FINAL REPORT

To

The Kootenai Indian Tribe of Idaho
P.O. Box 1269
Bonners Ferry, Idaho 83805

TITLE: Behavior and Habitat of Young Kootenai River White Sturgeon – 2009

by

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Summary

All studies that follow were conducted in artificial streams. Dispersing Kootenai sturgeon larvae did not prefer any of five substrate-velocity habitats (vegetation, cobble rocks, woody debris, clay terrace, or sand) in fast or slow velocities. These habitats are similar to habitats in the meandering reach of the river where larvae occur. The lack of an innate bottom habitat preference show wild larvae are bottom-habitat generalists. With no innate preference, there is likely no mismatch between fish habitat preference and the available bottom habitats. The present results on Kootenai sturgeon larvae are the only data on bottom habitat preference of dispersing larvae for any sturgeon species.

Dispersing larvae-early juveniles use of bedforms that simulated bottom habitat created by sand dunes, wood limbs, rocks, and vegetation found fish 1) tended to move downstream less when bottom structure was present, a result similar to that found in a previous study, and 2) used slow velocity (typically, < 0.15 cm/s) bottom areas created by any bedform. This result supports the conclusion from the substrate-velocity study that wild larvae use any bottom habitat providing a velocity refuge, and likely, forage. Further, larval behavior when on the bottom suggested fish were feeding on drift or on entrained or deposited benthos. Selection of slow velocity refuges also may conserve energy, which seems adaptive for small larvae resting from swimming above the bottom.

Behavior of dispersing larvae and early-juveniles was affected by bedform. Because artificial streams cannot duplicate the bottom habitat in the correct spatio-temporal occurrence and scale that it occurs during wild larval dispersal in the river, it is impossible to use these studies to build a dispersal model for Kootenai sturgeon larvae.

Wintering yr-0 juveniles observed for preference of the same five substrate-velocity habitats as larvae (described previously in the first paragraph) had the strongest preference for vegetation in fast velocity and the strongest avoidance of vegetation, woody debris, and clay terrace in slow velocity. The optimal wintering habitat may provide 1) an energy-saving velocity refuge, and 2) proximity to fast current bringing drifting food. Thus, yr-0 juveniles may have a different wintering strategy (and are in a different location) than older juveniles (yrs-3-4), which have been found in deep, slow velocity habitat. If yr-0 juveniles are in the lower river, the scarcity of vegetation may create a mis-match between habitat preference and available habitat in the altered river.

There is a strong ontogenetic shift in bottom habitat preference from summering larvae–yr-0 juveniles to wintering yr-0 juveniles. Summering fish prefer slow velocity created by any bottom structure, and wintering yr-0 juveniles prefer a velocity refuge in structure, particularly vegetation, in fast velocity.

Wintering yr-1 and yr-2 juveniles were solitary, but there was a significant tendency of yr-0 juveniles to aggregate more at colder temperatures, suggesting behavior may change during winter. Other observations found yr-0 juveniles selected wintering habitat that likely allowed access to drifting food; thus, the solitary behavior would reduce competition for limited drifting food resources in winter.

Activity of yr-0 juveniles in October–December (11–0 ºC) was significantly related to water temperature, with fish not becoming inactive until temperature decreased to ≤ 2 ºC. Libby Dam operations in winter create an artificially warmer Kootenai River than natural (temperatures only rarely are < 3 ºC). The altered warm temperature regime could create energetic problems for wintering young juvenile Kootenai sturgeons.
Introduction

The years of successful spawning by Kootenai River white sturgeon, *Acipenser transmontanus* (hereafter, Kootenai sturgeon) in the river between Kootenay Lake and Libby Dam has resulted in little recruitment of juveniles; indicating there is a bottleneck in spawning, survival of ELS (early life stages – eggs, free embryos, larvae), or both. The river environment has changed drastically due to anthropogenic activities (Anders et al. 2002); thus, the innate habitat preferences of ELS evolved in the pre-dam natural river environment may no longer be adaptive in the altered river; i.e., a mismatch may exist between the present rearing environment and the fish’s innate habitat preferences. Basic information on innate habitat preference behavior of ELS can help managers determine if this mismatch exists, and also identify the affected environmental factor(s) and if management can solve the mismatch. Because of the difficulties of studying ELS in the field, the only practical approach to revealing innate seasonal habitat preferences of ELS are studies in artificial stream tanks.

During 2005–2007, our artificial stream research on young Kootenai sturgeon movements during ontogenetic development found larvae have a dispersal style that likely takes them to the lower meandering reach of the Kootenai River, or even to the South Arm of Kootenai Lake (Kynard and Parker 2006, Kynard et al. 2007, 2008, 2009, 2010). Thus, these two areas are the likely rearing places for larvae and yr-0 juveniles after they disperse downstream from upstream spawning-free embryo rearing areas.

Much of the present study continues the study of innate habitat preferences of larvae and young juveniles, particularly their preference for bottom habitats. Studies on salmonids and sculpins provide a general approach for these studies (Heggenes 1988, Brown 1991). Sturgeons are benthic cruising foragers, so the relationship of juveniles to substrate type where food is located is probably strong, like it is with sculpins. However, larvae are dispersing and foraging for food on the bottom periodically, so their habitat preference is unknown. Data do not exist to answer this question for Kootenai sturgeon larvae or for any sturgeon larvae. Laboratory observations on young foraging Kootenai sturgeon larvae found they foraged on or just above the substrate, no matter whether the substrate was oriented horizontally or vertically, showing fish had a great ability to use bottom substrate.

Winter habitat of wild yr-0 & yr-1 juvenile Kootenai sturgeon has not been studied in the wild and only preliminary observations have been made in an artificial stream (Kynard et al. 2007). Wintering is a period of great physiological and energetic stress that likely lasts about 6 months. Thus, juveniles have likely evolved a survival strategy that includes a strong winter habitat preference. Wintering habitat preference of yr-0 Sacramento River white sturgeon juveniles in an artificial stream was cobble bottom, not sand, and they also selected a moderate water velocity of 20 cm/s (Parker et al. unpub. data). In addition, wintering yr-0 juveniles of some species are strongly photonegative, active only at night, e.g., green sturgeon, *A. medirostris* (Kynard et al. 2005). Because the Kootenai River has been so altered (Anders et al. 2002), it is possible that the existing
winter habitat and the evolved winter habitat preference of young juveniles are a mismatch.

In 2007-2008, using underwater video, we identified the dominant bottom habitats in the lower Kootenai River and the South Arm delta of Kootenay Lake during the foraging season (July-August) and during the wintering season (January; Kynard et al., 2008). This information was used to create artificial bottom foraging habitat for larvae and wintering habitat for juveniles to choose in an artificial stream. Also in 2008, we examined winter habitat preference of single vs. small groups of Kootenai sturgeon yr-0 and yr-1 juveniles. These tests showed no effect of the densities of our test fish on habitat preference; thus, in the present tests, we used the same group densities of fish.

We examined preference of larvae and yr-0 juveniles for several bottom habitat types that are available in the meandering reach of the Kootenai River where these life stages likely occur. Larval preference was observed during foraging-dispersing in summer and yr-0 juveniles were observed during wintering. In all tests, we conducted tests in an artificial stream that had a velocity gradient in order to evaluate the effect of velocity on fish choice of bottom habitat.

In a separate set of tests using a series of identical artificial streams, we observed how larvae and yr-0 juveniles respond to different bedforms and flow fields. These data were requested by researchers attempting to model downstream movement of larvae in the Kootenai River. Larvae do not disperse as passive particles; instead they pause dispersal and interact with the substrate to rest and forage (Kynard and Parker 2006, Kynard et al. 2007, Kynard et al. 2008, 2009). The objectives of the present study relative to bedforms were 1) to determine the feasibility of using behavioral studies in artificial streams to supply data for modeling downstream larval dispersal and, 2) to better understand young sturgeon’s use of the habitats created by various bedforms that were similar to those present in the river.

Additionally, in 2008 the KRWSRT embarked on a 5-year plan to stock free embryos in the Canyon Reach of the river, where spawning likely occurred historically and where there still seems to be abundant spawning habitat in some reaches (B. Kynard, pers. observation). Stocked fish surviving to recruit to the juvenile monitoring program will be detected after 5 years during sampling for juveniles in Kootenai Lake and the lower river. Five years is a long time to wait to learn if free embryo stocking is successful, and the stocking also provides larvae and yr-0 life stages in the river to study. Thus, we did two studies: one study to determine if it is possible to use underwater video to survey for young juveniles, and a second study, to determine the “time window” in fall when yr-0 & yr-1 juveniles cease activity (begin wintering behavior) as water temperature decreases and photoperiod shortens. We also analyzed yr-0 juveniles in these activity tests to determine if they are solitary or form aggregations, which would affect the ability to sample or detect with video or other survey techniques. Juveniles of some sturgeon species form strong winter aggregations (Kynard et al. 2005).
One of the most important unknown aspects of life history for all sturgeons is the importance of imprinting by early life intervals to river place(s) for a successful life history. This information has great significance for all restoration programs that involve rearing early life stages away from the natal reach, or rearing and stocking juvenile sturgeons, which are not allowed to complete natural movements and visit and be imprinted to foraging and wintering concentration areas. Studies on shortnose sturgeon, *A. brevirostrum*, indicate interruption of the natural movements and visits by juveniles to river reaches causes dysfunction later in life when fish are mature (Kynard et. al. unpub. data).

If free embryos imprint to their natal site, spawning adult sturgeons should show fidelity to their birthplace in at least two ways. First, they should search for and show preference to water from their birth reach. Second, their noses should show enhanced sensitivity to odors contained in water of the natal stream.

To test this hypothesis with Kootenai sturgeon is not time-efficient, but the most time-consuming work to test these hypotheses for shortnose sturgeon has already been done by B. Kynard and E. Parker, who in 1998 assembled several live boxes containing free embryos from the eggs of one female. Some live boxes were placed at a known spawning site with rocky bottom in the Connecticut River and others were placed in similar habitat in a nearby tributary (Deerfield River), far from where sturgeon go or spawn. Boxes were removed after free embryos developed into larvae, taken to the Conte AFRC, and fish from both groups were reared identically on city (well) water. The two groups were kept separate for 8 years, and then PIT-tagged, and combined into a common tank supplied with well water in 2006. The goal of the project is to determine if the two groups of fish show a significantly different olfactory response to the natal waters they were exposed to as embryos (eggs) and free embryos (<14 d old).

In 2008, Dr. Weiming Li, Michigan State University, an expert in olfactory studies on fish (Siefkes and Li 2004), and Mr. Andrew Willbill (research assistant), with us helping, constructed all the supporting systems for olfactory imprinting research. With great difficulty, we developed a Faraday cage, fish holding system, electrode system to determine olfactory bulb electrical signals, and water flow system for testing sturgeons at the Conte Lab. On the final day of work, we produced acceptable base-line levels of electrical activity in a test fish in response to odors. In 2009, we propose to continue tests with the two groups of fish, each reared in different natal waters as free embryos, using the equipment and expertise of Dr. Li.

**Objectives for 2009:**

1. Obtain 1,000-1,500 fertilized eggs of Kootenai sturgeon and rear them.
2. Determine bottom habitat preference for five substrate-velocity habitats of foraging-dispersing larvae in summer and of wintering yr-0 juveniles.
3. Determine if and when yr-0 & yr-1 juveniles aggregate in winter.
4. Determine activity level of yr-0 and yr-1 juveniles in relation to temperature decrease during late-fall and winter.
5. Determine if it is practical to use UW video to survey for wintering yr-0 and yr-1 juveniles.
6. Test hypotheses on olfactory imprinting and olfactory sensitivity of two groups of shortnose sturgeon.
7. Determine how dispersing-foraging larvae–early-juveniles respond to flow fields created by bedforms simulating natural bedforms (rocks, sand dunes, wood limbs-vegetation, and sand) in the lower Kootenai River.

Methods

Objective 1 and general procedures for rearing and holding sturgeons.— In 2007–2008, we reared fertilized eggs of Kootenai sturgeon sent to us by the hatchery in a McDonald hatching jar and transferred surviving hatchling free embryos (day-0 fish) to an outdoor stream channel for rearing in summer and fall. These are the yr-0 and yr-1 juveniles we studied in winter 2009. We also obtained an additional 1,500 eggs on 12 June 2009, which hatched on 28 June 2009 and these are the larvae used in all tests.

We used the number of days post-hatching to characterize age of fish, not the number of days post-fertilization, because we did not know how early egg-rearing (particularly water temperature) varied during shipping before we received eggs. We reared test fish in the artificial stream until the late fall, and then before freezing weather, moved fish into 1.5 m diameter tanks for rearing during wintering studies.

We used temperature-controlled dechlorinated city water (Montague, MA) for rearing and larval experiments during summer–early fall, and then, we switched to ambient Connecticut River water during winter, when tests were done inside a wet lab. Temperature during rearing and in experiments was the same (±1°C). We maintained the natural photoperiod for Turners Falls, MA, latitude (42.6°N) even during the winter when fish were inside the wet lab.

Objective 2. Determine bottom habitat preference for five substrate-velocity habitats of foraging-dispersing larvae in summer and of wintering yr-0 juveniles. — We conducted tests in a modified oval artificial stream tank that was used previously for observing larval dispersal (Fig. 1; Kynard and Parker 2006; Kynard et al. 2010). Dimensions of the tank follow: 5.7 m circumference in the channel x 28 cm wide with a water depth of 20 cm. The tank had two long, straight sides with 180° turns at each end. Underwater light intensity was no greater than that observed at 15 m depth on the river bottom in January 2008 (430 lx in the river) or August 2007 (about 400 lx). The in- and outflow of water was 1 L/min to keep temperature consistent.

The environment in the stream varied for velocity and bottom type. Two small propellers were placed at one end of each straight reach to create a velocity gradient down each straight reach (maximum of three fish body lengths/s (bl/s) to a minimum of 0.3 bl/s). This configuration created equal-size patches of the same pair of habitats along the length of each straight side with a different velocity pattern (fast to slow or slow to fast; Fig. 1). Thus, along each straight reach, there was a gradient of fast to slow velocity
habitat, and also, two substrate habitats. Coarse sand (1-2 mm diameter) covered the bottom of the tank, and the additional substrate was added on top of the sand in one-half of each test area. Habitat pairs tested follow: (1) sand vs. sand + clay terrace, (2) sand vs. sand +vegetation, (3) sand vs. sand + woody debris, (4) sand vs. sand + small cobble. On one test side of the tank the sand was closer to the propellers, and on the other side of the tank, the substrate habitat was closer; thus, creating areas of open sand and substrate with fast (about 3 bl/s to 1 bl/s) and slow (about 1 bl/s to 0.3 bl/s) velocities on each tank side and splitting the tank’s straight test areas into four sections or quadrants (Fig. 1).

We tested larvae in summer in groups of 15 and tested yr-0 juveniles in winter in groups of 10. Tests in 2008 found no density effect on habitat preference of these size groups and ages of Kootenai sturgeon. In all cases, fish were introduced and allowed to acclimate for 1 h, and then, observed for about 24 h (beginning about 1200 h) to identify diel patterns of habitat preference and allow a long-term recording of habitat preference. After 24 h, this group of fish was removed from the tank, a new group of fish was put in the tank, and observations repeated. We recorded fish over the two straight reaches for 10 min/h with four video cameras (with IR lights for nighttime viewing) over the tank. We also made direct visual observations three times/day in daylight, during which each fish was located and their habitat use recoVideo data were used for day vs. night comparisons. Direct observations were used for summer (larvae) vs. winter (yr-0 juveniles) daytime analysis because all individuals were located during each observation (a variable number of individuals were not visible to the video cameras for each video observation). Larvae were tested at days 30-60 (July – September, from early- to late-larval stage), and yr-0 juveniles were tested at months 6-9 (December – February). We tested two – six groups per substrate type during larval tests and eight groups per substrate type for wintering yr-0 juveniles.

During review of tapes, we identified fish that were stationary or had paused up- or downstream movement and assigned each fish to one of the four quadrants of the tank. Two-way analysis-of-variance (ANOVA) was used to analyze effects of the two substrates (sand and substrate of interest) and the two water velocities for daytime experiments with larvae and juveniles. The proportion of observations in each habitat was analyzed with n = number of groups of fish (two– six groups of larvae in summer; eight groups of yr-0 juveniles in winter). For juveniles, two-way ANOVA was used to analyze day vs. night and water velocity for the use of each substrate of interest (n = eight groups). Finally, one-way ANOVA was used to compare the four habitats of interest across equivalent winter experiments using only fast velocity treatments (which had the majority of use by fish).

**Objective 3. Determine if and when yr-0 and yr-1 juveniles aggregate during wintering.**—We used 1.5 m diameter circular flow-through tanks for these tests. We used two tanks for yr-0 juveniles and only one tank (no replicate) for yr-1 juveniles. We placed 20 yr-0 juveniles in each of two replicate tanks and 10 yr-1 juveniles in one tank (no replicate). We used a permanent ink pen to mark a grid (10 cm square) on the bottom of all tanks. Four times each day, we took point samples on fish locations, marking them
on the grid-map of the tank’s bottom. Fish were observed from 28 October to 21 December 2008. Water supply and temperature was ambient Connecticut River.

Each fish located in a grid cell was assigned to the center point of that cell. Distances (mm) between fish were then calculated using the Pythagorean equation. Mean nearest neighbor distance was calculated for all fish in a tank. We used computer simulations to determine expected spatial distributions of fish that were randomly located within the tank. These random distributions could then be compared to the actual results in order to determine whether greater aggregation was occurring than expected by chance.

Within the tanks, fish were more likely to be found in grid cells along the wall than in central grid cells. Approximately 50% of all fish locations were in wall-grid cells, but only about 30% of grid cells were found along the wall. Thus, we chose to give the simulated fish the same bias toward the wall; in this way, the randomized groups would show a similar spatial pattern, allowing us to evaluate aggregation alone. The same number of fish found in the tanks (20, 6, or 5 after one was removed) was modeled. Fish were randomized with a 50% chance of being placed in one of the 28 wall grid cells and a 50% chance of being placed in one of the 68 central grid cells; 1000 simulations were done for each number of fish.

Mean nearest neighbor distance (NND) was calculated for each simulation. We determined the range that included the middle 95% of simulated NNDs; any observed NND that occurred outside of this range were considered more or less aggregated than expected by chance. Additionally, we evaluated the number of pairs of fish that were found in the same grid cell. This value was calculated for each observation, and the distribution of these values over the entire experiment was compared to the randomized values.

We also determined the number of contiguous squares containing fish each day and plotted these in a regression against water temperature as another way to look at fish aggregation through time.

Objective 4. Determine activity level of yr-0 and yr-1 juveniles in relation to temperature decrease during late-fall and winter. —After we counted fish to determine aggregation, every 2 days we observed 10 fish in each tank for their activity level by counting the number of gridlines each fish crossed during a 3 min observation period. Counting was done from 29 October to 26 December 2008, during the gradual decrease in water temperature as would occur naturally. We used regression to analyze the correlation between activity (number gridlines crossed) and water temperature (ambient Connecticut River).

Objective 5. Determine if it is practical to use UW video to survey for wintering yr-0 & yr-1 juveniles in the Kootenai River and South Arm. —In July 2010, we released juveniles on the bottom of the Kootenai River: two fish at 20 ft depth, 12 fish at 3.5–9 ft depths to determine if we could observe and track juveniles with our video equipment. In the one deep water test, two yr-1 fish were released together and in the shallow water
tests, three yr-0 fish were released in each of four tests. Fish were placed in a glass jar with a foam stopper, and the jar and video camera were lowered together to the river bottom. When the jar was on or very near the bottom, and the observer (E. Parker) could see the jar on the video screen, the stopper was removed by pulling a string attached to it, and the fish were free to swim out. The observer scored how well the fish could be seen after they had left the jar.

We also surveyed the existing UW video technology and research literature to help determine the feasibility of video surveying for fish during late-fall or winter in the Kootenai River and South Arm of Kootenay Lake.

Objective 6. Test hypotheses on imprinting and olfactory sensitivity of shortnose sturgeon.—We proposed to continue electro-physiological tests with the two groups of fish, each reared in different natal waters as free embryos. General methods were outlined in the Introduction.

Objective 7. Determine how dispersing-foraging larvae-early-juveniles respond to flow fields created by bedforms simulating natural bedforms (rocks, sand dunes, wood limbs-vegetation, and sand) in the Kootenai River.—For these tests, we used eight identical circular 180-cm diameter artificial stream tanks (570 cm circumference; Fig. 14). Each tank had a mesh insert in the center to create a circular channel 35 cm wide x 330 cm long with a mostly uniform width and velocity, although in the widest part velocity near the inside wall was slower. Water depth in tanks was 30 cm, except in the dispersal video camera viewing area, where a ramp reduced depth to 13.5 cm for a short distance.

The water system for the artificial stream tanks and the rearing stream for other fish was a constant circulating system. Water constantly drained from a head tank, which regulated water flow through the system, to each tank and overflowed into the common drain, where the water was pumped through a chiller-heat pump back to the head tank. The chiller-heat pump within the water system maintained the same water temperature in all tanks. Individual pumps at each of the tanks provided the water velocity regime by pumping water through the flow outlet in the ramp. Each tank was covered with a fine-mesh netting to exclude insects and aerial debris from falling into the water. The entire system was outside on a platform and protected from weather by a tent cover.

We tested fish response to four bedforms (two replicate tanks of each bedform): 1) a control bottom with only smooth sand, 2) a straight-crested baffle weir (simulated sand dune, 3) rocks, and 4) vertical and horizontal square and cylindrical shapes (simulated wood limbs. The bottom of all tanks was covered with coarse sand (1-2 mm diameter). A simulated sand dune was created by vertically inserting into the sand a 5-cm high piece of clear sheet Plexiglas across the width of the tank. This baffle-weir structure created flow fields similar to the cross-river barrier created by a small sand dune in the river, which is a dominant bottom feature in the meandering reach of the Kootenai River. While the scale of our sand dune was small relative to most sand dunes in the river, the artificial dune enabled us to observe how the small test larvae responded to dune flow fields.
Rocks were 5 cm or 2.5 cm diameter pebbles placed 10 cm apart, with placement alternating between large, then small, in a grid pattern across the entire bottom. The vertical and horizontal shapes we tested were pieces of clear Plexiglas pipe, round (5 cm diameter) and square (5 cm diameter), filled with coarse sand with one round and one square pipe laid horizontally across the width of the channel, creating long weirs across the direction of flow and two round and two square pieces were positioned vertically to extend about half the water depth.

To plot position of fish, a grid was established on the bottom of each tank using rows of small nails driven into plastic strips under the sand. Only the nail heads, which were painted with fluorescent pink paint, were visible at sand level. Distance between the grid points depended on the test configuration. For the vertical shape tanks, where the bottom velocity was highly variable and complex, nails were placed every 5 cm apart along 15 radial transects 5 cm or 10 cm apart, depending on placement of the shapes. In tanks with rocks, nails were 5 cm apart along 21 radial transects that were 5 cm apart. In the baffle configuration and in the control tanks, nails were placed 10 cm apart along 11 radial transects that were 10 cm apart. During observations on fish, we recorded fish position and measured water velocity at the nearest nail (see next paragraph).

Water velocity in tanks ranged from 5 to 80 cm/s with a mean bottom velocity of 20 cm/s in the channel as determined in one tank of each substrate composition. To determine available velocities, velocity was measured at each grid point 2.5 cm above the bottom with a 2-dimensional small ball (2 cm diameter) velocity meter.

Test methodology was to introduce 10 fish into each tank, give them 24 h to acclimate, and observe them three times (see details below), and then, remove them and insert another group. This procedure was repeated every third day using fish 14 days old to fish 80 days old (days 14−65 larvae, and day 66+ juveniles). During an observation period, we recorded fish position every 30 sec for 2.5 min until fish were 22 days old. After that, fish positions were recorded every 30 sec for 5 min. We also recorded if fish were moving downstream, against the inside wall, on the bottom, or on the ramp. Histograms of available water velocities and water velocities used by fish were constructed and distributions of available and used habitat were tested with Kolmogorov Smirnov two-sample tests, as well as velocities used by fish in the control vs. those in the three other treatments.

**Results and Discussion**

**Objective 1. Obtain 1,000-1,500 fertilized eggs of Kootenai sturgeon and rear them.** — We obtained eggs and reared fish as described in the Methods. Reared fish were used in all tests in the present study of larvae and yr-0 juveniles. Yr-1 juveniles were reared from eggs obtained in 2008.

**Objective 2. Determine bottom habitat preference for five substrate-velocity habitats of foraging-dispersing larvae in summer and for wintering yr-0 juveniles.** —
Foraging-dispersing Kootenai sturgeon larvae did not significantly prefer any of five substrate-velocity habitats (vegetation, cobble rocks, woody debris, clay terrace, or sand in fast and slow velocities ($P > 0.10$; Fig. 2). These bottom habitats were similar to Kootenai River habitats observed in the meandering reach using UW video (Kynard et al. 2008, 2009). Day-night differences in habitat selection of larvae were not analyzed as only daytime observations were taken.

Because larvae are actively swimming and dispersing in the water column and only pausing to use the bottom, the lack of a substrate preference by larvae suggests they are bottom habitat generalists. Dispersing larvae are unlikely to be able to detect and select particular bottom habitats from their dispersal location in the water column as most are facing upstream; thus, they likely stop when needing to forage or rest at whatever habitat is present on the bottom, and thus, the conditions were not favorable to evolve a strong habitat preference. The length of time spent on the bottom may be directly related to food abundance and the energetic need to rest by larva, but data are not available.

The habitat preference results of Kootenai sturgeon larvae are the only available data on habitat preference of dispersing larvae for any sturgeon species. Because many other sturgeon species have dispersing-foraging larvae (Kynard and Horgan, 2002; Kynard and Parker 2004, 2005; Kynard et al. 2005; Zhuang et al. 2002) the results with Kootenai sturgeon suggest larvae of other sturgeons may also lack a strong preference for substrate habitat and are bottom-habitat generalists.

Experiments indicated wild Kootenai sturgeon larvae have no preference for bottom habitat and select many bottom velocity-foraging habitats. Thus, a mismatch is unlikely between the bottom habitat that is best for survival and fitness of larvae and the available bottom habitat of the altered Kootenai River.

Wintering yr-0 juveniles significantly preferred vegetation in fast velocity over sand in either fast or slow velocity (Fig. 2). Indeed, the preference for vegetation in fast velocity was the strongest preference for any habitat. In all other configurations, fish preferred (or had a strong preference trend for) open sand in slow velocity, suggesting if fish could not get the preferred habitat, they chose sand-slow velocity. Unlike the significant preference for vegetation in fast velocity, fish did not prefer other substrates (woody debris, cobble, or clay terrace) in fast velocity. Fish avoided cobble and significantly avoided it in fast velocity. They also avoided woody debris and clay terrace in slow velocity, suggesting fish prefer a fast velocity environment with structure. There was a significant interaction between water velocity and substrate in all treatments except cobble (Fig 2).

Wintering yr-0 juveniles used vegetation and cobble significantly more at night than during the day (Fig. 3). Also at night wintering fish significantly avoided woody debris in fast velocity and clay terrace in slow velocity.

Wintering yr-0 juveniles tended to use structure, particularly vegetation and clay terrace, more in fast velocity. Comparing only the types of structure (not sand), juveniles used vegetation significantly more than cobble ($P = 0.015$, Fig. 4).
The greater use of structure (vegetation, woody debris, and clay terrace; Fig 2) in fast velocity suggests fish prefer a velocity of several body lengths/s with a velocity refuge in which to hold position, forage on drift, and conserve energy. Selection of a fast velocity habitat could enhance feeding on drift, even in the winter. The results suggest wild yr-0 juveniles select a wintering habitat in vegetation with a current speed > 1 bl/s.

Where is the place in the lower meandering reach of the Kootenai River where this vegetated habitat is abundant and most likely exists? Aquatic vegetation is present in the lower meandering reach of the river, but is not abundant anywhere (B. Kynard pers. obs.). Perhaps, it was more abundant historically before many river alterations that reduced productivity of the river (Anders et al. 2002). Presently, UW video observations on bottom habitat in the lower river and South Arm of Kootenay Lake found aquatic vegetation is abundant along the river delta and the South Arm (Kynard et al. 2008; unpubl. data). Thus, these areas seem most likely to provide both vegetation and current. However, if the historical wintering habitat was abundant vegetation in the lower river, particularly in the many small drainages that previously existed in the river delta, and this habitat does not now exist, there could be a mis-match between the evolved winter habitat preference of yr-0 juveniles and present habitat availability.

We cannot exclude the possibility that yr-0 juveniles were selecting vegetation in our tests but in the wild, another structure provides the velocity refuge, not vegetation. Although, our UW video surveys do not indicate this situation exists (i.e., there is no other abundant structure like vegetation), if it does, then the focus on vegetation to locate likely wintering sites is an error.

The study results indicated when yr-0 juveniles did not have access to vegetation in fast current, they used open sand in slow current. Open sand with slow velocity is the dominant habitat in the meandering reach of the river where depths are typically > 25-30 m. In these deep areas, we have used UW video to view wintering juveniles estimated at yrs-3-4. If small yr-0 juveniles are presently wintering in the same deep reaches with older juveniles, it seems likely that many are eaten by older juveniles during the long wintering period. Perhaps, yr-0 juveniles evolved a strong preference for current and vegetation as an adaptation to avoid habitat used by older juveniles. Limited observations indicated the older Kootenai River juveniles use slow-zero current and a sand substrate, much like older juvenile and adult shortnose sturgeon do in winter (Kieffer and Kynard in press).

Objective 3. Determine if and when juveniles aggregate during wintering.—For yr-0 juveniles, most fish observations were within the 95% confidence intervals (CIs) of the nearest neighbor random distribution simulation, and therefore, were not considered aggregated (Fig 5). However, some juveniles early in winter (October) and a few fish in December were more aggregated than would be expected by chance. The count of contiguous squares containing fish showed a weak, but significant, trend toward more aggregation in colder water (Fig. 6), suggesting aggregation behavior may change during a wintering season.
For yr-1 juveniles, almost all fish observations were within the 95% CIs of the nearest neighbor random distribution simulation, and as many observations showed less aggregation than expected by chance as showed more aggregation than expected by chance. Yr-1 juveniles did not show a trend for aggregation at any temperature (Fig. 7).

Yr-0 and yr-1 juveniles did not aggregate, but instead were solitary. If wild yr-0 and yr-1 juveniles do similarly, this behavior should reduce competition for rare food resources that may drift to their position. A solitary wintering behavior is also consistent with a habitat preference strategy to prefer current, which could bring a rare food item to the fish’s position. The adaptive significance of the solitary behavior may be related to reducing competition for the rare food. Other sturgeons aggregate during wintering (Kynard et al. 2002, Kynard and Parker 2004), so there is much to learn about wintering strategy of young sturgeons, including how aggregation or non-aggregation fits into a total wintering strategy.

**Objective 4. Determine activity level of yr-0 and yr-1 juveniles in relation to temperature decrease during late-fall and winter.**—We had enough fish for two replicates of 20 fish for yr-0 juveniles with temperature but only one replicate of yr-1 juveniles. The two replicates of yr-0 juveniles were combined because they were not significantly different (Fig. 8).

The number of gridlines crossed decreased with the decrease in water temperature during the fall. When tests began at 11°C, yr-0 juveniles crossed an average of about 60 gridlines and yr-1 juveniles crossed an average of about 80 gridlines (Fig. 9). As temperature decreased steeply to 1.6°C in late December, fish activity in both groups also decreased steeply to an average of 0 gridlines crossed at \( \leq 2^\circ C \). In both groups, activity was significantly correlated with water temperature (Figs. 10, 11).

Although fish activity did eventually decline to 0 gridlines crossed, this only occurred at temperatures \( \leq 2^\circ C \). At temperatures > 3°C, fish were still active in both groups of juveniles (Figs. 8, 11). Due to Libby Dam operations, temperatures in the Kootenai River at the Bonner’s Ferry gage only rarely go below 3°C in the winter (Fig. 12). Winter temperature in the lower meandering reach, delta, or Kootenay Lake, where yr-0 juveniles are most likely to winter is poorly understood. The energetic and impact on survival of these juveniles of staying active during winter, when there is likely little forage available, is not known. Historically, these life stages would have been inactive in water \(< 2 \,^\circ C \) for most of the winter.

**Objective 5. Practicality of using UW video to survey for wintering yr-0 & yr-1 juveniles in the Kootenai River and Delta.**—Fish released in 20 ft of water were difficult to distinguish from the bottom if they were stationary, so we quickly concluded that surveying was not likely practical unless a superior video system was used. The twelve juveniles released in shallow water could be distinguished from the bottom and could be tracked easily by vision from an observer in the boat, but it was difficult to see and track the fish with our equipment. As in deeper water, the problem observing fish was the video’s resolution and small field of view.
Video surveying to determine fish location or habitat association appears to work best with fish larger than 5 cm (Morrison and Carbines 2006, Schaner et al. 2009), fish at high density (Yoshida et al. 2010), or fish which have daily periods of inactivity or defend territories and are spending long time periods holding station (Morrison and Carbines 2006, Schaner et al. 2009). However, even in the winter, juveniles are small. Further, behavioral results from the present study suggest wild yr-0 and yr-1 juveniles are active, solitary fish that are likely associated with vegetation or other structure making video detection difficult or impossible.

Mounting video cameras on a bottom sled with a tickler chain is effective for surveying for flatfishes buried in the substrate (Spencer et al. 2005). This likely would not work well for sturgeons, which swim away quickly when disturbed, even during wintering (B. Kynard, pers. obs.). The only UW video technology that may be effective at seeing small juveniles on the bottom in winter is not the boat-mounted and tethered camera we tested, but a remote-controlled bottom rover with video that can survey close to the bottom in reaches with a low water current. However, even this device will have difficulty surveying for yr-0 and yr-1 juveniles in vegetation or other bottom structure, which the present habitat preference tests indicates is the preferred wintering habitat.

**Objective 6. Test hypotheses on imprinting and olfactory sensitivity of shortnose sturgeon.**—The study was not possible as the research scientist cooperating with us failed to bring his equipment to the Conte Lab. Without this equipment and his expertise, we could not perform the study.

We searched for another cooperator in 2009 and 2010, but found none. We returned the remaining funds to the Tribe in 2010.

**Objective 7. Determine how dispersing-foraging larvae-early-juveniles respond to flow fields created by bedforms simulating natural bedforms (rocks, sand dunes, wood limbs-vegetation, and sand) in the Kootenai River.**—In all four tank configurations, distribution of velocities at fish locations was significantly different from distribution of available velocities with fish in all configurations choosing slower velocities more often than expected \(P < 0.05\). A higher proportion of fish also chose slower velocities in tanks with structure than in the control tanks \(P < 0.01\), Fig. 13). The greatest proportion of larvae was in velocity < 0.15 cm/s.

Areas of the tank where fish concentrated also changed slightly depending on the type of bedform structure available. In the control tank, fish spread out throughout the bottom area (Fig 14), but they concentrated in the most downstream, slower velocity reach created by the ramp across the downstream end. Fish essentially avoided the upstream fast velocity reach of the control tank.

In the tank with the two sharp-crested baffle weirs (simulated sand dune flow fields), a few fish aggregated just upstream of the most upstream baffle in fast velocity, but 84% used the downstream baffle, particularly the large slow velocity area just downstream of it (62%; Fig. 15). Zero fish in control tanks used the fast upstream reach where the
upstream baffle was located, so placement of the upstream baffle in fast velocity did create a small slow velocity habitat that 5% of the fish used. This result suggests that if slow velocity bottom habitats ever are found to be limiting for larvae, management could easily create artificial habitat using a configuration similar to our baffle-weirs.

In the tank with pebbles, fish clustered at the downstream end of the group of pebbles (Fig. 16). Fish were also clustered in rocks on the inside, not the outside, of the curve.

In the tank with the simulated wood limbs laid across the channel and vegetation downstream, most fish clustered between the two pieces of PVC pipe or just downstream of the most downstream piece (Fig. 17). Few fish used the vertical pipes or vegetation.

The results found Kootenai sturgeon larvae and early yr-0 juveniles use velocity refuges created by benthic structures or bedforms when they pause to use the bottom during dispersal. Fish may be feeding on drift or on benthos, which is entrained or deposited in these slower current areas. They are also likely taking advantage of the velocity refuge to rest and conserve energy during the pause in dispersal swimming, which occurs above the bottom (Kynard et al. 2010).

Fish tended toward less downstream dispersal in tanks with a structural bedform than in control tanks, although this difference was not significant (Fig. 18). A previous study found larger patches of rock or larger rocks had a significant effect on dispersal with fewer fish dispersing per unit time in tanks with larger rocks than in tanks with small rocks (Kynard et al. 2008). The slow velocity provided by some bedforms (including large rocks) may provide more food, more refuge from velocity, or both, and thus, cause larvae to spend more time in these areas than in areas with no structure (smooth sand). In our tests, structures at a right angle to the current and horizontal orientation (not vertical) to the bottom provided the largest habitat area for larvae (Figs. 14–17). This suggests the abundant sand dunes in the meandering reach, which are mostly oriented across the river channel, may be a major source of foraging-resting habitat for dispersing larvae.

This study gives insight into how young Kootenai larvae and young juveniles interact with various bedforms during dispersal. The results also show the complex relationships between dispersing-foraging fish and substrate bedforms. Because artificial streams cannot duplicate the bedform-forage abundance occurrence that wild fish would encounter, it is not possible to use these studies to build a dispersal model for Kootenai sturgeon. Artificial stream studies are most useful for examining the effect of one or two factors at a time on fish behavior, which can be useful for modeling. However, in the case of foraging-dispersing Kootenai sturgeon, no artificial stream can adequately address all the interactions between environmental factors and sturgeons that occur in the river.

The study revealed the bottom habitat preference of young Kootenai sturgeon switches with ontogeny: larvae-early-juveniles in summer-early-fall prefer slow velocity (created by any structure) and wintering yr-juveniles prefer fast velocity in structure, particularly in vegetation. This is the only thoroughly studied example of this ontogenetic habitat switch by young sturgeons, and has major implications for identifying preferred
habitat types for these early life stages in the river and for management of Kootenai sturgeon.

As found in previous artificial stream studies (Kynard and Parker 2006, Kynard et al. 2007, 2008, 2009, 2010), larvae and early-yr-0 juveniles continued to disperse until observations ceased, in this study on day 81. This is many days after larvae developed into juveniles (about day 66), showing both life stages are dispersing. Thus, although the present stream environments differed from any previous stream configurations, fish dispersal was similar in length and in having peaks and valleys (Kynard et al. 2010).

During ontogeny, fish became more benthic with increased age (Fig. 19). The majority of days 20-40 larvae spent almost all the time swimming and dispersing above the bottom. But during days 40-50, most fish switched and typically only a maximum of 20% were in the water column, with the remainder on the bottom. After day 50 is the time period when late-larvae were developing into juveniles. This result is consistent with previous artificial stream observations suggesting a similar increase in benthic use with age of dispersing larvae, and also, supports the long dispersal of Kootenai sturgeon larvae and early-juveniles (Kynard et al. 2007, 2008, 2010) that would take fish to the lower meandering reach or to Kootenay Lake in Canada.

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Literature cited


Fig. 1. Plan view of artificial stream tank (5.7 m circumference, 28 cm wide channel, 20 cm deep water) used to observe larvae and yr-0 juveniles for selection of bottom habitat. The two X squares at the upstream end of the two test habitats (grey color) are small electric propellers that generated a fast to slow velocity gradient in each test habitat. Placement of a substrate type on the sand bottom created four major habitat areas for that substrate: A = (fast velocity over sand and slow velocity over test substrate) and B = (fast velocity over test substrate and slow velocity over sand). Test substrates were vegetation, cobble rocks, woody debris, and clay terrace. I inside circle = inflow water source; o inside circle = drain. Grey area = test habitat where fish were observed by video.
Fig. 2 Proportions of larvae or juvenile observations in each habitat type during daytime based on direct visual observations. Data are mean proportion of observations in each habitat type and bars are 95% confidence intervals (CIs). A line is included at 25% for the proportion expected if habitat use is random; CIs that do not include this line indicate significant preference or avoidance. No bars are presented for woody debris and clay terrace in summer as these data were based on only two groups. Results (p-values) of two-way ANOVA are presented with: S = substrate effect, V = velocity effect, and I = interaction.
Fig. 2

Proportion of observations

Winter

Summer

\( S_p = 0.89 \)
\( V_p = 0.001 \)
\( L_p < 0.001 \)

\( S_p = 0.25 \)
\( V_p = 0.74 \)
\( L_p = 0.11 \)

\( S_p = 0.064 \)
\( V_p = 0.57 \)
\( L_p = 0.99 \)

\( S_p = 0.14 \)
\( V_p = 0.37 \)
\( L_p = 0.08 \)

\( S_p = 0.06 \)
\( V_p = 0.43 \)
\( L_p = 0.03 \)

\( S_p = 0.30 \)
\( V_p = 0.56 \)
\( L_p = 0.30 \)

\( S_p = 0.05 \)
\( V_p = 0.93 \)
\( L_p = 0.02 \)

\( S_p = 0.54 \)
\( V_p = 0.32 \)
\( L_p = 0.25 \)
Fig 3. Proportions of yr-0 juvenile observations in each habitat type day and night in winter based on video observations. Data are mean proportion of observations in each habitat type and bars are 95% CIs.
Fig 4. Number of observations on wintering yr-0 juveniles per day in each habitat type (fast velocity only). Habitats sharing a letter were not significantly different. Bars are SE.
Fig 5. Nearest neighbor distances (cm) for wintering yr-0 juveniles during October–December. Upper panel shows means (± SE); other two panels show individual observations. Dashed upper and lower lines are 95% CIs from 1000 computer simulations; observations with a mean nearest neighbor distance < these CIs are considered aggregated. Replicate tanks 1 & 3 are displayed separately.
Fig 6. Linear regression of the number of contiguous grid squares in tank containing yr-0 juveniles and water temperature. $R^2 = 0.148, P = 0.047, y = -0.129x + 6.380$ indicating a weak (but significant) behavioral tendency to aggregate more at colder temperatures.
Fig 7. Nearest neighbor distances (mm) for wintering yr-1 juveniles. Upper panel shows means (± SE); lower panel shows individual observations. Upper and lower dashed lines on panels are 95% CIs from 1000 computer simulations; observations with a mean nearest neighbor distance less than these CIs are considered aggregated. Confidence intervals increase in December as the number of fish in the tank was reduced from six to five fish.
Fig 8. Number of gridlines crossed by yr-0 juveniles as temperature decreased in each replicate test, showing great overlap and NS difference between replicates.
Fig 9. Seasonal trend in activity as indicated by the mean number of gridlines fish swam across during decreasing water temperature, October–December. Regression of number of gridlines crossed and temperature was highly positively significant ($R^2 = 0.790; P = 0.0001; y = 9.112x – 22.914$).
Fig 10. Activity of yr-0 wintering juveniles during decreasing temperatures as indicated by the linear regression of the number of gridlines fish swam across and water temperature, October–. Regression was positive and highly significant ($R^2 = 0.904; P = 0.0001; y = 6.666x – 6.681$), showing a strong relationship between wintering activity and temperature.
Fig 11. Linear regression of the number of gridlines yr-1 juveniles swam across and water temperature, October–December. $R^2 = 0.790; P = 0.001; y = 9.112x - 22.914$, showing a significant positive relationship between activity and temperature.
Fig 12. Water temperature in the Kootenai River from 2006 to 2009 measured at the Bonners Ferry USGS gage. Note water temperature rarely and only briefly decreases to 2 °C.
Fig 13. Histograms of larval use vs. availability for bottom velocities present in the various bedform treatments.
Fig. 13

Control

- Fish locations (n = 853)
- Available

Rocks

- Fish locations (n = 682)
- Available

Shapes

- Fish locations (n = 431)
- Available

Baffle-Weir

- Fish locations (n = 683)
- Available

Proportion of observations

Velocity (cm/s)
Fig 14. Plan view of sand-only (no bedform) control tank showing the distribution of larvae to early-juveniles (days 14–80 fish). The area in the channel surrounded by the solid line delineates a concentration of fish and the number inside represents the proportion of total fish in the tank observed in that area.
Fig 15. Plan view of tank with straight-crested baffle-weirs (simulated sand dunes) showing the distribution of larvae to early-juveniles (days 14–80 fish). The areas in the channel surrounded by the solid line delineate a concentration of fish and the number inside represents the proportion of total fish in the tank observed in that area.
Fig 16. Plan view of tank with pebbles showing the distribution of larvae to early-juveniles (days 14–80 fish). The area in the channel surrounded by the solid line delineates a concentration of fish and the number inside represents the proportion of total fish in the tank observed in that area.
Fig 17. Plan view of tank with simulated wood limbs and vegetation showing the distribution of larvae and early-juveniles (days 14–80 fish). The areas in the channel surrounded by the solid line delineate a concentration of fish and the number inside represents the proportion of total fish in the tank observed in that area.
Fig 18. Daily mean number of larvae and early-juveniles moving downstream in each of the four bedform configurations.
Fig 19. Proportion of time larvae and early-juveniles were on the bottom of artificial streams with bedforms with age during visual observations. Fish were typically almost always on the bottom or always off the bottom, therefore the proportion that were on the bottom for > 95% of the time are given as well as the proportion that were off the bottom for > 95% of the time. The remaining proportion is in “Other”.