

## Behavior of early life intervals of Klamath River green sturgeon, *Acipenser medirostris*, with a note on body color

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### Synopsis

We studied ontogenetic behavior, migration, and wintering behavior of young Klamath River green sturgeon, *Acipenser medirostris*, in the laboratory to provide insight into likely behavior of wild sturgeon. Hatchling free embryos preferred cover but were poor swimmers and could not move farther than a few centimeters to cover. The poor swimming ability and cover preference of hatchlings suggests evolution for habitat selection of females to place eggs in habitat with cover for eggs (and hatchlings), and for egg characteristics (large, dense, and weakly adhesive) to cause rapid sinking into cover without drifting. A day or so after fish developed into larvae (first life interval feeding exogenously), day-12 larvae initiated a 12-day downstream nocturnal migration. A totally nocturnal migration is unlike other *Acipenser* migrants yet studied. Migrant larvae had a dark-colored body typical of other *Acipenser* species that migrate as larvae. Tail color was a dark black (black-tail phenotype) only during the early larva period, suggesting a morphological adaptation for migration, foraging, or both. Post-migrant larvae and early juveniles to day 84 foraged diurnally with a nocturnal activity peak. Day 110–181 juveniles moved downstream at night until water temperature decreased to about 8°C, indicating wild juveniles migrate downstream to wintering habitat. Habitat preference of month 9–10 wintering juveniles suggests wild juveniles are in deep pools with low light and some rock structure. Wintering juveniles were only active at night. Initiation and cessation of daily activity was at dusk and dawn during illumination changes of < 1.0 lx. This sensitivity to illumination has not been found before in sturgeons. During the first 10 months of life, nocturnal activity of early life intervals is a dominant feature of migration, foraging, and wintering.

### Introduction

Green sturgeon, *Acipenser medirostris*, is an anadromous sturgeon species with a maximum size of 2.1 m fork length (FL) and 159 kg body weight (Lee et al. 1980, Moyle et al. 1994, Moyle 2002). The species inhabits the Northeastern Pacific biogeographic province, i.e., rivers of the United States, Canada, Russia, Korea, and Japan (Bemis & Kynard 1997). Abundance of green sturgeon has decreased and many populations are protected (Birstein 1993, Moyle 2002). Small populations

still occur in the Sacramento and the Klamath-Trinity Rivers in California, in the Rogue River, Oregon, and possibly in the Columbia River, Oregon (Moyle et al. 1994, Moyle 2002).

Green sturgeon life history in fresh water is among the least understood of any sturgeon species in North America. Spawning occurs during mid-April to mid-June in the Klamath River (Moyle 2002) and in June–July in Russia (Art-yukhin & Andronov 1990). Capture of a larva in drift nets at river km (rkm) 317 in the Sacramento River shows that adults move far upriver to spawn

(Kohlhorst 1976). Spawning in the Klamath River likely occurs at several mainstem locations <100 km, and in a tributary, the Trinity River (Moyle 2002, Scott Turo personal communication). Recent tracking in the Rogue River found adults spent longer in fresh water than previously thought (Erickson et al. 2002).

Some information on behavior of early life intervals with life history implications was gathered during fish culture. Green and white sturgeon, *A. transmontanus*, differ in egg size, i.e., green sturgeon eggs are large (4.2–4.5 mm diameter), white sturgeon eggs are average-size (3.4–3.6 mm diameter), and hatchlings differ in swim-up behavior (white sturgeon swim-up, green sturgeon do not; Van Eenennaam et al. 2001, Deng et al. 2002). Asian green sturgeon also do not swim-up after hatching (Artyukhin & Andronov 1990), suggesting this behavior is widespread within the species.

Field collections and laboratory studies indicate that early life intervals of anadromous and amphidromous sturgeons remain in fresh water and juveniles (months 9–12) are the youngest life stage to enter salt water. The smallest juvenile green sturgeon captured in the Sacramento River estuary during extensive surveys was 20–22 cm FL, a yearling or almost so (Radtke 1966). Three small juveniles (lengths unknown) were also captured in the Suisun Marsh, San Francisco estuary (Matern et al. 2002). Cultured Klamath River yearlings in a large endless stream moved downstream at night during July–November, suggesting wild yearlings have an innate drive to migrate downstream to the estuary (Kynard et al. in press).

Factors that affect the survival of sturgeon early life intervals are poorly understood, yet the first weeks of life are the period when most mortality occurs for each generation (Gross et al. 2002, Parsley et al. 2002). To evaluate effects of natural and anthropogenic factors on survival of early life intervals, it is important to understand ontogenetic behavior, particularly migration, habitat preference, and initiation of foraging. It is possible to capture early life intervals in rivers, but it is impossible to study most behaviors because the small fish are in large rivers during moderate to high flows and turbidity. Thus, a laboratory approach is the only method to study ontogenetic behavior. Ontogenetic behavior is largely innate;

thus behavior in the laboratory can provide insight into likely behavior of wild fish (Brannon 1972, Taylor 1990, Kynard & Horgan 2002, Kynard et al. 2002a, in press, Kynard & Parker in press).

Recent comparative research on ontogenetic behavior of many *Acipenser* species found each goes through major behavioral changes during the free embryo, larva, and juvenile intervals (Richmond & Kynard 1995, Gisbert & Willot 1997, Kynard & Horgan 2002, Kynard et al. 2002a, b, 2003, Zhuang et al. 2002a, b). The free embryo interval (hereafter, embryo) begins at hatching and lasts until the larva interval, when exogenous feeding begins, and the juvenile interval begins when fish resemble adults (Balon 1999). Field sampling of 10 sturgeon species from three continents confirmed that the directed downstream movement of young sturgeon in a laboratory stream reflects a downstream migration by wild fish (Kynard & Horgan 2002, Kynard et al. 2002a, Zhuang et al. 2002a, b; Gisbert & Ruban 2003, Kynard & Parker in press, Kynard et al. unpublished data).

To make a conceptual model of the ontogenetic behavior of green sturgeon to compare with other *Acipenser* species, we repeated the same behavioral tests used for the other species. Tests examined migration, diel behavior, and habitat preference. We also noted the body and tail color of life intervals to compare with other sturgeons.

## Methods

We conducted tests on Klamath River green sturgeon during 2000 and 2002 using 200 fertilized eggs yearly that hatched on 18 May 2000 and 4 May 2002. Eggs were from one female and fertilized by several males. Fertilized eggs were shipped to us (see Acknowledgements) and we reared and tested fish at the S.O. Conte Anadromous Fish Research Center. We reared eggs in McDonald hatching jars and transferred hatchlings to rearing tanks. Early larvae were fed a starter diet (see Acknowledgements) 6–8 times daily using a timed feeder and four times daily with live *Artemia* nauplii. We used dechlorinated city water (Montague, Massachusetts) for rearing and all experiments, except for the wintering study. Temperature in rearing and test tanks was similar

( $\pm 1^\circ\text{C}$ ). The photoperiod was natural and similar to that of the Klamath River.

We used the number of days post-hatching to characterize age of fish, and to link daily behavior and development, we scaled fish development to the daily cumulative water temperature. We used the mean hourly temperature ( $^\circ\text{C}$ , temperature recorded each 15 min) in the stream tank to calculate the mean daily temperature. The mean daily temperatures were sequentially totaled to obtain the total cumulative temperature units (CTU) for each day post-hatch. For example, day-0 fish accumulated 0 degree days of temperature, day-1 fish accumulated the mean temperature for day 1; day-2 fish accumulated the degree days of day 1 plus the mean temperature on day 2. The 24-h cycle for calculation of CTU began at 24:00 h of each day, not at 12:00 h as was done by Kynard et al. (2002a, b). This procedural change was needed because 24:00–24:00 h, not 12:00–12:00 h, best included the first 24 h of development by green sturgeon.

At rearing temperatures of 15.5–18.5 $^\circ\text{C}$  for green sturgeon cited by Deng et al. (2002), fish develop into larvae on day 10 after about 176.5 CTU, and develop into juveniles on day 45 after about 824 CTU. We calculated these CTU values using the daily mean temperatures of Deng et al. (2002) to compare with our data, which uses CTU to characterize development. Temperature in our tanks was slightly higher (17.4–23.5 $^\circ\text{C}$ ) than the temperatures used by Deng et al. (2002). For body color characterization we used Hype's Color for Netscape v. 3.

In habitat preference tests, we randomly selected fish from 200 rearing tank fish, tested fish singly, and returned all to the rearing tank. No individual was tested twice in a replicate, but there was a chance a fish could be tested in other tests. The haphazard selection process should provide a group of fish with a set of random tests.

#### *Illumination and substrate color preference*

Aquaria used in illumination and substrate choice were two 20-l rectangular glass tanks with black plastic covering the four sides to exclude outside light. Two 20-W fluorescent lights with light diffusers were placed above the tanks to provide light. A black cover over one-half of the illumi-

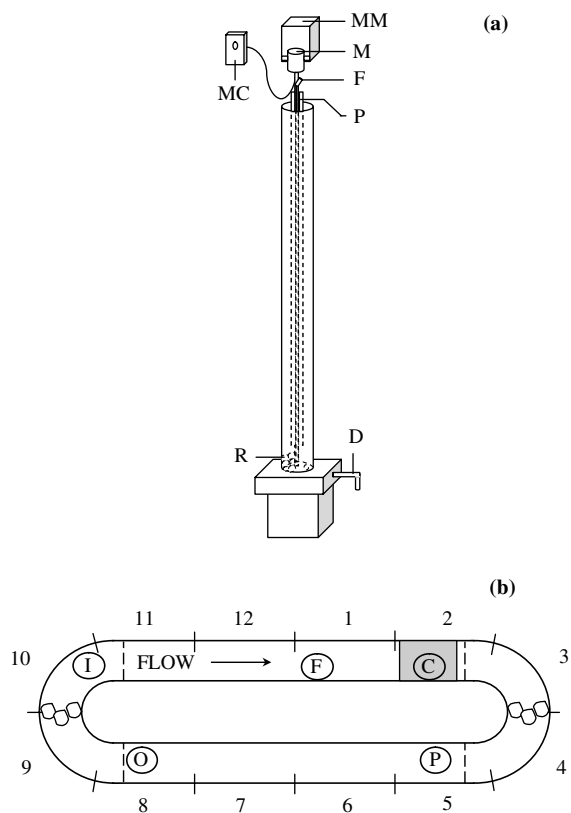
nation aquarium's top divided the tank into equal areas of bottom illumination (8.2–3.0 lx) and dark (2.2–0 lx) over a uniform tan bottom. The bottom of the substrate tank was divided equally by black and white squares. Underwater illumination intensity just above the bottom was: 4.3–2.6 lx (white) and 3.3–3.0 lx (black). Habitat position was reversed after each fish to avoid side bias of fish.

For illumination preference, we daily tested fish in 2000 ( $n = 5$ ) and in 2002 ( $n = 8$ ), and for substrate preference, we tested fish in 2000 ( $n = 5$ ). We mixed rearing tank fish and selected test fish by beaker brailing, placing all test fish in a bucket. We tested fish singly, placing each at the water surface in the tank's center. After 1 min, we continuously observed fish movement for 1 min (illumination test – dark vs. illuminated; substrate test – white vs. black). We calculated the percent of time each fish spent in illuminated or white habitat and the daily mean percent of time all fish spent in this habitat, and plotted this as a daily time series. We transformed the daily percentages to arcsine values and calculated binomial 95% confidence intervals to determine if the percent of time fish spent in the selected habitat was significant (confidence intervals that included 50% were not significant).

#### *Swimming height above the bottom*

In 2000, we daily tested eight fish for swimming height above the bottom in a stream tube that simulated a vertical section of stream with horizontal water flow, like in a natural stream (Figure 1a). Underwater illumination measured inside the tube (top to bottom) was 300–50 to 30–5 lx depending on time of day. The tube was partially drained after each test to remove fish, replace water, and maintain water temperature.

We captured fish for daily tests like during illumination tests. A single test fish entered the bottom of the tube after transport down the introduction tube. Upward swimming and cover seeking were noted for 1 min (acclimation period); then at 5–6 min, we visually recorded swimming height of fish each 10 s for 60 s (total measurements = 7) using a depth scale (1-cm marks with 0 = bottom) on the outside of the tube. We calculated the mean of the seven measurements for



**Figure 1.** Panel a shows the stream tube (water depth, 150 cm; diameter, 15 cm) used to determine swimming height and cover use of green sturgeon. Key to components: M = motor, MM = motor mount, MC = motor control, F = fish introduction tube, P = paddlewheel, R = rocks, and D = drain. The rotating paddlewheel created horizontal water current with a velocity of  $2\text{ cm s}^{-1}$ . Panel b shows the oval stream (32 cm wide, 7.3 m circumference, water depth = 20 cm) cover at each end with three rocks for used to observe migration and habitat use. The stream circumference was marked at 62 cm intervals dividing it into 12 sections. Dashed lines are seams. Mean bottom velocity was  $3.5\text{ cm s}^{-1}$  (range,  $1\text{--}9\text{ cm s}^{-1}$ ). Feature notations are: flow direction = arrow, water inflow = I, water outflow = O, submerged pump = P, feeder = F, and video camera and infrared light = C. The shaded area in section 2 is the ramp and camera field of view.

each fish and present the grand mean for all fish as a daily time series.

#### *Migration and diel activity*

We observed up- and downstream movements of sturgeon in an oval endless stream tank colored green-blue (Figure 1b). Underwater light intensity

was 20 lx (maximum). The water in- and outflow was  $11\text{ min}^{-1}$  of dechlorinated city water. Water temperature was  $17\text{--}23^\circ\text{C}$  and was monitored each 15 min with a data-logger. Three rocks (each 10–15 cm diameter) at each turn provided cover. The ramp reduced water depth to 13 cm, placing fish close to the camera. Fish were observed with a Cohu camera and infrared (IR) light over the stream to see fish at night (Figure 1b). Silver reflective tape covered the ramp and sides of the stream in the video field of view to enhance seeing fish at night. We recorded fish for 5 min per hour each 24 h, then reviewed the videotapes and counted the number of up- and downstream fish passes for every other hour.

We introduced 15 hatchlings into the stream in 2000, but the video system failed. We visually counted the number of fish moving up- and downstream past section 2 about mid-day during days 0–34.

In 2002, we introduced 15 day-0 embryos into the stream and viewed fish with the video system previously described. Few fish died during observations, but we replaced dead fish so there were 15 in the stream on all days. After day 56, we videotaped the same 15 juveniles for 48 h each 4 weeks until day 154 to determine if juveniles migrated during summer or fall. We present the mean number of day and night up- and downstream fish passes during each day as a time series.

During 6–16 November 2000, we determined the movements of day 171–181 juveniles in Connecticut River water. The number of fish observed was 30–50, so we scaled the number of fish passes to the number of fish present (number of fish passes divided by the number of fish). We plotted the daily number of day and night up- and downstream fish passes with daily water temperatures and day length as a time series.

#### *Habitat preference*

During 2000, we recorded habitat use in the stream tube and in the oval stream, and during 2002 we tested embryos for choice of cover vs. open. In stream tube tests, one-half of the bottom was covered with two layers of gray rocks (5 cm diameter); the other one-half was open. We recorded the number of eight fish using four habitats: water column, swimming on open bottom, resting on open bottom, and in rocks. For each

day, we determined the total percent of fish using each habitat.

In the oval stream, we visually counted the number of fish in four habitats five times each day: on the bottom under rocks, on the bottom in the open, in the water column (>3 cm above the bottom), and at the water surface. We calculated the grand total of fish using each habitat type during each day, converted this number to percent of total fish in each habitat, and present the percent in each habitat as a daily time series.

Day 1–4 embryos were tested for choice of cover vs. open in a 20l aquarium like the one used for substrate tests. Rocks (5 cm diameter) placed around the tank wall created equal areas of open and cover on the bottom. We introduced a single fish into the center of the open area about 5 cm from rocks, and without acclimation, for 2 min recorded the number of seconds fish spent in the open vs. cover.

#### *Juvenile wintering behavior*

During February–March 2001, we tested month 9–10 juveniles for preference of substrate texture, substrate color, and structure, and determined use of illuminated habitat. The substrate and structure choice tests used section 12 of the oval stream (Figure 1b), which was divided at mid-length to form two 22 cm wide × 30 cm long test areas. Barrier screens isolated test fish in this section, but permitted water to flow through. Test #1 gave fish ( $n = 20$ ) a choice of dark (area covered by black plastic) vs. illumination (area with ambient underwater illumination of 7.0–8.0 lx). Fish choosing the illuminated area had a further choice of open (not touching structure) vs. structure (body touching a wall or brick). The bottom was smooth in these tests. For substrate preference tests, a tray in each test area contained a different substrate. Test # 2 gave fish ( $n = 10$ ) a choice of white sand (2–3 mm diameter) vs. white coarse gravel (5–10 mm diameter) and test # 3 gave fish ( $n = 15$ ) a choice of black sand vs. black gravel (same size as for white color). Test # 4 tested fish ( $n = 20$ ) choice of white vs. black sand. In all tests, we introduced a single fish into the middle of the two habitats, and with no acclimation, recorded fish for 15 min with video (fish in tests using all black

substrate were observed visually). After one-half the fish were tested, position of the two habitats was reversed to control for side bias. For all tests, we reviewed tapes and determined the percent of total seconds fish spent on each habitat type. Total percent of time on a habitat was transformed to arcsine values and binomial 95% confidence intervals were used to determine significance (confidence intervals that included 50% were not significant).

We examined preference of wintering juveniles for illuminated vs. dark habitat and determined diel activity in a large rectangular tank (2.4 m long × 0.7 m wide with water 16 cm deep) (Henyey 2002). Eight pumps, positioned equidistant apart along the length of the tank, created a directional flow across the length of the tank, like in a stream. Water velocities were 0–14  $\text{cm s}^{-1}$ , with the highest velocities along the inflow wall. Water was Connecticut River water at ambient temperature (3–4 °C). The tank's walls and bottom were opaque and a black plastic curtain around the perimeter of the tank excluded outside light. One-half of the tank's top was covered lengthwise with black plastic to create total darkness (0 lx) and the other one-half was illuminated by a mixture of natural and artificial light (<2.5 lx underwater). The natural photoperiod was maintained by setting on and off times of the artificial light to fall within the natural period of daylight. Underwater light intensity was recorded each 15 min by a datalogger with the sensor centered on the bottom of the illuminated section.

During each of six 24-h tests, we tested groups of five fish (total, 30 fish), introducing each group into the tank at 1400 h. A fish only participated in one replicate. Movement of fish on the illuminated side of the tank was recorded using a video system for the first 5 min of each hour, 24 h per day. An overhead IR light was used with the camera to see fish during the night. Fish had no startle response when the IR light was turned on or off. During review of videotapes, we counted the total number of fish on the illuminated side of the tank during 5 min per hour and determined the mean number of fish on the illuminated side per hour for 6 days. The data is a 24-h time-series of mean number of fish on the illuminated side each hour and the mean hourly light intensity (lx).

## Results

Body color of day-1 embryos on 19 May 2000 was light gray (gray 81). The tail developed a dark black color by day 9. Fish developed into larvae on day 11 (CTU = 175.5). On day 14, body color was a uniform medium gray (gray 31) and the tail was still dark black (black-tail phenotype).

On 4 May 2002, day-0 hatchling body and tail color was a light gray (gray 81). Body color of embryos on all days was light gray, but by day 3, the tail was dark gray (gray 11). The tail was dark black on day 7, i.e., the black-tail phenotype. On day 9 (CTU 174.1), fish were larvae. Early larva body was a dark gray (gray 31) with black-tail. On day 16, the tail was a dark gray (gray 11) and only the tip was black. On day 23, the body was a medium gray (gray 31) as was most of the tail (only the tip was black). By day 30, tail and body color were medium gray.

In 2002, day-0 embryos were 13.5 mm total length (TL,  $n = 2$ ) and day-9 larvae were a mean of 25.5 mm TL ( $n = 2$ ). The CTU per mm of growth to day-9 larvae was  $174.1 \text{ CTU} \div 25.5 \text{ mm TL} = 6.8 \text{ CTU per mm TL}$ . Day-32 late larvae were a mean of 47 mm TL ( $n = 2$ ). On day 40, fish were exposed to 829.9 CTU and were early juveniles.

### *Illumination and substrate color*

During 2000, day 0–8 embryos showed great variability for response to illumination, and fish did not prefer illuminated or dark habitat (Figure 2a). As embryos developed into larvae, fish briefly strongly preferred dark habitat on day 9 and days 11–14, then larvae showed a trend with age to day 35 for no preference. Day-42 early juveniles were similar to late larvae with no preference for illuminated vs. dark habitat.

In 2002, embryos preferred dark habitat on all days, except on day 0 and day 5 (Figure 2b). This significant preference for dark continued after day 6 with a few exceptions when fish developed into larvae (day 9) and continued to day 19. Like in 2000, the trend in older larvae was no preference for either habitat. Juveniles on day 40 showed no preference for illuminated vs. dark habitat.

During substrate tests, embryos used black substrate more than white substrate, but only day

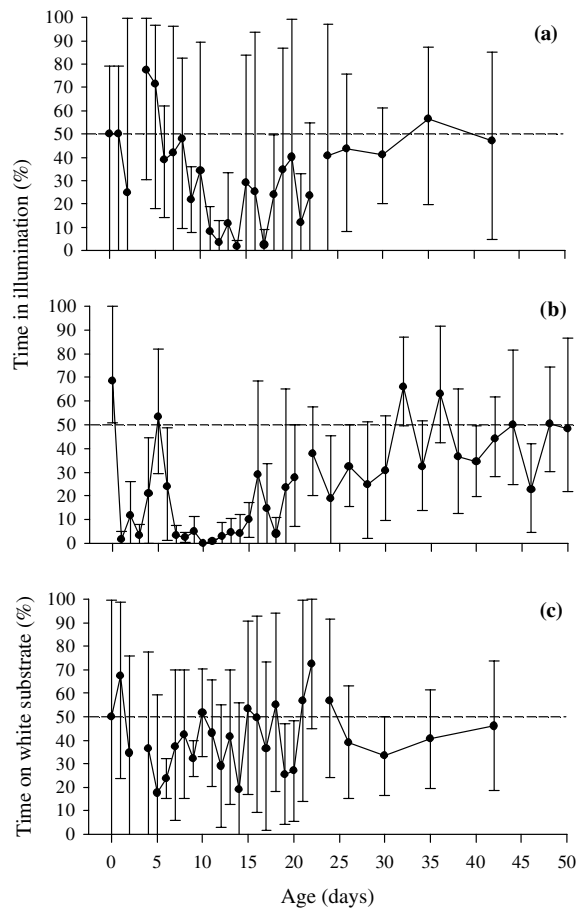


Figure 2. Mean percent of time ( $\pm 95\%$  CI) green sturgeon spent on the illuminated side of the illuminated vs. dark tank (panels a and b), and on the white side of the white vs. black substrate tank by age (panel c). Panel a shows fish observed in 2000 ( $n = 5$  per day), panel b shows fish observed in 2002 ( $n = 8$  per day), and panel c shows fish observed in 2000 ( $n = 5$  per day). Significant preference = 95% CI does not include 50% (dashed line).

6 and day 9 embryos had a significant preference for black color (Figure 2c). Day 9 and older larvae also spent more time on black than white, but preference for black was not significant, except for day 19–20 larvae. Day-42 juveniles spent more time on black than white, but the preference was not significant.

Poor swimming ability was a dominant behavioral feature of early embryos. The lack of a significant preference for dark habitat by day-0 embryos in choice tests during both years was the result of fish immobility and not preference. Other

observations show a strong preference of hatchlings for dark habitat (see Habitat Selection section).

Results from illumination tests show that tests with eight fish produced significant results, whereas tests with five fish did not. Eight fish, or even 10 fish, are needed to show a significant preference of young sturgeon for habitat when behavior is variable.

#### *Swimming height*

Single day 0–4 embryos introduced into the stream tube in 2000 lay on the bottom, making no effort to swim above the bottom, so we did not test 8 fish per day until day 5. Day 5–10 embryos tested daily swam around the bottom 0 cm high. Day 11–14 larvae tested daily were like embryos and none swam higher than 0 cm. Of the 56 larvae tested during days 15–21, only 10 fish swam above 0 cm, but they indicate a slight trend for increasing numbers of fish to swim above the bottom. The day and number of fish swimming above the bottom (in parenthesis) follows: day 15 (1), day 16 (1), day 17 (1), day 19 (3), and day 21 (4). Mean swimming height of the 10 larvae was 13.7 cm (range, 0.3–79.4 cm), so fish were still near the bottom.

Observations on swimming height in the previous paragraph do not provide information on swimming height at night, when the larval migration occurs (see Migration Section). Video observations in 2002 of migrant larvae in the stream found fish were mostly on the bottom, but some swam to the surface, showing they would swim higher if possible. Many of the migrants remained in contact with the wall, suggesting a strong drive by many fish to remain in contact with structure.

#### *Migration and diel behavior*

During 2000, visual observations found zero embryos moved up- or downstream during the daytime of days 0–10. Fish were under rocks. Day 11–18 larvae were also mostly under rocks during the day and none moved up- or downstream. There was a trend with age for larvae to leave rocks in the day, i.e., between days 19 and 25 about 50% of the fish were in the rocks and after day 25, only about 25% were in rocks. Visual observations

found no up- or downstream movement of larvae during the day.

During video observations of fish in 2002, embryos did not move up- or downstream during the day or night (Figure 3). When fish developed into larvae on day 9, none migrated, but day-10 larvae initiated a nocturnal downstream migration. The migration gradually peaked on days 14–18 (5 days) and ended on day 21 (12 day migration). During downstream migration, many fish also moved upstream (about 30% of the number of downstream fish passes). Upstream movement occurred when migrants swimming headfirst downstream turned and moved upstream, foraging on the bottom as they went. These foraging bouts would continue until fish were out of sight of the video. Thus, migrants interrupted downstream movement with foraging bouts. Migrants moved downstream with faster than the water speed, but moved upstream very slowly while foraging. Downstream movement occurred on the bottom; foraging occurred on the bottom, tank sides, or water surface. Fish did not forage when moving downstream. During migration there was no daytime movement, and fish hid in rock cover during the day (see Habitat Section). After the larval migration ceased on day 21, for a few days of

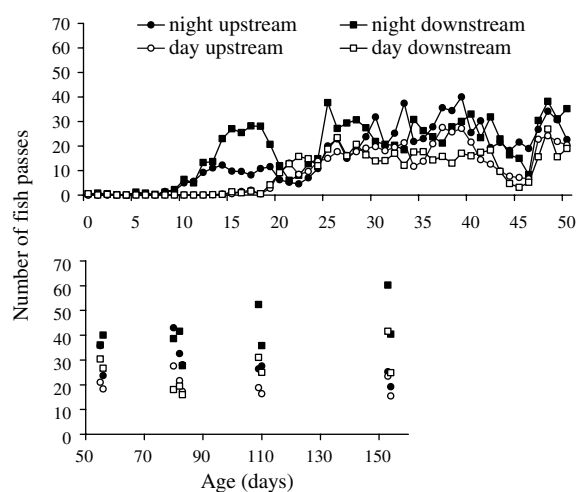


Figure 3. Number of up- and downstream passes of green sturgeon ( $n = 15$ ) in the oval stream during the day and night by age. A video camera recorded up- and downstream fish passes during 5 min per hour, 24 h each day, and we counted the passes every other hour. Daily means were calculated from day and night hourly totals.

transition, most up- or downstream movement was during the day. Then from day 25 to day 85, fish moved up- and downstream mostly at night. Behavior of fish indicated they were foraging, i.e., fish swam slowly, searching and looping, with no fast, straight, directed up- or downstream movement. Day 109–110 (22–23 August) and day 153–154 (4–5 October) juveniles showed a trend for increased downstream fish passes with a peak at night. The downstream movement was similar to the larval migration with directed downstream movements interrupted by upstream foraging.

Day-171 juveniles on 6 November 2000 in the stream had a peak in daily movement that was nocturnal and downstream (Figure 4). This was similar to the nocturnal downstream movement peak by day 153–154 juveniles in 2002 (Figure 3). During the 10 days we observed fish, day and night downstream movement decreased about one-half, water temperature decreased from 9.8 to 8.1°C, and day length decreased from 10 h 6 min to 9 h 44 min (22 min decrease in 10 days). However, upstream movement during the day and night did not change much during the 10 days. During downstream movement, fish swam close to the bottom, not high in the water column, which was similar to larvae. Observations ceased at 8.1°C, just before juveniles exhibited wintering behavior, i.e., daytime movement ceased and fish aggregated in a pile if no dark habitat was present.

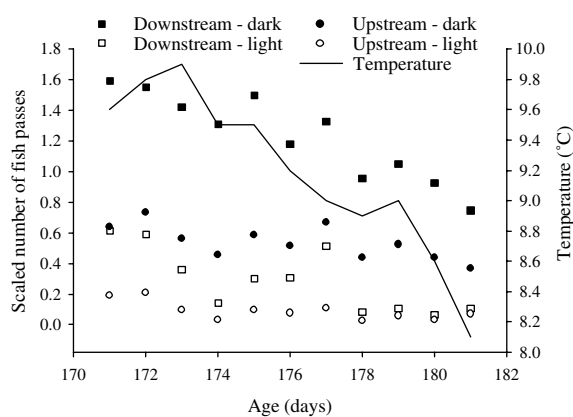


Figure 4. Scaled number of fish passes by day 171–181 juvenile green sturgeon in the oval stream as water temperature and day length decreased. Day length decreased from 10 h 6 min to 9 h 44 min (22 min total) during the 10-day test (6–16 November 2000).

### Habitat preference

During cover vs. open preference tests, day 1–4 embryos placed 5–6 cm from cover strongly preferred cover on days 1–3 (8 of 8 or 7 of 8 test fish preferred cover). Day-4 embryos moderately preferred cover (6 of 8 fish in cover and two fish had no preference). Day 1–3 embryos moved the 5–6 cm to cover in a mean of 1.0–7.8 s (range) and day-4 fish moved in a mean of 23.2 s.

In the stream tube, 95–100% of the day 5–10 embryos and day 11–15 larvae swam directly into the rocks and remained. A rare embryo or larva rested or swam on the open bottom. During days 16–21, the percent of larvae in the water column increased to 25% (day 18) and 35% (day 21) and the percent on the open bottom increased to 12%.

In the oval stream, the 15 day 0–1 embryos introduced during the day into section 1 (about 1 m upstream from rocks) lay immobile on the bottom, did not fan their tails, and resembled dead fish (Figure 5). Fish seldom moved at all, and fish only weakly swam a few centimeters before stopping. Even when prodded, fish only weakly moved a few centimeters. By day 2, 12 of 15 (80%) were in the rocks and by day 3, 100% were in rocks. Thus, it took 96 h for all 15 embryos to move 1 m to cover. Fish moved into cover at night. Embryos

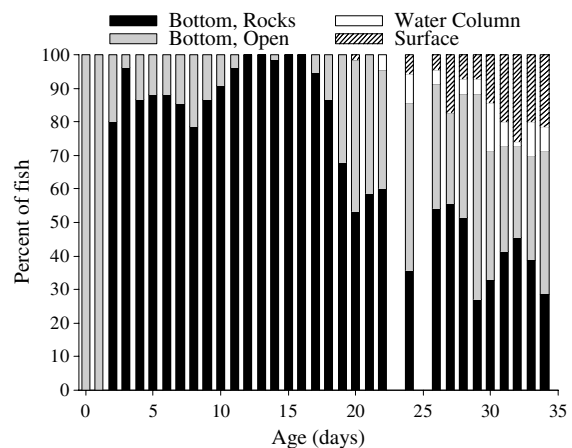


Figure 5. Percent of fish using four habitats in the oval stream by age. Total number of fish in the stream was 15. The daily percentages of fish in each habitat were calculated from the total number of fish in each habitat during five daily observation periods.

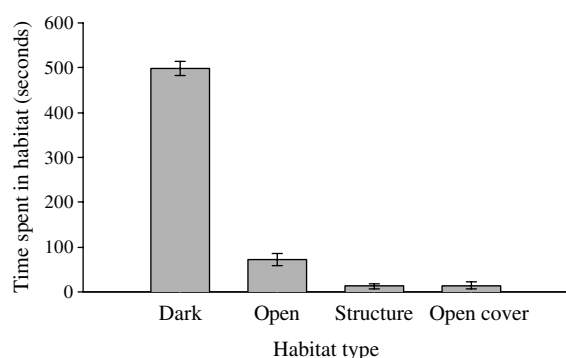


remained in the rocks or briefly swam between rocks on the open bottom until day 10 (Figure 5). Day 11–16 early larvae behaved similarly to embryos and remained in the rocks. Early larvae often had their heads under rocks and fanned their tails. After day 17, the trend with age of larvae was increased swimming on the open bottom and swimming in the water column, even to the surface. On day 34, 25% of the larvae were in the rocks, so larvae continued to use cover during the day.

#### *Wintering habitat and diel activity*

Day 290–305 wintering juveniles chose dark, not illuminated, habitat in the day (Figure 6). Fish choosing the illuminated side spent most of the time in the open, not next to the wall or bricks (structure). Fish had no preference for wall vs. bricks. However, in the oval stream most wintering juveniles clustered in the rocks during the day, indicating 15 min was too brief to evaluate preference for structure.

Juveniles spent more total time on sand than on gravel (67% for tests with white sand vs. white gravel and 55% for tests with black sand vs. black gravel), but in both tests the 95% confidence intervals included 50% (no significant preference). Juveniles also spent more time on white sand



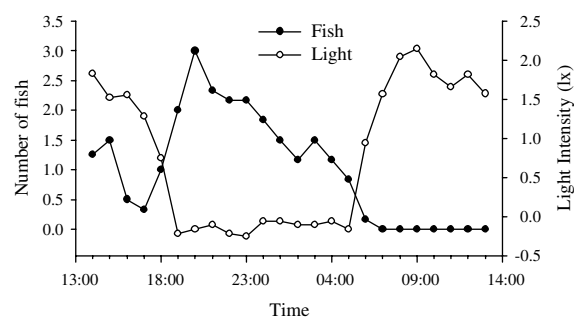
*Figure 6.* Mean number of seconds (out of 600,  $\pm$  SE) wintering green sturgeon ( $n = 20$ ) spent in each of four habitat types in a test arena built in section 12 of the oval stream. One-half of the test arena was covered with black plastic and one-half was open to ambient light ( $<10$  lx). Fish choosing the illuminated side had a choice of structure (touching wall or two bricks), open (not touching any structure), and open cover (between a brick and the wall).

(55%) than on black sand, but use of white substrate was not significant. Day 290–305 juveniles had no significant preference for white vs. black substrate, which was similar to the behavior of early juveniles (Figure 2c).

Day 310–322 juveniles in the large rectangular tank only used the illuminated side from dusk to dawn when illumination was  $<1.0$  lx (Figure 7). Fish introduced at 1400 h took about 1 h to acclimate, explore, and avoid the illuminated side. Juveniles avoided the daytime illumination of 2.5 lx, and moved onto the illuminated side at 1800 h, when illumination was  $<1.0$  lx. Fish activity on the illuminated side peaked at night (2000 h), then slowly declined as light increased to 1.0 lx at dawn (05:00–06:00 h).

#### **Discussion**

Hatchling embryos preferred cover, but they were the poorest swimming hatchling of any sturgeon we have studied and were incapable of moving very far to cover. This situation suggests that natural selection may have selected for



*Figure 7.* Effect of illumination on activity and habitat preference of days 310–322 wintering juvenile green sturgeon. We observed fish in a rectangular stream tank 0.7 m  $\times$  2.4 m  $\times$  16 cm deep (Heney 2002) with one-half the length covered to create dark habitat (0 lx) and one-half a weakly illuminated habitat (maximum, 2.5 lx in the day). Underwater illumination intensity (lx) was recorded on the illuminated side at 15 min intervals, 24 h per day. We observed six groups of fish ( $n = 5$  fish per group) during 24 h for each group after introduction at 1400 h. A video system recorded fish that swam on the illuminated side for 5 min per hour, 24 h per day. For each hour, we determined the mean number of fish on the illuminated side and present the data as a 24-h time-series along with the mean hourly illumination intensity.

adaptations of females and eggs to enhance survival of helpless hatchlings. Females may have evolved a preference for spawning in eddies with large rocks or boulders that would provide a low velocity cover habitat. This habitat exists in the Klamath River (S. Turo personal communication) and Rogue River (D. Erickson personal communication). Females may deposit eggs close to the substrate, similar to shortnose sturgeon, *A. brevirostrum*, which minimizes egg drift (Kynard et al. unpublished data). Also, eggs of green sturgeon have three characteristics (large size, high density, and poor adhesiveness) that may be adaptations to reduce egg drift and place eggs deep among rocks. Green sturgeon eggs are among the largest for sturgeons and are weakly adhesive (Deng et al. 2002, present study). We did not directly measure egg density, but the eggs sank quickly and the water current in the hatching jar, which was the same current used to suspend eggs of six North American sturgeons above the bottom, failed to move green sturgeon eggs off the bottom. Dense eggs that are weakly adhesive should sink quickly into rocks without attaching. Van Eenennaam et al. (2001) also speculated that green sturgeon eggs might not attach to rocks. This same situation may exist for Chinese sturgeon, *A. sinensis*, which have large, weakly adhesive eggs and spawn in deep water over large boulders in the Yangtze River (Wei 2003).

In *Acipenser*, the size of hatchling embryos and larvae is no guide for whether the initial migration from the spawning area is by embryos or larvae. For example, green and Chinese sturgeons have large eggs, embryos, and larvae, but green sturgeon migrate as larvae (present study) and Chinese sturgeon migrate as hatchling embryos (Zhuang et al. 2002a). Sturgeons with average-size eggs, embryos, and larvae have also evolved a great diversity of migration styles, i.e., migration as hatchling embryos (Russian sturgeon, *A. guedenstaedtii*, Kynard et al. 2002b), as larvae (shortnose sturgeon, Kynard & Horgan 2002), or as juveniles (Dabry's sturgeon, *A. dabryanus*, Kynard et al. 2003). Thus, size of eggs, embryos, or larvae does not determine evolution of migration style.

During the first 181 days of life, movements of young green sturgeon in the oval stream indicated wild sturgeon have two downstream migrations,

i.e., an initial larval migration from the spawning area and a fall migration by juveniles to wintering habitat. The initial dispersal of young sturgeon from the spawning area may be related to predation risk of embryos: if predation risk is high, hatchling embryos migrate, if risk is low, larvae or juveniles migrate (Kynard & Horgan 2002, Zhuang et al. 2002a, Kynard et al. 2003). The lack of migration by green sturgeon hatchlings suggests that predation risk of embryos is low. In the species yet studied, if embryos migrate, the migration begins at hatching; whereas, if larvae migrate, the migration is delayed a few days, like the situation for green sturgeon. A downstream movement by juveniles was first evident on day 110 (22 August), increased by day 153 (4 October), and still occurred on day 171 (6 November), then decreased rapidly by 16 November. Water temperature on day 153 was the same as on day 110 (23°C), so decreasing day length, not temperature, may be the trigger for downstream movement. Because movement occurred during the late summer and fall, it likely reflects a movement of wild juveniles to wintering habitat. Shortnose sturgeon move to wintering areas during the same time period (Kynard et al. 2000).

Green sturgeon larvae and early juveniles had the weakest response to bright habitat (illumination and white substrate) of any species we have examined. No life interval preferred illumination, even when larvae began to feed, as frequently occurs with other species (Richmond & Kynard 1995, Gisbert & Willot 1997, Gisbert et al. 1999, Kynard & Horgan 2002, Kynard et al. 2002a, b, Zhuang et al. 2002a, b, Kynard & Parker in press). The absence of a preference for bright habitat by early larvae suggests a different foraging strategy than for other sturgeons yet studied.

Migration of green sturgeon larvae was different from other *Acipenser* migrant larvae in two ways: other species are nocturnal early, and then diurnal (active day and night), and other species have much less upstream movement (Kynard & Horgan 2002, Zhuang et al. 2002b, Kynard & Parker in press). Green sturgeon migrants were nocturnal and hid in rocks during the day. There was no daytime movement. Larvae moved downstream with a high number of fish passes, but often stopped and moved upstream while foraging (Figure 3). Thus, green sturgeon did not combine

downstream movement with simultaneous foraging, but alternated downstream movements with upstream movement to forage. This may be because fish swim too fast (faster than the water current) when going downstream. The moderate level of upstream movement during migration suggests that green sturgeon larvae spend more time feeding during migration than other sturgeons. Further, wintering juveniles in the laboratory actively foraged nightly, even when water temperature was 3°C. Green sturgeon larvae and juveniles are the most voracious feeders of any sturgeon we have reared.

During the first 10 months of life, green sturgeon were the most nocturnal of any North American sturgeon yet studied, and this was the case for all life intervals during any activity (migration, foraging, or wintering). Migrant larvae were nocturnal, post-migrant larvae and early juveniles foraged diurnally with a nocturnal peak, late summer and fall juveniles migrated downstream (and foraged) nocturnally, and movements of wintering juveniles were nocturnal. Additionally, the response of wintering juveniles to illumination shows they limit daily activity in response to very low light levels (<1.0 lx), a sensitivity not previously reported for any sturgeon. The nocturnal activity of green sturgeon during all early life intervals and activities suggests a unique ecological relationship between green sturgeon and the environment. The nocturnal activity of green sturgeon could be related to predation, foraging, competition with other species (Helfman 1993), or any combination of factors. Many migrating riverine fishes are believed to be less susceptible to predation at night (Lucas & Baras 2001). The nocturnal behavior of green sturgeon during all activities suggests early life intervals may be visually limited in an illuminated environment.

Habitat preference of wintering year-0 juveniles in the laboratory suggests that wild juveniles should be in deep pools with some rock structure. The 10 wintering juveniles spent 67% of the time on sand, not gravel habitat, but the preference was not significant, likely because only 10 fish were tested. Wintering juveniles of six North American sturgeons prefer sand (Parker et al. unpublished data), and wintering juvenile (and adult) Connecticut River shortnose sturgeon, *A. brevirostrum*,

use deep, sandy habitat (Kynard et al. 2000). Thus, sandy deep-water habitat may be typical winter habitat of many sturgeons. Deep pools are present in the lower Klamath River (Scott Turo personal communication), so some of these areas may contain juveniles in winter. Wintering juveniles were only active at night, so passive netting for them should be most successful in the early evening (activity peaked at 20:00 h).

Green sturgeon required about 174 CTU or 6.8 CTU per mm TL to develop into larvae, which is average for many sturgeon species (Kynard et al. 2003). Using temperature data of Deng et al. (2002) to rear Klamath River green sturgeon, we calculated a similar CTU (176.5) to develop to the larva interval in the fish they reared. The CTU per millimeter length for green sturgeon larvae was similar to the 7.2 CTU per mm larva TL for Chinese sturgeon, another species with large eggs and hatchlings (Zhuang et al. 2002a, Kynard et al. 2003). Although green and Chinese sturgeons require a similar CTU to develop into larvae, this common developmental characteristic did not result in the evolution of the same migration style, i.e., green sturgeon migrate as larvae, Chinese sturgeon as embryos.

A conceptual model of early behavior and migration of green sturgeon early life intervals based on the present study follows. Females deposit eggs at sites with large rocks and moderate or eddy water flow that keeps the large, dense, poorly adhesive eggs from drifting, so eggs sink deep within the rocks. Hatchling embryos seek nearby cover, and embryos remain under rocks. After about 9 days and 174 CTU, fish develop into larvae and initiate exogenous foraging up- and downstream on the bottom. After a day or so, larvae initiate a downstream dispersion migration that lasts about 12 days (peak, 5 days). All migration and foraging during the migration period is nocturnal. Post-migrant larvae are benthic, foraging up- and downstream diurnally with a nocturnal activity peak. Foraging larvae select open habitat, not structure habitat, but continue to use cover in the day. When larvae develop into juveniles, there is no change in response to bright habitat, and no preference or avoidance of bright habitat. In the fall, juveniles migrate downstream mostly at night to wintering sites, ceasing migration at 7–8°C. During winter, juveniles select low

light habitat, likely deep pools with some rock structure. Wintering juveniles forage actively at night between dusk and dawn and are inactive during the day, seeking the darkest available habitat.

Several studies on early life intervals of *Acipenser* suggest body color is related to migration style. Kynard et al. (2002a) described three lateral body and tail colors of sturgeon embryos and larvae: dark body and tail, light body with black-tail, and light body and tail. Green sturgeon did not migrate as embryos with a light gray body, but as larvae with a medium gray body and a black tail. All North American sturgeons that migrate as larvae have dark bodies (medium or dark gray, brown, or black): shortnose, Atlantic, Gulf, and lake sturgeons (Kynard & Horgan 2002, Kynard & Parker in press, Kynard & Parker unpublished data). Body color of the larval migrants of green sturgeon was similar to the body color of larval migrants of these other species.

The tail of green sturgeon developed a black tip during the embryo interval, but the posterior one-third of the body and tail was dark black only during the early larval interval when fish initiated feeding and migration. The black-tail phenotype was gradually lost after migration ceased. The occurrence of the black-tail phenotype on early larvae suggests this morphological character is linked to behavioral characteristics and is an adaptation for migration, early foraging, or both. The wigwag movement of the black tail is a good visual signal and is possibly used for social signaling, predator avoidance, or both (Kynard et al. 2000). Green sturgeon were distributed randomly among the sections of the oval stream without rocks, a result suggesting they are not social and black-tail is not a social signal for green sturgeon. The black-tail phenotype occurs during the late embryo and larval intervals of many sturgeon species (Kynard et al. 2002a, b, Zhuang et al. 2002a, b, Kynard & Parker in press), suggesting a similar adaptation among species.

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