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Contaminant Uptake and Survival of White Sturgeon Embryos

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Abstract.—The goal of this preliminary experiment was to determine if treatments of various de-adhesion media affect contaminant uptake and survival of embryos of the endangered Kootenai River white sturgeon *Acipenser transmontanus*. Fertilized eggs (embryos) from a single mating were divided into three groups differing in de-adhesion and rearing media: 1) de-adhesed with suspended solids from the river and reared in unfiltered river water (SS-UFW), 2) de-adhesed with Fuller's Earth and reared in filtered river water (FE-FW, experimental control), and 3) de-adhesed with river bottom sediment and reared in filtered river water (SED-FW). Uptake of organochlorine pesticides, Aroclor 1200 series PCBs and metals and survival of embryos were assessed. Eight metals and two organochlorine compounds (DDE and PCB Aroclor 1260) were detected in embryos. Uptake of some metals was significantly different between and among treatments and controls (Kruskal-Wallace, Mann–Whitney *U*-tests, $P < 0.05$). The PCB Aroclor 1260 was not detected in embryos from the SS-UFW treatment, but it was detected in some replicates from the SED-FW treatment and the experimental controls (FE-FW). All embryos from the SS-UFW treatment developed heavy fungal growth and died within 8 d. Fungal growth was not associated with embryos in the SED-FW or FE-FW samples; however, the SED-FW embryos displayed significantly higher mortality (20.6%) than the FE-FW embryos (12.6%; $P = 0.036$). It was concluded that copper and Aroclor 1260 in the rearing media are potentially decreasing survival and incubation time of white sturgeon embryos. However, because mortality rate in relation to contaminant exposure is not excessive, more controlled laboratory studies with more parental fish are needed to establish relationships between actual bioavailable and bioaccumulated contaminant concentrations and survival of embryonic sturgeon.

Introduction

The Kootenai River white sturgeon *Acipenser transmontanus*, a genetically and geographically distinct, endangered fish stock, suffers from low population size, poor reproduction, and negligible recruitment (Duke et al. 1999; USFWS 1999). Intensive research over the past 10 years has focused on identifying factors and mechanisms inhibiting stock recovery (Duke et al. 1999; USFWS 1999). In addition to physical habitat changes in the Kootenai River, introduction of contaminants in the river has been implicated as a possible cause of poor reproductive success. Exposure of fish eggs or sperm to contaminants during fertilization and early development can result in disruption of developmental processes that normally establish gender, determine growth and development, and ensure reproductive capabilities in successive generations (Raloff 1994; McKim 1994; Heath 1995). The degree of contaminant bioaccumulation from parental stock coupled with environmental exposure can also affect survival rate of progeny and eventual juvenile recruitment within a population (Hall et al. 1993).

Contaminant accumulation may be significant in rivers such as the Kootenai where potential sources include mining and mineral extraction, agriculture, logging and lumber processing, recreation, hydroelectric power generation, urban development, and transportation (Kootenai River Network 2000). Continual or periodic redistribution of these contaminants by fluctuating flows and shifting substrates may render them repeatedly bioavailable for absorption and ingestion.

The adhesiveness and permeability of white sturgeon eggs following fertilization (Detlaff et al. 1993) increases their susceptibility to contaminants associated with water, sediment, and organic matter. The primary spawning substrate for the Kootenai River white sturgeon consists of sand and silt (Paragamian et al. 2001). Fertilized eggs may therefore become coated with sediment and organic

matter (suspended or settled) to different degrees during the adhesive phase of incubation, depending on the nature of the adhesed material and the rearing medium.

As part of the recovery plan for the Kootenai River white sturgeon population (USFWS 1999), artificially-spawned embryos from a single mating became available for use in contaminant investigations. In a preliminary step toward assessing the role of contaminants as a limiting factor in Kootenai River white sturgeon recovery, the objectives of this study were to 1) determine if rearing media contributed significant concentrations of organochlorine pesticides, Aroclor polychlorinated biphenyls (PCBs), and metals to developing Kootenai River white sturgeon embryos during incubation, and 2) determine if uptake and adhesion of organochlorine pesticides, PCBs, and metals by developing embryos varied with exposure to different embryo de-adhesing and incubation media.

Methods

De-adhesion and Rearing Treatments

White sturgeon embryos were obtained from a single mating at the Kootenai Tribal conservation aquaculture facility in Bonners Ferry, Idaho (Ireland 2000). An adhesive jelly layer surrounds white sturgeon eggs throughout early development, and contact with freshwater causes the jelly layer to hydrate, causing the egg to become adhesive (Conte et al. 1988). Because of this adhesion, artificially fertilized eggs must be de-adhesed to avoid suffocation within clumps of adhered eggs. This adhesive nature of hydrated white sturgeon eggs provided the opportunity to test the effects of different de-adhesion treatments on contaminant uptake and survival of white sturgeon embryos.

Two distinct de-adhesion and incubation combination treatments and one control were applied to white sturgeon embryos: 1) de-adhesion in suspended solids from the river water column and rearing in unfiltered river water (hereafter called SS-UFW), 2) de-adhesion in Fuller's Earth and rearing in filtered river water (experimental control, hereafter called FE-FW), and 3) de-adhesion in river-bottom sediment and rearing in filtered river water (hereafter called SED-FW). The experimental control conditions (FE-FW) represented standard rearing protocol for white sturgeon at the Kootenai Tribal conservation aquaculture facility (Ireland et al., this volume). Survival rates of embryos reared under these conditions have been good (Ireland 1997–1999; Ireland et al., this volume).

Two hundred and fifty mL of white sturgeon embryos (from one female, fertilized with sperm from one male) were separated into the three experimental groups consisting of approximately 83 mL each. All experimental embryo groups were de-adhesed for 2–3 h (Conte et al. 1988) in 12°C water. River bottom (substrate surface) sediment and suspended solids used for de-adhesion were collected from known sturgeon spawning areas in the Kootenai River (rkm 229–230; Paragamian et al. 2001), during June 1999. Sediment (SED) was collected with a Ponar dredge, and suspended solids (SS) were collected with an 80-µm mesh plankton net.

Following de-adhesion, embryos from each of the two treatment groups (SS-UFW, SED-FW) and the control group (FE-FW) were subdivided into eight rearing containers per group (approximately 10 g of eggs per container), for a total of 24 containers. Containers consisted of clear 456 g polycarbonate jars with perforated polypropylene lids and a hose that extended into the bottom of each jar. Water was circulated into the bottom and out the top of each jar in order to gently agitate the embryos during incubation. Filtered river water (FW) was circulated through a sand trap and an AMIAD automatic filter to remove particles larger than 10 µm before it was sterilized by ultraviolet light. Unfiltered river water (UFW) was pumped directly from the Kootenai River at the hatchery intake.

Water temperature in rearing containers was allowed to fluctuate consistent with in situ river water temperatures during de-adhesion and incubation periods. Water temperatures during embryo incubation ranged from 10.9°C to 12.0°C. Water quality parameters (including alkalinity, total dissolved solids, total suspended solids, ammonia, nitrate + nitrite, ortho-phosphorous, calcium, copper, magnesium, manganese and zinc) at the hatchery intake and from the head holding tank in the hatchery were recorded one day per month during June and July as part of standard procedure for hatchery operations. Incubation tanks were kept in the dark, and jars were checked daily to count and remove dead or dying embryos. Dead embryos were placed into sample jars and frozen for analysis of lipid content, organochlorine pesticides, PCB Aroclors, and metals (Appendix 1).

Rate of contaminant uptake varies greatly between the embryonic and larval phases

(Lukyanenko 1980), so it was necessary in this experiment to remove the larvae at, or immediately prior to, hatching, when the chorion was still intact. As a result, the contaminant residues in embryos from this experiment included imbibed and adhesed concentrations. Within 13 d after fertilization, the majority of surviving embryos began to hatch. All remaining embryos and larvae were counted, removed from containers, and frozen for contaminant analysis. Percent egg mortality for all containers was determined by dividing the total number of dead embryos by the initial total number of embryos for each jar.

Data Analysis

The null hypothesis tested for each contaminant was that contaminant uptake and embryo mortality did not significantly differ between and among treatment and control groups (Kruskal-Wallace; Mann–Whitney *U*-test; $P < 0.05$). Data were tested for normality of distribution with the expression 2(SE)±K (where SE is the standard error, and K is a measure of kurtosis), and homogeneity of variances (Cochran's C-test; Kirk 1995). Data displayed heterogeneity of variances and were not normally distributed. Therefore, nonparametric statistical analyses were used to test the hypotheses.

Results

Embryo and Water Quality Analysis

The null hypothesis of no significant difference in contaminant uptake between and among the two treatments and the control was rejected (Table 1). Embryos reared in the SED-FW treatment sorbed higher concentrations of most metals and organochlorine compounds than SS-UFW treatment and control (FE-FW) embryos.

Eight metal compounds were detected in embryos from this experiment (Table 1). Uptake of arsenic (Kruskal-Wallace; $P = 0.041$), cadmium (Kruskal-Wallace; $P = 0.032$), copper (Kruskal-Wallace; $P = 0.001$), iron (Kruskal-Wallace; $P = 0.001$), magnesium (Kruskal-Wallace; $P < 0.001$), and lead (Kruskal-Wallace; $P = 0.002$) were significantly different between and among the treatment and control groups (Table 2; Figure 1). Concentrations of selenium (Kruskal-Wallace; $P = 0.787$) and zinc (Kruskal-Wallace; $P = 0.052$) were not significantly different among treatment and control groups. Concentrations of copper and zinc in embryos were much higher than those detected in water samples collected at the hatchery (Table 3).

Two organochlorine compounds (DDE and Aroclor 1260) were detected in embryos in this experiment (Table 1). Concentrations of the organochlorine compound DDE were not significantly different between and among treatment and control groups (Kruskal-Wallace; $P = 0.962$; Table 1). Although DDE was present in all groups, the PCB Aroclor 1260 was not detected above 360 ppb in embryos from the SS-UFW treatment. There was no significant difference between Aroclor 1260 concentrations in the SED-FW and FE-FW comparisons (Kruskal-Wallace; $P = 0.052$; Table 2). However, concentrations of Aroclor 1260 in SED-FW and FE-FW groups did differ significantly from each other (Wilcoxon rank-sum test; $P = 0.008$; Table 2). Lipid concentrations in embryos ranged from 3.0% to 9.0% and were not significantly different between and among treatment and control groups (Kruskal-Wallace; $P = 0.519$; Table 1).

Embryo Mortality

The null hypothesis of no significant difference in mortality rates among treatments and the controls was also rejected. Percent mortality was significantly higher (Mann–Whitney *U*-test; $P = 0.036$) in the embryos from the SED-FW treatment (Figure 1; mean = 20.6%; range 6–36%) than from the FE-FW controls (Figure 1; mean = 12.6%; range 10–16%). All embryos from the SS-UFW treatment died within the first 7 d (Table 1). Mortality of embryos in all 3 experimental groups peaked at days 5–6 of the experiment (Figure 2).

Discussion

Significantly higher concentrations of arsenic, cadmium, copper, iron, magnesium and lead in embryos from the SED-FW treatment than from the SS-UFW treatment and the FE- FW controls indicated that river-bottom sediments may be a more significant route for uptake of these metals compared with water and suspended solids. Ramamoorthy and Ramamoorthy (1997) noted that although metals also partition into organic material in the water column, they are more likely associated and bound with sediments. However, contaminant levels in water do play a role in sediment toxicity. Spacie et al. (1995) reported that contaminant uptake from sediments was affected by the sediment-water mixing that generally occurs near the substrate surface.

No evidence was found from other studies to indicate that concentrations of arsenic, cadmium,

Table 1. Treatment, percent mortality, contaminants detected, number of samples contaminants were detected in and percent of total samples, concentration range, mean and standard deviation by group for white sturgeon embryos from SS-UFW and SED-FW treatments, and the FE-FW control groups. The difference in number of decimal places for concentration range and mean indicate variation in method detection limit. Asterisks indicate significant differences among treatment and control means for individual contaminants (Kruskal-Wallace and Mann-Whitney *U* tests; $P < 0.05$).

Treatment (mortality)	Contaminant (ppm)	Number of samples (% of total sample)	Concentration range	Mean concentration (SD)
SS-UFW (100%)				
	Arsenic	8 (100)	0.26–0.97	0.42 (0.25) *
	Cadmium	8 (100)	0.01–0.03	0.02 (0.01) *
	Copper	8 (100)	1.30–2.50	1.96 (0.38) *
	Iron	8 (100)	540–1200	881 (205) *
	Magnesium	8 (100)	400–860	630 (141) *
	Lead	8 (100)	1.00–3.00	1.78 (0.62) *
	Selenium	8 (100)	0.46–1.00	0.68 (0.20)
	Zinc	8 (100)	12.0–24.0	18.4 (3.74)
	PCB (ppb)	0 (0)	<52	<52
	DDE (ppb)	6 (75)	39.0–60.0	48.3(7.12)
	Lipid (%)	8 (100)	5.80–9.00	7.06 (1.28)
FE-FW (Control) (12.6%)				
	Arsenic	2 (25)	0.17-0.29	0.23 (0.09) *
	Cadmium	3 (38)	0.01-0.01	0.01 (0.001) *
	Copper	8 (100)	0.79-1.50	1.15 (0.22) *
	Iron	8 (100)	16.0-140	38.30 (41.4) *
	Magnesium	8 (100)	96.0-170	137 (24.7) *
	Lead	6 (75)	0.08-0.63	0.24 (0.21) *
	Selenium	8 (100)	0.48-0.94	0.71 (0.17)
	Zinc	8 (100)	9.50-18.0	19.9 (2.93)
	PCB (ppb)	3 (38)	92.0-100	95.7 (4.01)*
	DDE (ppb)	8 (100)	34.0-71.0	51.6 (13.6)
	Lipid (%)	8 (100)	3.90-7.70	5.96 (1.35)
SED-FW (20.6 %)				
	Arsenic (ppm)	8 (100)	0.36-0.82	0.61 (0.15) *
	Cadmium (ppm)	8 (100)	0.002-0.04	0.03 (0.01) *
	Copper (ppm)	8 (100)	1.90-2.20	2.01 (0.10) *
	Iron (ppm)	8 (100)	17.0-2800	1889 (843) *
	Magnesium (ppm)	8 (100)	810-1400	1050(185) *
	Lead (ppm)	8 (100)	1.80-3.30	2.71 (0.51) *
	Selenium (ppm)	8 (100)	0.63-0.89	0.74 (0.08)
	Zinc (ppm)	8 (100)	22.0-26.0	24.0 (1.20)
	PCB (ppb)	4 (50)	120-160	140 (23.1)*
	DDE (ppb)	8 (100)	38.0-65.0	47.8 (9.51)
	Lipid (%)	8 (100)	4.60-8.70	6.60 (1.37)

iron, magnesium, lead, or DDE at levels observed in tissues in this study would be expected to reduce embryonic survival in fish. However, concentrations of Aroclor 1260 and copper in all treatment and control groups fell within ranges reported in several other studies to disrupt normal embryonic survival and development in other fish species. Halter and Johnson (1974) and Roberts et al. (1978) reported results from studies in which embryonic and early life stage mortality in fishes were in-

Table 3. Water quality parameters from the Kootenai Tribal hatchery during June and July 1999. Water samples were taken from the intake point in the river (unfiltered) and from the hatchery head tank (filtered).

	Intake		Head tank	
Parameter (mg/l)	June	July	June	July
Alkalinity ($CaCO_3$)	42	74	42	66
Total Dissolved Solids	61	84	64	85
Total Suspended Solids	10	4.3	<1.1[1]	<1.0[1]
N-Ammonia	0.032	0.021	0.044	<0.010[1]
Nitrate+Nitrite (NO2+NO3)	0.067	0.45	0.076	0.11
Ortho-Phosphorous	<0.004[1]	<0.004[1]	<0.004[1]	<0.004
Calcium	13.3	22.1	13.2	22.5
Copper	0.002[1]	0.002[1]	0.002[1]	0.002
Magnesium	3.65	6.09	3.54	6.11
Manganese	0.010	0.006	0.002	0.001[1]
Zinc	0.009	0.006	0.004[1]	0.004[1]

[1] Undetected at reported detection limit

ing the later stages of development, after the SS-UFW embryos died. Peterson and Kristenson (1998) suggested that embryos with a longer incubation period have higher uptake of lipophilic substances such as PCBs. The significantly higher Aroclor 1260 concentrations in SED-FW than in FE-FW embryos suggested that sediment was a greater source of PCB uptake for the embryos than water. Although most research indicates that PCB uptake per concentration by aquatic organisms is higher from water than from other media, sediments generally contain higher PCB concentrations and higher resulting bioavailability than water (Reynoldson 1987). Although the PCB Aroclor 1260 was not detected in sediment samples collected from the lower Kootenai River it has been detected above EPA standards in a water sample collected from the Kootenai River near Flemming Creek (rkm 225.0, Kruse 2000).

The significant difference between mortality rates and copper concentrations in SED-FW and

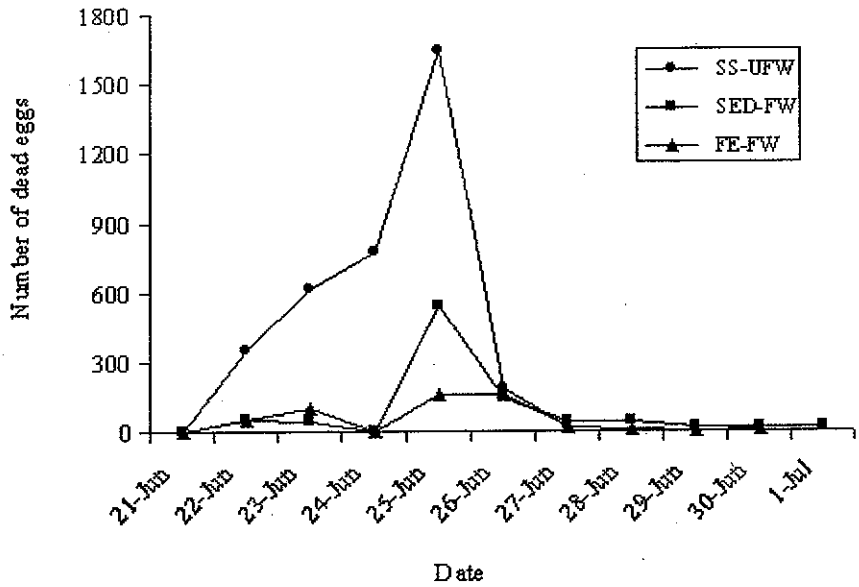

Figure 2. Mortality over time for embryos coated with suspended solids and reared in unfiltered river water (SS-UFW treatment), embryos coated with river bottom sediment and reared in filtered river water (SED-FW treatment) and embryos coated with fullers Earth and reared in filtered river water (FE-FW treatment).

FE-FW embryos (Table 1) suggested that copper associated with adhered river-bottom sediments may be reducing survival of white sturgeon embryos. Research by Scudder et al. (1988) indicated that the chorion is variably permeable to copper at specific developmental stages. Although embryos appear to be less sensitive than fry to copper, copper is more toxic to small fish than larger ones and any changes to egg permeability during embryogenesis allow for increased entry of copper into the chorion (Sorenson 1991). According to McKim and Benoit (1974), uptake of aqueous copper by brook trout embryos magnified up to five times from baseline exposure levels. Eddy and Talbot (1983) found that the water-hardening process in Atlantic salmon eggs could be inhibited or prolonged by the presence of divalent metal ions. Therefore, exposure of the developing embryo to copper in rearing media may be prolonged and uptake increased because the hard, protective and otherwise relatively impermeable chorion is not promptly formed. Although copper concentrations in sturgeon embryos from this experiment were lower than concentrations found in sediment samples, they were higher than concentrations found in water samples collected from the Kootenai River (Bauer 1999; Kruse 2000).

High early mortality in the SS-UFW treatment probably resulted from fungal and bacterial growth within the first three days of incubation. Exposure to fungal growth during early gastrulation can result in total mortality of sturgeon embryos (J. Siple, Kootenai Tribe of Idaho, personal communication). As fungus appeared, embryos in the SS-UFW treatment died. Organic matter in the suspended solids used for de-adhesion, and the organic material in unfiltered river water were suggested sources of bacteria and fungi.

One difficulty in our study was determining the proportion of contaminants actually incorporated into the eggshell from that merely attached to the outside of the shell or associated with the specific de-adhesion medium. Although the water-hardening process may imbibe contaminants into the egg, some contaminants inevitably remain bound with the outer shell or de-adhesion medium where they are not necessarily bioavailable to the developing embryo (Rosenthal and Alderdice 1976). It has not been possible to rear viable embryos without de-adhesion medium (J. Siple, Kootenai Tribe of Idaho, personal communication) so the best measure of effects is a comparison among treatments of contaminant concentrations and mortality rates. More elaborate, controlled laboratory studies are needed to establish relationships between actual bioavailable (from rearing media) and bioaccumulated (by embryos) contaminants and their effects on performance and survival of white sturgeon embryos.

The results of this preliminary experiment suggest that although the mortality rate on Kootenai River white sturgeon embryos in relation to contaminant exposure is not excessive, it may be an additional stress on viable reproduction in this already endangered sturgeon population. More definitive results will require a larger number of matings for more genetic diversity of the test organisms, and a more extensive experimental design.

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