (and dioxin-like) toxicity (Cook et al. 2003; Elonen et al. 1998). Thus, concentrations of maternally transferred PCDDs and PCDFs unlikely caused early life stage toxicity in Willamette River NPM.

Based on the literature, it was unclear whether any of the concentrations of POPs detected in Willamette River NPM ovary/oocyte tissue likely caused skeletal deformities. However, no significant differences in maternally transferred POP concentrations were observed for fish from the NP versus CV study site (Table 3). Even if early life stage exposure to POPs was causing some disruption of early development, leading to skeletal deformities, it unlikely accounted for 2-3 fold greater rates of skeletal deformities among NP fish. As a whole, these results provided no compelling evidence that supported the hypothesis that greater maternal transfer of POPs was a likely cause for the greater prevalence of skeletal deformities in fish from the NP region of the Willamette River.

Sediment POPs

A small number (n=3 per site, per year) of surficial sediment samples collected from NP and CV sites were analyzed for persistent chlorinated pesticide residues, PCBs and organophosphate pesticides to determine whether tropic transfer of these compounds from sediment, or direct exposure of embryo-larval fish (particularly for broadcast spawners; Cunningham et al. 2004) accounted for differences in deformity loads at the two sites. Chlorinated pesticides were not detected in samples collected from CV or NP in either 2002 or 2003. In 2002, PCB 8 was detected in 2/3 CV samples and 1/3 NP samples. Concentrations of PCB 8 ranged from 1.3-6.6 ng/g. Additionally, PCB 128 was detected in a single CV sample and PCBs 18, 101, and 153 were detected in a single NP sample. Concentrations of these congeners ranged from 0.5 (PCB 101) to 3.8 (PCB 18). In 2003, only 2 congeners, PCB 101 and PCB 110, were detected. PCB 101 was found in one CV sample and two NP samples, at concentrations

ranging from 0.37-1.2 ng/g. PCB 110 (1.1 ng/g) was detected in a single sample from CV. Organophosphate pesticides were not detected in sediments from NP or CV. Samples were not collected at identical locations each year, so it was not possible to determine whether differences in the congeners detected were primarily the result of spatial versus temporal differences. Overall, however, the results did not provide compelling support for the hypothesis that chlorinated pesticide residues, PCBs or organophosphate pesticides present in surficial sediments were a likely cause for the greater skeletal deformity load in fish from the NP region, relative to those from CV.

Skeletal Deformities Bioassay

Laboratory exposures of FHM to Willamette River water extracts from d.2-d.6 post-hatch with subsequent grow-out to d. 28-30 post-hatch provided no evidence that unknown compounds or chemical interactions caused greater deformity loads observed in fish from certain regions of the Willamette River. Survival to d 6 post-hatch ranged from 83-100% in all trials and there were no significant differences among treatments (p=0.202-0.754), indicating that the extracts were not acutely toxic to larval FHM. Survival during the grow-out period was variable among replicates and among trials, ranging from 5-19 fish per replicate (17-63%). In all cases, a minimum of 20 fish, per treatment group were examined for deformities. It was not possible to determine whether fish that died during grow-out were deformed. None the less, the lack of a significant treatment-related effect on survival to the examination day (p=0.425-0.980) suggested mortality during grow-out was randomly distributed among replicates and did not obscure a treatment effect.

When simple dorsal-ventral curvature was included as a deformity, 5-25% of the fish examined were classified as deformed, although no treatment-dependent effect was observed (p=0.834-0.929). When the analysis was restricted to only those deformities characterized as "qualitatively similar to those observed in Willamette River fish" (as per categories defined by Cunningham et al. 2004), the incidence of deformities ranged from 0.8%-2% for the entire population surveyed in each trial. Given the total sample sizes of 210-397 fish per trial, this represented 2-8 individual fish. In all cases, the

deformities were spread across treatments, such that no association with any particular treatment was evident.

The distribution of developmental scores was unaffected by treatment in the NP/CV and AI/CV trials (p=0.255, 0.470), with most fish having developmental scores greater than 3. In the WF/CV trial, fish from the 4XC group were significantly more developed than those from all other treatment groups (p=0.024). However, no concentration-dependence was evident. As a whole, there was no evidence that the Willamette River water extracts induced skeletal deformities or otherwise adversely affected larval fathead minnows exposed for 96 h from d 2 to d 6 post-hatch.

A negative response in the skeletal deformities bioassay can not rule out the possibility that chemicals in the extracts had the potential to induce deformities in cyprinid fish. As designed, the assay provided a reasonable screen for the potential of the river water extract to disrupt early life-stage developmental processes important for later formation of the ossified vertebral column. The assay was not designed as an effective screen for chemicals that acted through acute neuromuscular damage or disruption of vertebral calcification. A time-series for skeletal development in FHM held under assay conditions showed that as early as d 5 post-hatch nearly all fish had ossified skulls and initial vertebral formation as indicated by ossification of the anterior-most centra (unpublished results). Deformities were not induced at non-toxic concentrations of Cd, Se, and chlorpyrifos as positive controls (unpublished results). However, the assay detected dexamethasone-induced deformities, suggesting effectiveness for screening for some mechanisms of action (Warner et al. unpublished). Robust application of the method will require additional characterization of the detectable mechanisms of action, and further optimization to reduce mortality-related variability during grow-out.

Parasites

Skeletal lesions in fish from the Willamette River were strongly linked with metacercariae of a diagenean treamatode, likely *Apophallus donicus* (Kent et al. 2004, Cunningham et al. 2004, Niemi and

Macy 1974). An analysis of cleared and stained specimens collected from four locations along the Willamette River (including the NP, WF, and CV study sites) concluded that the probability of a precaudal skeletal deformity was strongly dependent on the number of trematode cysts in the body ($p<0.0001$) and the area collected ($p=0.006$) (Cunningham et al. 2004). Species and fish size were not significant predictors (Cunningham et al. 2004). Trematodes were directly associated with 86.5% of 592 primary precaudal deformities detected in chiselmouth examined as part of the parasite investigation (Cunningham et al. 2004, Kent et al. 2004). In NPM, trematodes were directly associated with 46.3% of the lesions observed (Cunningham et al. 2004). Additionally, a *Myxobolus* sp., likely *Myxobolus cyprini*, was associated with a significant portion (36%) of NPM with histologically verifiable skeletal lesions (Kent et al. 2004). These results suggested that parasites likely caused skeletal lesions observed in Willamette River fish. However, based solely on examination of field collected specimens, it was not possible to determine whether parasites actually caused the lesions or whether fish with lesions were simply more vulnerable to infection.

Results of the laboratory infection studies convincingly demonstrated that vertebral deformities, consistent with those observed in Willamette River fish, were caused by trematode cercariae. Five separate exposure trials were conducted with FHM (a cyprinid species) ranging from 8-17 days old (post-hatch). Mortality was variable and often high in both cercariae-exposed (14-71%) and control fish (5-91%). None the less, conclusions were drawn. A high incidence of infection (80-100%) was observed in cercariae-exposed fish from all trials (Table 4). Infected fish exhibited a high incidence of vertebral deformities (70-93%; Table 4). Most of the lesions were directly associated with metacercariae (Figure 2), and nearly all trematodes were directly located along the vertebral column. The types of deformities observed were identical to those observed in field collected specimens (Kent et al. 2004, Cunningham et al. 2004) including extra spines, lordosis, fused vertebrae, and increased vertebral density (Figure 2). Metacercariae occurred directly appressed to or deep within vertebrae and were often associated with bone hypertrophy. In contrast to cercariae-exposed fish, only 7% of the control fish examined exhibited skeletal deformities (Table 4). Control deformities were characterized

as curvature of the spine or fused vertebrae. The incidence of skeletal deformities observed in control fish for the infection studies was consistent with background rates of skeletal deformities determined for lab-reared fathead minnows examined by fluorescence micrscopy.

Replication of the vertebral deformities, observed in fish collected from the field, by exposure to *Apophallus donicus* under controlled laboratory conditions further demonstrated that this parasite was likely a major cause of the lesions observed in cyprinid fish from the Willamette River. This heterophyid digenean trematode exhibits broad host specificity, infecting many species in the family Cyprinidae as well as fish from several other families (Niemi and Macy 1974; Kent et al. 2004; Cunningham et al. 2004). As observed in both our laboratory and field studies, the parasite exhibited remarkable affinity for bone (Kent et al. 2004, Cunningham et al. 2004). Most of the metacercariae were associated directly with skeletal structures and were not found in the viscera. Similar to *Apophallus* sp. in the present study, *A. brevis* in yellow perch apparently does not infect the visceral organs (Pike and Burt 1983). Taylor et al. (1994) described bony ossicles in yellow perch caused by *A. brevis*. Infections by other metacercariae types were linked to vertebral anomalies. Muscle infections by *Bucephalus polymorphus* caused vertebral deformities in cyprinid fishes (Baturo 1980), and *Riberiora sp*. was a suspected to be a major cause of supernumerary limbs and other vertebral changes in frogs in North America (Kaiser 1999). Thus, both empirical evidence and literature reports supported the conclusion that trematode parasites caused the skeletal deformities observed in Willamette River fish.

Future Investigation

Although parasitic infection was established as the likely cause of skeletal deformities in Willamette River fish, questions remain as to whether the spatial differences observed were due to natural factors or anthropogenic influences. Increased occurrence of trematode infections were linked to anthropogenic pollution and physical alteration of aquatic habitats caused by human activities (Lardans and Dissous 1998). Potential synergism between exposure to herbicides and pesticides and

susceptibility of frogs to infection by metacercariae of *Ribeiroia sp.* and *Telochris sp*. was reported (Kiesecker 2002). None of the chemical contaminants detected in this study were known to cause immune suppression or increase susceptibility to infection at the concentrations observed. However, the biological assays used in this study were not designed to test the interaction between exposure to parasites and exposure to complex mixtures of chemicals present in Willamette River water and/or sediment (i.e. Willamette River water or sediment extracts). This would be a useful step toward determining the potential role of chemicals in promoting susceptibility to the parasites. Alternatively, it is equally possible, that the spatial difference in deformity loads was reflective of natural phenomenon. Given the life cycle of *Apophallus* sp. (Niemi and Macy 1974), habitat characteristics favoring either the intermediate host (snails such as *Fluminciola* sp.) or the definitive host (fish-eating birds) could result in greater *Apophallus* sp. abundance, resulting in more infections. Natural factors influencing the viability, numbers, and microbial and other infectious agents, such as ORP, may also play a role. Additional study of the parasite ecology and potential interactions with anthropogenic influences could help determine appropriate management actions for affected regions of the Willamette River basin.

ACKNOWLEDGMENT

This work was supported by a grant from the Oregon Watershed Enhancement Board (#201-562). Additional support for some aspects of the study was provided by NIEHS Center Grant P30 ES03850 through Oregon State University's Marine & Freshwater Biomedical Center and by the Oregon Agricultural Experiment Station. Specimens were collected under Oregon scientific taking permits OR 2002-116 and OR2003-1176 and a NOAA Fisheries 4(d) authorization letter. We also thank Mark Williams and Robnetts.

Supporting Information Available: (1) List of all target analytes and parameters analyzed as part of *in situ* monitoring with a YSI 6920 Sonde probe and *in situ* sampling of bioavailable organic compounds and metals using PSDs and DGTs, (2) diagram of float-cable-cage-cable-anchor setup, (3) 2002 and 2003 pH and ORP trends, (4) list of target analytes analyzed in ovary/oocyte tissues and surficial sediments and the concentrations detected in each sample, (5) developmental scoring criteria used for fathead minnow skeletal deformities assay, and (6) examples of deformities observed in fathead minnow skeletal deformities assay.

REFERENCES

1. Bengtsson BE. 1979. Biological variables, especially skeletal deformities in our fish for monitoring marine pollution. Phil. Trans. R. Soc. Lond. B 286:457-464.

2. Valentine DW, Soulé M, Samallow P. 1972. Asymmetry analysis in fishes: a possible statistical indicator of environmental stress. Fish. Bull. NOAA 71:357-370.

3. Lemly AD. 1997. A teratogenic deformity index for evaluating impacts of selenium on fish populations. Ecotoxicol. Environ. Saf. 37:259-266.

4. Slooff W. 1982. Skeletal anomalies in fish from polluted surface waters. Aquat. Toxicol. 2:157-173.

5. Bauman PC, Hamilton SJ. 1984. Vertebral abnormalities in white crappies, *Pomoxis annularis* Rafinesque, from Lake Decatur, Illinois, and an investigation of possible causes. 25:25-33.

6. Bengtsson A. 1991. Effects of bleached pulp mill effluents on vertebral defects in fourhorn sculpin (*Myxocephalus quadicornis L.*) in the Gulf of Bothnia. Arch. Hydrobiol. 121:373-384.

7. Moore CJ, Hixson JH. 1977. Incidence of crooked vertebral columns in adult Potomac River white perch, *Morone-Americana.* Copeia. 2:384-387.

8. Lindesjöö E, Thulin J. 1992. A skeletal deformity of northern pikes (*Esox lucius*) related to pulp mill effluents. Can. J. Fish. Aquat. Sci. 49:166-172.

9. Ellis SG. 2000. Characterization of juvenile fish from the Willamette River Basin. Report for the Oregon Department of Environmental Quality (ODEQ) by EVS Environmental Consultants, Project No. 2/839-02, Feb. 11, 2000. http://www.deq.state.or.us/wq/willamette/wqstudies/willfishskeldeformrpt.pdf

10. Markle DF, French R, Naman S, Demarest J, Hatfield M. 2002. Historical patterns of skeletal deformities in fishes from the Willamette River, Oregon. Northwestern Naturalist. 83:7-14.

11. Hughes RM, Gammon JR. 1987. Longitudinal changes in fish assemblages and water quality in the Willamette Basin, Oregon. Trans. Amer. Fish. Soc. 116:196-209.

12. Gill CD, Fisk DM. 1966. Vertebral abnormalities in sockeye, pink, and chum salmon. Trans. Amer. Fish. Soc. 129:754-770.

13. Wells DE, Cowan AA. 1982. Vertebral displasia in salmonids caused by the herbicide trifluralin. Environ. Pollut. (Series A). 29:249-260.

14. Holcombe GW, Benoit DA, Leonard EN, McKim JM. 1976. Long-term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 35:1084-1088.

15. Hodson PV, Hilton JW, Blunt BR, Slinger SJ. 1980. Effects of dietary ascorbic acid on chronic lead toxicity to young rainbow trout (*Salmo gairneri*). Can. J. Fish. Aquat. Sci. 37:170-176.

16. Bengtsson BE, Larsson A. 1986. Vertebral deformities and physiological effects in fourhorn sculpin (*Myxocephalus quadricornis*) after long-term exposure to a simulated heavy metal-containing effluent. Aquat. Toxicol. 9:215-229.

17. McCann JA, Jasper RL. 1972. Vertebral damage to bluegills exposed to acutely toxic levels of pesticides. Trans. Amer. Fish. Soc. 101:317-322.

18. Cheng SH, Wai AWK, So CH, Wu RSS. 2000. Cellular and molecular basis of cadmium-induced deformities in zebrafish embryos. Environ. Toxicol. Chem. 19:3024-3031.

19. Teraoka H, Dong W, Ogawa S, Tsukiyama S, Okuhara Y, Niiyama M, Ueno N, Peterson R, Hiraga T. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity in the zebrafish embryo: altered regional blood flow and impaired lower jaw development. Toxicol. Sci. 65:192-199.

20. Olsson PE, Westerlund L, Teh SJ, Billsson K, Berg AH, Tysklind M, Nilsson J, Eriksson LO, Hinton DE. 1999. Effects of maternal exposure to estrogen and PCB on different life stages of zebrafish (*Danio rerio*). Ambio 28:100-106.

21. Mayer FL, Mehrle PM, Crutcher PL. 1978. Interactions of toxaphene and vitamin C in channel catfish. Trans. Am. Fish. Soc. 107:326-333.

22. Mehrle PM, Haines TA, Hamilton SJ, Ludke JL, Mayer FL, Ribick MA. 1982. Relationship between body contaminants and bone development in east-coast striped bass. Trans. Am. Fish. Soc. 111:231-241.

23. Henry TR, Spitsbergen JM, Hornung MW, Abnet CC, Peterson RE. 1997. Early life stage toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in zebrafish (*Danio rerio*). 142:56-68.

24. Frojnar JR. 1977. Egg and larval survival of white suckers (*Catostomus commersoni*) at low pH. J. Fish. Res. Board Can. 34:262-266.

25. Beamish RJ. 1972. Lethal pH for the white sucker (*Catostomus commersoni*) (Scapede). Trans. Am. Fish. Soc. 101:355-358.

26. Alderdice DF, Wickett WP, Brett JR. 1958. Some effects of temporary exposure to low dissolved oxygen levels on Pacific salmon eggs. J. Fish. Res. Board Can. 15:229-249.

27. Garside ET. 1959. Some effects of oxygen in relation to temperature on development of lake trout embryos. Can. J. Zool. 37:689-698.

28. Gabriel ML. 1944. Factors affecting the number and form of vertebrae in *Fundulus heteroclitus*. J. Exp. Zool. 95:105-143.

29. Kwain W. 1975. Embryonic development, early growth, and meristic variation in rainbow trout (*Salmo gairdneri*) exposed to combinations of light intensity and temperature. J. Fish. Res. Board Can. 32:397-402.

30. Halver JE, Ashley LM, Smith RE. 1969. Ascorbic acid requirements of coho salmon and rainbow trout. Trans. Am. Fish. Soc. 98:762-771.

31. Kloppel TM, Post G. 1975. Histological alterations in tryptophan-deficient rainbow trout. J. Nutr. 105:861-866.

32. McKay LR, Gjerde B. 1986. Genetic variation for a spinal deformity in Atlantic salmon, *Salmo salar*. Aquaculture. 50:263-272.

33. Ponyton SH. 1987. Vertebral column abnormalities in brown trout, *Salmo trutta* L. J. Fish Dis. 10:53-57.

34. Prion RD. 1978. Spontaneous skeletal deformities in the zebra dani (*Brachydanio verio*) bred for fish toxicity test. J. Fish. Biol. 13:79-83.

35. Kent ML, Groff JM, Morrisson JK, Yasutake WT, Holt RA. 1989. Spiral swimming behavior due to cranial and vertebral lesions associated with *Cytophaga psychrophila* infections in salmonid fishes. Dis. Aquat. Org. 15:115-121.

36. LaPatra SE, Batts WN, Overturf K, Jones GR, Shewmaker WD, Winton JR. 2001. Negligible risk associated with the movement of processed rainbow trout, *Oncorhynchus mykiss* (Walbaum) from an infectious haematopoietic necrosis virus (IHNV) endemic area. J. Fish. Dis. 24:399-408.

37. Hedrick RP, El-Matbouli M, Adkison MA, MacConnell E. 1998. Whirling disease re-emergence among wild trout. Immunol. Rev. 166:365-376.

38. Matthews JL, Brown AMV, Larison K, Bishop-Stewart JK, Rogers P, Kent ML. 2001. *Pseudoloma neurophilia* n.g., n.sp., a new genus and species of Microsporidia from the central nervous system of the zebrafish (*Danio rerio*). J. Euk. Microbiol. 48:229-235.

39. Cunningham ME, Markle DF, Watral V, Kent ML, Curtis LR. 2004. Patterns of fish deformities in the Willamette River, Oregon, and their association with trematode cysts. Submitted.

40. Du SJ, Frenkel V, Kindshci G, Zohar Y. 2001. Visualizing normal and defective bone development in zebrafish embryos using the fluorescent chromophore calcein. Develop. Biol. 238:239-246.

41. Dingerkus G, Uhler LD. 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Technol. 52:229-232.

42. Potthoff T. 1984. Clearing and staining techniques. Pp. 35-37. In: Moser HG (ed). Ontogeny and systematics of fishes. Spec. Publ. No.1, American Society of Ichthyology and Herpetology, Allen Press, Lawrence, KS 760 p.

43. Kent ML, Watral VG, Whipps CM, Cunningham ME, Criscione CD, Heidel JR, Curtis LR, Spitsbergen J, Markle DF. 2004. A digenean metacercaria (*Apophallus* sp.) and a myxozoan (*Myxobolus* sp.) associated with skeletal lesions in cyprinid fishes from the Willamette River, Oregon. –submitted.

44. Georgacakis E, Khan MAQ. 1971. Toxicity of the photoisomers of cyclodiene insecticides to freshwater animals. C.R. Hebd. Seances Acad. Sci. Ser. D. 233:120-121.

45. Mayer FLJ, Ellersieck MR. 1986. Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. Resour. Publ. No. 160, U.S. Dept. Interior, Fish Wildl Serv., Washington DC.

46. Giesy JP, Solomon KR, Coats JR, Dixon KR, Giddings JM, Kenaga EE. 1999. Chlorpyrifos: ecological risk assessment in North American aquatic environments. Rev. Environ. Contam. Toxicol. 160:1-129.

47. Urich MA, Wentz DE. 1999. Environmental setting of the Willamette basin, Oregon. Water Resources Investigations Report 97-4023. U.S. Geological Survey, Portland, OR, USA.

48. Wentz DE, Bonn BA, Carpenter KD, Hinkle SR, Janet ML, Rinella FA, Uhrich MA, Waite IR, Laenen A, Bencala KE. 1998. Water quality in the Willamette basin, Oregon 1991-1995. Circular 1161. U.S. Geological Survey, Portland, OR, USA.

49. Altman B, Hensen CM, Waite IR. 1997. Summary of information on aquatic biota and their habitats in the Willamette basin, Oregon, through 1995. Water-Resources Investigations Report 97-4023. U.S. Geological Survey, Portland, OR, USA.

50. Ellis SG, Deshler ST, Miller R. 1997. Characterizing fish assemblages in the Willamette River, Oregon, using three different bioassessment techniques. In Laenen A and Dunnette DA, eds. River Quality Dynamics and Restoration. Lewis Publishers, NY, NY, pp. 347-366.

51. Huckins JN, Tubergen MW, Manuweera GK. 1990. Chemosphere. 20:533-552.

52. Huckins JN, Manuweera GK, Petty JD, Mackay D, Lebo JA. 1993. Environ. Sci. Technol. 27:2489-2496.

53. Sethajintanin D, Johnson E, Loper B, Anderson KA. 2004. Bioaccumulation profiles of chemical contaminants in fish from the Willamette River Portland Harbor Superfund site, Oregon. (submitted).

54. Usenko S, Simonich SL. 2004. In preparation…….

55. Harris GE, Metcalfe TL, Metcalfe CD, Huestis SY. Embryotoxicity of extracts from Lake Ontario rainbow trout (*Oncorhynchus mykiss*) to Japanese medaka (*Oryzias latipes*). Environ. Toxicol. Chem. 13(9):1393-1403.

56. Villalobos SA, Papoulias DM, Pastva SD, Blankenship AL, Meadows J, Tillitt DE, Giesy JP. 2003. Toxicity of o,p'-DDE to medaka d-rR strain after a one-time embryonic exposure by in ovo nanoinjection: an early through juvenile life cycle assessment. Chemosphere. 53(8):819-826.

57. PAN Pesticide Database. http://www.pesticideinfo.org/Index.html

58. Walker MK, Peterson RE. 1991. Potencies of polychlorinated dibenzo-*p*-dioxin, dibenzofuran, and biphenyl congeners, relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin for producing early life stage mortality in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 21:219-238.

59. Zabel EW, Cook PM, Peterson RE. 1995. Toxic equivalency factors of polychlorinated dibenzo-*p*-dioxin, dibenzofuran, and biphenyl congeners based on early life stage mortality in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 31:315-328.

60. van den Berg M, et al. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs, for humans and wildlife. Environ. Health Perspect. 106:775-792.

61. Elonen GE, Spehar RL, Holcombe GW, Johnson RD, Fernandez JD, Erickson RJ, Tietge JE, Cook PM. 1998. Comparative toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to seven freshwater fish species during early life stage development. Environ. Toxicol. Chem. 17(3):472-483.

62. Cook PM, Robbins JA, Endicott DD, Lodge KB, Guiney PD, Walker MK, Zabel EW, Peterson RE. 2003. Effects of aryl hydrocarbon receptor-mediated early life stage toxicity on lake trout populations in Lake Ontario durin the 20th Century. Environ. Sci. Technol. 37:3864-3877.

63. Niemi DR, Macy RW. 1974. The life cycle and infectivity to man of *Apophallus donicus* (Skrjabin and Lindtop, 1919) (Trematoda: Heterophyidae) in Oregon. Proceed. Helminthol. Soc. Wash. 41:223-229.

64. Pike, A.W., and M.D. Burt 1983. The tissue response of yellow perch, *Perca flavescens* Mitchell to infections with the metacercarial cyst of *Apophallus brevis* Ransom, 1920. Parasitology 87: 393-404.

65. Taylor, L.H., Hall, B.K., T. Miyake, and D.K. Cone. 1994. Ectopic ossicles associated with metacercariae of *Apophallus brevis* (Trematoda) in yellow perch, *Perca flavescens* (Teleostei): development and identification of bone and chondroid bone. Anatomical Embryology 190: 29-46

66. Baturo, B. 1980. Pathological changes in cyprinid fry infected by *Bucephalus polymorphos* Baer, 1827 and *Rhipidocotyle illensis* (Ziegler, 1883) metacercariae (Trematoda, Bucephalidae). Acta Parasitologica Polonica 27: 241-246.

67. Kaiser, J. 1999. Link to parasites grows stronger. Science 24: 286: 2434.

68. Lardans V, Dissous C. 1998. Snail control strategies for reduction of schistosomiasis transmission. Parasitol. Today. 413-417.

69. Kiesecker JM. 2002. Synergism between trematode infection and pesticide exposure: A link to amphibian deformities in nature. Proc. Natl. Acad. Sci. 99:9900-9904.

FIGURE CAPTIONS

Figure 1. Diagram of the Willamette Basin depicting the general location and course of the Willamette River and primary study locations.

Figure 2. Photos showing various types of skeletal deformities and associated trematode metacercariae, observed in fathead minnows exposed to *Apophallus donicus* cercariae in the laboratory. *A. donicus* cercariae were isolated from *Fluminicola virens* snails collected from the NP region of the Willamette River.

Table 1. Frequency of occurrence of precaudal deformities and average deformity loads for the five species most commonly collected from three Willamette River study areas in 2002-2003.

Species	NP			Wheatland Ferry			CV		
	%	DL	N	%	DL	N	%	DL	N
Ptychocheilus oregonensis Northern pikeminnow	23.6	0.37 ± 0.016 A	2314	22.8	0.35 ± 0.021 A	1205	6.9	0.08 ± 0.01 B	928
Richardsonius balteatus Redside shiner	14.2	0.24 ± 0.029 A	515	13.0	0.20 ± 0.018 A	1091	6.3	0.09 ± 0.020 B	349
Catostomous macrocheilus Largescale sucker	14.8	0.21 ± 0.015 A	1394	21.3	0.34 ± 0.111 A	47	17.3	0.22 ± 0.050 A	127
Mylocheilus caurinus Peamouth	14.1	0.20 ± 0.032 AB	305	24.2	0.34 ± 0.033 B	442	8.4	0.10 ± 0.038 A	96
Acrocheilus alutaceus chiselmouth	39.6	0.70 ± 0.061 A	268	55.1	0.97 ± 0.103 B	109	12.8	0.17 ± 0.030 C	251

%: Frequency of occurrence of precaudal deformities

DL: deformity load; number of deformity categories present per individual; mean ± SE (SE is individual rather than pooled)

N: sample size

A,B,C: Different letters indicate significant difference between sites ($p \leq 0.05$) based on Bonferroni multiple range test.

Table 2. Mean concentrations (ng/L) of bioavailable organics estimated from concentrations accumulated in passive sampling devices (PSDs) exposed for 21 d at two sites (2 locations per site) along the Wilamette River.

Bioavailable	**CV**		**NP**	
	2002	**2003**	**2002**	**2003**
Σ PAH [c]	3.17 ± 1.47	3.22 ± 1.22	2.01 ± 0.34	1.72 ± 0.54
Phenanthrene	1.15 ±0.60	0.58 ± 0.22	0.93 ±0.27	0.375 ± 0.107
Anthracene [a]	1.32 ± 0.52	1.18 ± 0.90	0.43 ±0.12	0.04 ± 0.02
Fluoranthene [c]	0.70 ± .40	0.43 ± 0.13	0.66 ±0.07	0.29 ± 0.07
Σ PCB [c]	0.035 ± 0.054	0.043 ± 0.020	0.067 ± 0.074	0.074 ± 0.026
Σ DDT [a]	0.102 ± 0.127	0.160 ± 0.018	0.222 ± 0.086	0.211 ± 0.051
p,p'-DDT [b]	0.013 ± 0.020	0.046 ± 0.004	0.045 ± 0.023	0.050 ± 0.009
p,p'-DDE	0.028 ± 0.036	0.067 ± 0.011	0.071 ± 0.026	0.092 ± 0.030
p,p'-DDD [c]	0.069 ± 0.011	0.045 ± 0.007	0.106 ± 0.040	0.069 ± 0.018
Dieldrin [a]	0.037 ± 0.041	0.064 ± 0.009	0.103 ± 0.030	0.112 ± 0.028
Chlorpyrifos	NA	0.74±0.51	NA	1.38 ± 0.33

Target analytes with concentrations < detection limit not shown. See supplementary materials for complete list of analytes.

[a] Significant difference between sites, both years

[b] Significant difference between sites, 2002 only

[c] Significant difference between sites, 2003 only

Table 3. Concentrations of chlorinated pesticides, polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) detected in oocyte/ovary tissue from northern pikeminnow (Ptychocheilus orgeonensis) collected from NP (NP) and CV (CV) study sites.

Compound	Mean ± SE (ng/g)[a]		Median (ng/g)[a]		P
	NP	CV	NP	CV	
Endrin	3.94±1.62	*1.62±0.01*	*1.65*	*1.62*	0.191
4,4'-DDD	3.63±1.34	*1.62±0.01*	*1.65*	*1.62*	0.171
4,4'-DDE	46.9±13.4	48.0±15.9	35.0	31.0	0.942
PCB-8 [2,4']	3.26±1.74	10.8±4.68	*1.65*	7.70	0.144
PCB-18 [2,2',5]	*1.55±0.07*	16.7±15.1	*1.65*	*1.65*	0.999
PCB-101 [2,2',4,5,5']	3.52±0.90	*2.21±0.06*	4.16	*1.65*	0.402
PCB-110 [2,3,3',4',6]	*1.98±0.46*	*1.62±0.01*	*1.65*	*1.62*	0.674
PCB-118 [2,3',4,4',5]	*2.48±0.96*	*1.62±0.01*	*1.65*	*1.62*	0.674
PCB-138 [2,2',3,4,4',5']	*2.80±1.27*	*1.62±0.01*	*1.65*	*1.62*	0.674
PCB-153 [2,2',4,4',5,5']	4.20±0.92	*2.65±0.65*	4.20	*1.65*	0.163
$TEQ_{[PCDD/DFs]}$ (pg/g wet wt)	0.84±0.20	0.85±0.31	0.99	0.67	0.959

[a] For the purposes of calculating means, medians, and statistics, non-detects were assumed to be equal to ½ the method detection limit (MDL). All concentrations reported in ng/g wet wt. except TEQ which are reported as pg/g wet wt.

Italics indicate that mean or median estimate was less than the MDL.

Table 4. Incidence of vertebral deformities and metacercariae in fathead minnows (Pimephales promelas) exposed to cercariae of Apophallus donicus.

Trial #	Concentration of Exposure Cercariae/fish	Age of Exposure (days)	Days Post Exposure when Examined	Number Examined	% Deformed	% Infected	Abundance	% lesions associated with parasites	% worms asso. With lesions
1	30	8	55	11	8/11 (73)	9/11 (82)	1.0	9/10 (90)	9/11 (82)
1C	0	8	55	7	0	0	0	NA	NA
2	10	8	70	14	12/14 (86)	13/14 (93)	1.9	17/17 (100)	20/27 (74)
2C	0	8	70	18	1/18 (6)	0	0	0	NA
3	30	5	70	10	7/10 (70)	8/10 (80)	1.2	8/9 (88)	8/12 (67)
3C	0	5	70	12	0	0	0	NA	NA
4	30	17	70	14	13/14 (93)	14/14 (100)	4.5	31/33 (94)	38/64 (59)
5	30	24	70	21	19/21 (91)	20/21 (95)	4.0	37/42 (88)	58/88 (66)
4/5C	0	17/24	70	18	1/18 (6)	0	0	0	NA
totals	exposed			70	84	91	2.5	93	66
	controls			55	4	0	0	0	0