

## Effects of Sediment Cover on Survival and Development of White Sturgeon Embryos

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**Abstract.**—A simple, inexpensive apparatus (embryo incubation unit [EIU]) was developed and used to assess the relationship between sediment cover (Kootenai River sediments, 97% by weight in the 0.83-mm- to 1.0-mm-diameter range) and survival of white sturgeon *Acipenser transmontanus* embryos in the laboratory. An apparatus-testing trial assessed the effects of two sediment depths (5 and 20 mm), three EIU ventilation hole sizes (4.8, 6.8, and 9.5 mm) providing three levels of intrasubstrate flow, and EIU location (upstream or downstream in laboratory troughs) on embryo survival at two above-substrate flow velocities (0.05 and 0.15 m/s). A second trial assessed the effects of sediment cover duration (5-mm sediment cover for 4, 7, 9, 11, or 14 d, with a ventilation hole size of 9.5 mm and a flow velocity of 0.17 m/s) on mean embryo survival and larval length and weight. In the apparatus-testing trial, embryo survival was reduced ( $P < 0.0001$ ) to 0–5% under sediment covers of either 5 or 20 mm in both the higher-flow and lower-flow troughs; survival in control EIUs without sediments exceeded 80%. Survival was not significantly affected by ventilation hole size but was weakly affected by EIU location. In the second trial, embryo survival was negatively correlated ( $P = 0.001$ ) with increasing duration of sediment cover and was significantly higher for embryos covered for 4 d (50% survival) or 7 d (30% survival) than for those covered for 9, 11, or 14 d (15–20% survival). Sediment cover also delayed hatch timing ( $P < 0.0001$ ) and decreased mean larval length ( $P < 0.0001$ ). Our results suggest that sediment cover may be an important early life stage mortality factor in rivers where white sturgeon spawn over fine-sediment substrates.

The white sturgeon *Acipenser transmontanus* population of the Kootenai River, Idaho, has been declining since the 1950s (USFWS 1994; Duke et al.

1999). Between 1965 and 1980 (with the exception of 1974), natural recruitment to the population was estimated to be only one to three individuals annually (Paragamian et al. 1996). By the mid-1990s, the population was estimated to consist largely of individuals 21 years of age or older (Paragamian et al. 1996), and the population was listed as endangered under the U.S. Endangered Species Act in 1994 (USFWS 1994, 1999).

Spawning of white sturgeon in the Kootenai River in recent years (1991–1998) has been confirmed by the collection of over 1,000 eggs from filter mats anchored in the river (Paragamian et al. 2001). Sampling with proven gear and techniques over the past decade has, however, failed to capture naturally produced larvae, suggesting that the recruitment bottleneck may be occurring during the embryonic or early larval periods. This hypothesis is further supported by a relatively high postrelease survival rate for juvenile white sturgeon released from the Kootenai Tribal Hatchery at Bonner's Ferry, Idaho. Surveys have shown that these hatchery-reared fish, which are released at a size of approximately 20 g, survive at rates of approximately 60% the first year and 90% in each subsequent year (Ireland et al. 2002).

Of the many factors implicated in the failure of white sturgeon production in the Kootenai River, the potential effects of sedimentation on embryo survival are of particular interest (Duke et al. 1999; Anders et al. 2002; Ireland et al. 2002). Annual surveys have shown that the substrate in the thalweg of the spawning reach is predominantly sand (Paragamian et al. 1996). During a recent 8-year study, only 15 white sturgeon eggs were collected over gravel and cobble substrates, while 1,193 eggs were collected over sand substrate (Paragamian and Kruse 2001). The fine-sediment spawning substrate used by Kootenai River white sturgeon differs considerably from the gravel, cobble, boulder, and

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bedrock spawning substrates used by some other white sturgeon populations (Parsley and Beckman 1994; Anders 2002; Perrin et al. 2003). White sturgeon embryos, which are adhesive and demersal, may be buried by aggrading fine sediments in Kootenai River spawning areas characterized by large mobile sand dunes on the river bottom (Paragamian et al. 1998).

Field and laboratory studies have established a negative correlation between sedimentation and the survival of salmonid embryos (Phillips et al. 1975; Tappel and Bjornn 1983; Witzel and MacCrimmon 1981; Young et al. 1991). Similar studies have not, however, been conducted with incubating sturgeon embryos. Because of uncertainty associated with early life stage mortality in white sturgeon (Parsley et al. 2002) and the importance of habitat-based early life mortality for many imperiled sturgeon populations (Birstein et al. 1997; Secor et al. 2002), research on this topic could be of scientific and practical value. Consequently, we conducted a laboratory study to assess the effects of sediment cover on the survival and development of white sturgeon embryos. The objectives of this study were to (1) design and test an apparatus for evaluating the effects of sediment on the survival and development of replicated groups of embryos incubated in the laboratory, (2) assess the effects of sediment depth on embryo survival and development using sterilized sediment collected from white sturgeon spawning areas in the Kootenai River, and (3) determine the effect of sediment cover on embryo survival and development during all stages of embryonic development.

### Methods

*Source of embryos.*—On June 7 and again on June 21, 2002, ova and sperm were obtained from one wild female white sturgeon and two wild male white sturgeon held at the Kootenai Tribal Hatchery. Different animals were spawned on the two dates, and the embryos produced were used in two separate trials. Females were injected with priming and resolving doses of lutenizing hormone (LHRH-A) by hatchery staff during the 48 h prior to spawning as per standard methods (Conte et al. 1988; Ireland et al. 2002). On each occasion, spawned eggs were divided into two groups, and each group was separately fertilized with milt from one of the two males. The two lots of embryos produced were subsequently combined. After fertilization, embryos were treated in a Fuller's Earth solution for approximately 2 h with constant stirring ("de-adhesion") according to standard hatchery practice (Conte et al. 1988; Ireland et al. 2002). A portion of each fertilized egg lot was incubated at the Kootenai Tribal Hatchery and served

as a supplemental control to determine the survival of nontransported embryos.

*Source and composition of sediments.*—Sediments used in the embryo incubation units (EIUs) were obtained by Ponar dredge samples from the Middle Shorty's Island reach of the Kootenai River (river kilometers 229.6–231.5) in early May 2002. This reach is currently the major spawning area for white sturgeon in the Kootenai River (Duke et al. 1999; USFWS 1999; Paragamian et al. 2001). Sediment particle size was estimated by rinsing samples (20 g each) from six locations through a series of brass sieves (1.00, 0.83, 0.50, and 0.25 mm) with a gentle flow of water. The sediments collected by each sieve were dried and weighed, and the combined mean percent by weight of sediments collected by each sieve from the six samples was estimated. The 1.00-, 0.83-, 0.50-, and 0.25-mm sieves collected 0.5, 1.0, 15.0, and 82.0% of the sediments by weight, respectively; the remaining portion (approximately 1.5%) passed through the 0.25-mm sieve. Sediments were pooled, mixed thoroughly, and sterilized in an autoclave 72 h prior to use in each trial.

*Experimental apparatus.*—Trials were conducted in fiberglass hatchery troughs (66 cm wide × 29 cm deep × 243 cm long; maximum water depth, 12 cm) provided with single-pass well water at 12–15°C. Artificial lighting approximated the ambient photoperiod. Perforated metal sheets at the head of each trough spread the incoming flow evenly across the width of the trough. A 12-cm-high weir was placed at the downstream end of the trough, just upstream from the outlet, to control the water level and reduce variation in flow velocities.

Water velocities were measured with a portable flowmeter (Model 2000, Marsh-McBirney, Inc.) at the beginning, middle, and end of each trial. Water velocity measurements were taken perpendicular to the flow across five randomly selected transects in each trough. Measurement points along each transect included velocities above embryo incubation units (EIUs), between EIUs, and between EIUs and the trough sides.

The EIUs had four basic components: (1) unit body, (2) insert sleeve, (3) base screen, and (4) cap screen (Figure 1). The unit body was constructed from a standard 10.7-cm-inside-diameter polyvinyl chloride (PVC) pipe cap (nominal size, 4 in [1 in = 2.54 cm]), with 16 evenly spaced ventilation holes drilled around the base. A section of polypropylene woven screen cloth (250-micron mesh) was placed inside the cap to cover the ventilation holes and retain sediments. The insert sleeve was a section of 10.7-cm outside-diameter PVC pipe (inside diameter, 10.3 mm; nominal size, 4

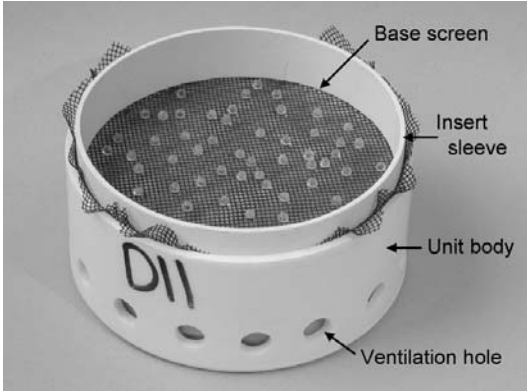


FIGURE 1.—Embryo incubation unit. In use, the embryos (represented here by 3-mm glass beads) were covered with sediments to the top of the insert sleeve, and a cap screen (not shown) was secured over the top of the insert sleeve. The unit shown has 9.5-mm ventilation holes (“large”) and a 20-mm insert sleeve. Embryo density in the two trials was about one-half that shown here.

in) that fit inside the unit body. The height of the insert sleeve (5 or 20 mm) determined the depth of sediments over the incubating embryos.

Sediments were loaded into the unit body to a depth of 40 mm (about 5 mm below the top). The base screen (a piece of 1.5-mm mesh plastic window screen) was placed over the top of the unit and the insert sleeve pressed firmly down into the unit body, securing the base screen over the sediment layer (Figure 1). White sturgeon embryos (approximately 24) were gently transferred onto the base screen and evenly dispersed with a fine-bristled brush. After a digital photograph was taken to record the number of embryos present within each unit, sediments were placed over the embryos with a sterile plastic spoon until flush with the top of the insert sleeve (control EIUs received no sediment treatments). Finally, the cap screen (1.5-mm mesh plastic window screen) was placed over each unit and secured with a plastic cable tie.

At the end of each trial, embryos or larvae were removed from each EIU and preserved in a 10% solution of buffered formalin. When removed from the EIUs, live embryos and larvae were free of fungus, but dead embryos and larvae were covered by masses of fungal filaments. Fungus-free preserved embryos and larvae were later measured and weighed. Embryo diameters (measured at the widest point) and total lengths of larvae were measured to the nearest 0.01 cm with digital calipers (Control Company, Model 3415, Friendswood, Texas) under a dissecting microscope (8×). Larvae were placed on a dissecting tray, straightened, and measured from the tip of the tail to

the snout. Embryos and larvae were weighed on an electronic balance to the nearest 0.001 g.

*Experimental design.*—Because the EIU had not been previously used, the sensitivity of experimental results to variation in experimental conditions was not known. Therefore, the first trial, which lasted 7 d, was an apparatus-testing trial that tested the interactive effects of water velocity, EIU ventilation hole size, and depth of sediment on the survival and development of white sturgeon embryos. Two troughs were used: water velocities were maintained at approximately 0.15 m/s in one trough and at 0.05 m/s in the other. Mean water column velocities in the reach used by spawning white sturgeon in the Kootenai River have been reported to range from 0.19 to 0.67 m/s (1994–1998; Paragamian et al. 2001); however, because velocities decrease logarithmically as the streambed is approached, velocities a few centimeters above the streambed, corresponding to the scale of velocity measurements in our laboratory troughs, would be lower than mean velocities. Assuming a water depth of 10 m in the Kootenai River spawning reach (Paragamian et al. 2001), a roughness height of 0.4 mm (fine sand), and a flat streambed, estimated velocities (Prandtl–von Karman universal velocity distribution law; Jowett 2003; equation 2) at 1–2 cm above the streambed would be about one-half the mean velocity, or 0.10–0.34 m/s. In the actual field situation, ova released over submerged sand dunes such as those in the Kootenai River spawning reach would tend to settle in protected areas on the leeward side of the dunes, where flow velocities would be lower. Three EIU ventilation hole sizes (9.53 mm, large; 6.75 mm, medium; and 4.76 mm, small), each doubling the open area of the next smaller size, were tested to determine the sensitivity of embryo survival and development to different levels of intrasubstrate flow. The two sediment depths tested in the first trial were 5 and 20 mm.

Fifty-two EIUs were placed in each of the two troughs. The EIUs were placed in a 4-column (parallel to flow) × 13-row (perpendicular to flow) configuration. The upstream row (row 0) received no embryos and served as a velocity break to standardize downstream flow conditions. Downstream rows were numbered consecutively from 1 to 12; rows 1–6 were designated as “upstream” and rows 7–12 as “downstream.” The three EIU ventilation hole sizes were tested in combination with the two sediment depths, yielding six ventilation depth treatments. Each of the six treatments was randomly assigned one position in every two rows of each trough, providing three upstream and three downstream replicated blocks. In addition, an EIU with large ventilation holes and no sediment cover over the embryos (control EIU) was

randomly assigned a position in each row, providing six upstream and six downstream replicated controls.

The second trial, which lasted 15 d, determined the effects of sediment cover duration on the survival and hatch timing of white sturgeon embryos. As for the first trial, the embryos used were pooled from the matings of one female with two males. Based on the results of the first, apparatus-testing trial, experimental conditions were selected that were expected to be relatively favorable for the survival and development of embryos: a nominal water velocity of 0.17 m/s (slightly greater than the higher of the two velocities tested in the earlier trial), a ventilation hole size of 9.53 mm (the largest of the three diameters tested earlier), and a sediment depth of 5 mm (the shallower of the two depths tested earlier). The EIUs were placed in a single trough in a 4-column  $\times$  13-row configuration, as in the apparatus-testing trial, the upstream row of four units receiving no embryos and serving as a velocity break. Eight EIUs were randomly assigned to each of five time intervals: 4, 7, 9, 11, or 14 d. For each time interval, embryos in four randomly selected EIUs were covered with sediment, the remaining four units receiving no sediments and serving as controls. At each time interval, the eight assigned EIUs were removed individually from the trough, and sediments and embryos were rinsed into a fine-mesh aquarium net. Embryos from three of the four sediment EIUs were returned to the appropriate EIU and placed back in the trough to complete incubation without sediment cover. Embryos in control EIUs were handled in the same manner. Eight additional EIUs served as undisturbed controls to assess potential effects of the apparatus on the growth and survival of embryos and larvae. Undisturbed controls were randomly distributed throughout the trough, received no sediments, and were not handled for the duration of the study. All EIUs were observed twice daily to monitor hatch timing. When hatching began, the number of emerged larvae visible in each EIU was recorded twice daily, and the emerged larvae were removed and preserved for later examination.

*Statistical analysis.*—Data for the apparatus-testing trial were analyzed by analysis of variance (ANOVA) in a randomized block design. Blocking consisted of six, two-row blocks per trough. Dependent variables were percent survival, embryo diameter, or embryo weight. The two test velocities were unreplicated, limiting the assessment of velocity effects to the visual inspection of data plots. Data for the sediment cover duration trial were analyzed by ANOVA in a randomized factorial design; percent survival, day of hatch, embryo length, or embryo weight served as dependent variables. Data for both trials were analyzed with the

SAS statistical package (SAS 2000). In each trial, a one-way ANOVA was used to estimate the significance of factor effects for each dependent variable, with  $P < 0.05$  required for statistical significance. For analyses of diameter, length, or weight differences, means for each replicate were weighted by the number of embryos or larvae counted. Specific hypotheses were tested via a priori contrasts (Cody and Smith 1997). For each trial, contrasts were used to assess whether specific factors affected dependent variables within the factorial study design. Study design included balanced treatments to satisfy a priori assumptions. Plots of residuals versus fitted  $Y$ -values and normal probability plots of the residuals were examined to assess potential violation of ANOVA assumptions. Because percentages based on categorical data form binomial rather than normal distributions, percentage survival data were transformed to arcsine square roots before analysis.

## Results

In the apparatus-testing trial, survival was higher for all treatments in the higher-flow trough (1.8–5.0%) than in the lower-flow trough (0–1.8%; Figure 2). In both the higher- and lower-flow troughs, embryo survival differed ( $df = 6, 36$  for both analyses;  $F = 106$  and  $387$ , respectively;  $P < 0.0001$  for overall models) among combinations of sediment depth, ventilation hole size, and EIU position. The survival of embryos incubated under sediments was reduced in both the higher- and lower-flow troughs ( $df = 1, 36$  for both tests;  $F = 635$  and  $2,316$ , respectively;  $P < 0.0001$ ), with low survival rates under both 5- and 20-mm sediment covers (Figure 2). At the higher flow, no significant differences in survival were found by comparisons between 5- and 20-mm sediment depths or between small, medium, and large ventilation hole sizes. The numbers of embryos surviving sediment treatments in the higher-flow trough were small ( $n = 24$  under 5-mm sediment cover;  $n = 8$  under 20-mm sediment cover), and the statistical power of the analyses correspondingly low. In the lower-flow trough, only three embryos survived under sediment cover, precluding comparisons of survival between sediment depths or ventilation hole sizes. In the higher-flow trough, embryo diameter and weight were not significantly affected ( $P > 0.50$ ) by sediment cover; corresponding analyses were not performed with data for the lower-flow trough because of the small number of surviving embryos. The location of EIUs (upper trough or lower trough) affected embryo survival in both the higher-velocity and lower-velocity troughs ( $df = 5, 36$  for both tests;  $F = 3.00$  and  $2.66$ , respectively;

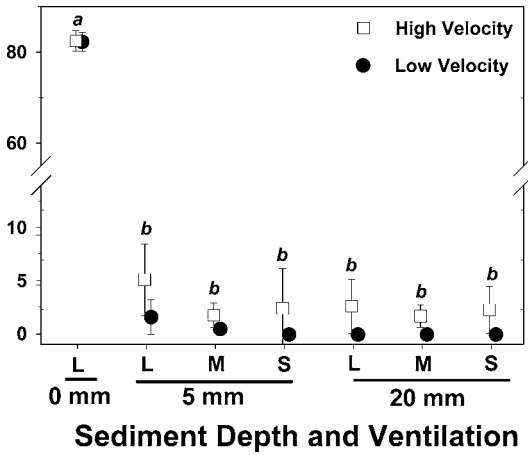


FIGURE 2.—Mean ( $\pm$ SD) percentage survival of white sturgeon embryos incubated under fine sand (0-, 5-, or 20-mm depth) in embryo incubation units with ventilation hole diameters of 9.5 mm (large [L]), 6.8 mm (medium [M]), or 4.8 mm (small [S]) in the apparatus-testing trial. Tests were performed at above-substrate flow velocities of 0.15 m/s (higher) or 0.05 m/s (lower). Different letters denote embryo survival rates that differed significantly ( $P < 0.05$ ).

$P = 0.02$  and  $0.04$ , respectively), with slightly higher survival rates in upstream positions.

In the sediment cover duration trial, embryo survival differed ( $df = 10, 37; F = 14.6; P < 0.0001$  for overall model; Figure 3) among combinations of sediment cover, duration of sediment cover, and handling, as did day of embryo hatch ( $df = 10, 43; F = 10.1; P < 0.0001$  for overall model) and larval length ( $df = 10, 43; F = 9.01; P < 0.0001$  for overall model) but not larval weight ( $df = 10, 43; F = 0.65; P = 0.76$  for overall model). As a single factor, the presence of cover sediments reduced embryo survival ( $df = 1, 37; F = 120; P < 0.0001$ ; Figure 3), increased day of hatch ( $df = 1, 43; F = 38.1; P < 0.0001$ ), and decreased larval length ( $df = 1, 43; F = 34.9; P < 0.0001$ ). Increasing duration of sediment cover resulted in decreased embryo survival ( $df = 4, 37; F = 5.54; P = 0.001$ ); survival rates were significantly higher for embryos in units that had the sediment cover removed at 4 and 7 d than for embryos in units that had the sediment cover removed at 9, 11, or 14 d (Figure 3). The duration of sediment cover also significantly affected the day of hatch ( $df = 4, 43; F = 4.06; P = 0.007$ ); however, a simple relationship between the two variables was not apparent, the earliest mean day of hatch (11.2 d) occurring for embryos covered for 11 d and the latest mean day of hatch (13.3 d) for embryos covered for 14 d. Duration of sediment cover did not

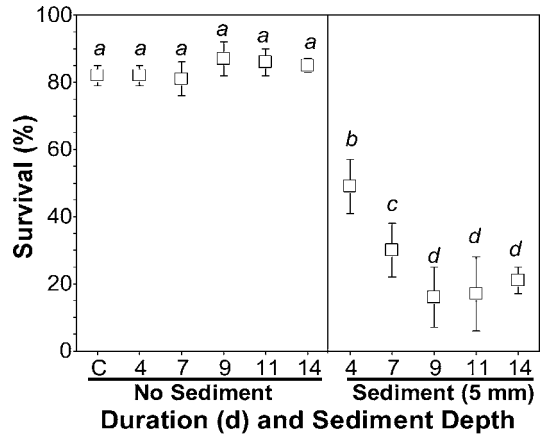


FIGURE 3.—Mean ( $\pm$ SD) percentage survival of white sturgeon embryos incubated under fine sand (0- or 5-mm depth) in embryo incubation units and handled (if present, sediment removed) at specific time intervals (4, 7, 9, 11, or 14 d) in the sediment-cover duration trial. Control units (C) were undisturbed for the study duration. Tests were performed at above-substrate flow velocities of 0.17 m/s. Different letters denote embryo survival rates that differed significantly ( $P < 0.05$ ).

significantly affect larval length or weight. Handling of embryos did not affect any of the dependent variables.

### Discussion

The results of this study clearly indicated that incubating white sturgeon embryos are highly sensitive to sediment cover. Sterilization of the native river sediments used in this study reduced the likelihood of fungal infection of the embryos and also reduced the microbial use of dissolved oxygen in the interstitial water (see below), both factors that might add to sediment-induced mortality in the river setting. Nevertheless, all combinations of sediment depth and duration of sediment cover resulted in low rates of embryo survival. Exposure to sediment depths of only 5 mm, with the upper surface of the 3-mm-diameter embryos at the most 2 mm below the sediment-water interface, reduced survival to 50% after 4 d and to less than 20% after 9 d or longer. In contrast, the mean survival of white sturgeon embryos incubated without sediment cover exceeded 80% in both trials, closely approximating the survival rates of same-family embryos reared under optimal hatchery conditions at the Kootenai Tribal Hatchery (85%; John Siple, Kootenai Tribe of Idaho, personal communication). These results suggest that Kootenai River white sturgeon embryos may experience high mortality rates in their current spawning habitat, which is characterized by a fine-sand substrate. Although sedimentation

has been suggested as a likely source of mortality for white sturgeon embryos in the Kootenai River (Paragamian et al. 1996; Anders et al. 2002; Ireland et al. 2002), the present study provides the first empirical evidence for sediment cover as a cause of embryo mortality.

High embryo mortality under sediment cover, as observed in this study, is presumably due to restricted rates of water renewal around the embryos, resulting in a reduced exchange of respiratory gases (oxygen and carbon dioxide). Finer substrates have lower rates of intrasubstrate flow; furthermore, in unsterilized sediments microbial respiration results in progressively lower oxygen concentrations as intrasubstrate flow decreases (Coble 1961). Because we were uncertain how the design of the experimental apparatus (EIU) would affect intrasubstrate flow and embryo survival, we tested the effects of three levels of ventilation of the sand layer below the embryos. Embryo survival was not, however, sensitive to variation in the level of ventilation. A three-fold increase in above-substrate water velocity (0.05–0.15 m/s) appeared to have a small effect on embryo survival (the significance of this difference could not be confirmed because velocities were not replicated). Renewal of interstitial water was apparently primarily by way of the substrate–water interface.

Newly fertilized sturgeon ova are highly adhesive and cannot be easily handled unless they are coated with suspended clay particles (Fuller's earth). A coating of clay particles would be expected to impair respiratory gas exchange to some degree. Although the lots of embryos used in our experiments were treated with Fuller's earth, survival was high (>80%) in the hatchery and, for control embryos, in our experiments. Similarly, Paragamian et al. (2001) remarked that many of the embryos collected in the Kootenai River were coated with sand, but that development did not seem to be affected.

Sediment cover delayed timing of hatch for white sturgeon embryos in our study and resulted in a smaller size at emergence. Differences in mean length were not apparent between units that were uncovered at time intervals ranging from 4 d to the initiation of hatching at 14 d, suggesting that the negative effect of sediment cover on length occurred primarily during the first 4 d of incubation. The growth, development, and oxygen uptake of salmonid embryos incubating under fine sediments is also reduced (Silver et al. 1963).

White sturgeon researchers have speculated for some time that sedimentation may be one of the factors contributing to reduced early life survival and recruitment of white sturgeon in altered large-river

systems (Anders 1991; Duke et al. 1999; USFWS 1999; Anders et al. 2002; Parsley et al. 2002). The results of the present study support this hypothesis with respect to the Kootenai River, where white sturgeon spawn over fine sand substrates in areas with mean velocities of 0.2–0.7 m/s (Paragamian et al. 2001). In contrast, white sturgeon spawning has been documented in Columbia and Snake River dam tailraces (Parsley et al. 1993, 1994; Parsley and Kappenman 2000) over bedrock and larger-diameter substrates of boulders, cobble, or gravel in areas of relatively high velocity ( $\geq 0.8$  m/s). In the Fraser River, white sturgeon spawn over gravel and sand substrates (Perrin et al. 2003) in relatively high-velocity side channels (1.3–2.2 m/s). Water temperatures in the Kootenai River during spawning (8.5–12.0°C; Paragamian et al. 2001) are also several degrees cooler than for several other northwestern white sturgeon populations (10–18°C in the lower Columbia River, Parsley et al. 1993; 15°C in the Fraser River, Perrin et al. 2003), which would be expected to lengthen the incubation period (and the duration of susceptibility to moving sediments) by several days.

Future research is needed to assess the degree of similarity between the incubation conditions experienced by embryos in this laboratory study and in the wild. Although it is reasonable to speculate that embryos might be buried by moving sand deposits in Kootenai River spawning areas, this has not been actually observed. Testing of the effects of early life mortality factors such as sedimentation and predation on embryo survival is inherently difficult at the river scale, and particularly so when the population of interest is small, with few spawning adults.

In summary, the present study provided empirical evidence of the degree to which the development and survival of white sturgeon embryos is negatively affected by a sediment cover. Our results indicated that (1) the incubation of white sturgeon embryos in replicated embryo incubation units under controlled laboratory conditions is a useful technique for assessing the effects of sediment cover on embryo survival, size, and emergence timing; (2) embryo survival and larval length were reduced and the timing of emergence delayed when embryos were covered by a thin (5-mm) layer of fine sand; and (3) sediment deposition in spawning areas may be responsible for high early life stage mortality of white sturgeon in the Kootenai River and in other, similarly altered rivers with high bedload movements of fine sediments.

#### Acknowledgments

The Bonneville Power Administration and the Kootenai Tribe of Idaho funded this research. Sue

Ireland, Charlie Holderman, the Kootenai Tribal Hatchery staff, David Smith, John Drennan, Joe Evavold, Michelle Kock, Darin Jones, Ben LaFrentz, Boling Sun, and Tyler Wagner provided valued technical support and assistance. Chris Williams assisted with the statistical analyses. This is contribution number 85 from the College of Natural Resources Experimental Station, University of Idaho. The Idaho Cooperative Fish and Wildlife Research Unit is supported by the U.S. Geological Survey, the University of Idaho, the Idaho Department of Fish and Game, and the Wildlife Management Institute. Reference to trade names does not imply endorsement by the U.S. Government.

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