BIOLOGICAL ASSESSMENT OF GREEN STURGEON IN THE SACRAMENTO-SAN JOAQUIN WATERSHED (PHASE 2)

(Project # 99-F105)

FINAL REPORT

to:

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Submitted by:

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Phase 2, Task 1: Temperature and Ration Effects on Green Sturgeon Bioenergetics

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Temperature Effects on Green Sturgeon (*Acipenser medirostris* Ayres) Bioenergetics: An Experimental Lab Study

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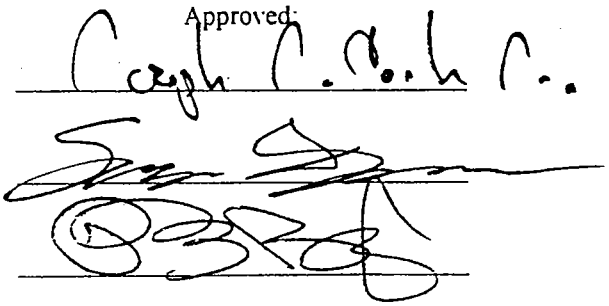

Committee in Charge

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Introduction

Temperature is an important environmental factor that directly or indirectly affects physiological and behavioral processes of fishes as well as their distribution (Cech et al. 1990, Schmidt-Nielsen 1999). Energetic (metabolic) demands of fish typically increase with temperature (Moyle and Cech, 2000). As a result, food consumption (energy ingested) often increases with increasing water temperature to satisfy increasing body maintenance demands and, often, increasing growth and reproduction demands (reviewed by Jobling 1994). These factors can be modeled as:

$$I = G + A + M + R + E + SDA, \quad (1)$$

where the energy ingested (I) is balanced by energy allocated to: growth (G), activity (A), maintenance (M), reproduction (R), excretion (E), and specific dynamic action (SDA, which is energy associated with digestive and anabolic processes following feeding). Thus, if an increase in M associated with a temperature increase is not balanced by increases in I (i.e., ration size or quality), decreases in A (e.g., foraging, migrating), G, or R (in mature fish) would be predicted.

The green sturgeon (*Acipenser medirostris*) is an anadromous chondrostean with a distribution ranging from the Bering Sea to Ensenada, Mexico (Moyle et al. 1995). Allopatric green sturgeon are also found in the Western Pacific in Asia but are a separate species, *Acipenser mikadoi* (Birstein and Bemis 1997). An area where north American green sturgeon are known to spawn is in California's Sacramento and Klamath rivers, located near the southern boundary of this species' distribution. These rivers show temperature variations due to natural (e.g., seasonal and precipitation-related cycles), as well as human-induced (e.g., impoundments) effects. The San Francisco Estuary green

sturgeon population size is unknown, but is believed to be significantly smaller than that of white sturgeon, *A. transmontanus* (Schaffter and Kohlhorst 1999). Green sturgeon are considered an "at risk" species by the CALFED consortium of state and federal natural resource agencies, and a species of special concern by California Fish and Game. Basic life history information is considered critical to its protection and because the variables from the equation above are important to fishes' life history, habitat changes (e.g., due to dams, loss of riparian cover, or thermal pollution) leading to temperature increases can have serious ramifications on resident fish populations (Chart and Bergesen 1992).

The purpose of this study was to determine how temperature affects the food consumption rate, resting routine metabolic rate (maintenance), growth rate, food conversion efficiency (growth/consumption), swimming performance, and thermal preference of juvenile green sturgeon. All were hypothesized to increase with increasing water temperature.

Materials and Methods

Age-0 and age-1 green sturgeon used in this study were progeny of wild-caught Klamath River sturgeon that had been artificially spawned during late May, 1999 (Van Eenenaam et al. 2001). The eggs were incubated at UC Davis and juveniles reared in aerated water at temperatures similar to those in the Klamath River (11-15°C) during late spring. The age-0 fish were fed commercial Silvercup trout pellets at 3-5% body weight ration per day based on a feeding table for white sturgeon. Fish (age 31 d post-hatch) were placed into round, 284-L fiberglass holding tanks receiving continuous flows of air-equilibrated, 19°C well water and held until needed for experiments.

Experiment 1: Food Consumption, Growth, and Food Efficiency

At age 144 days post-hatch, fish (age 0, n = 144) from a holding tank were randomly distributed into 24, 110-L round tanks (6 fish/tank) and either held at 19°C or acclimated to 11 or 15°C at a maximum rate of 1°C per day. There were 4 replicate tanks for each of the six treatments (3 temperatures x 2 ration levels) for the 30-d experiment. Tanks were indoors and were maintained on a natural photoperiod (latitude: 38.55°N; October-December) using both natural (translucent roof panels) and artificial (fluorescent lights) sources. Incoming water flow (4 L/ min) current was adjusted, using angled spray bars, to 10 cm/ sec current, and current direction was reversed every 5 d to uniformly exercise the fish.

Food Consumption

Half of the fish (tanks selected randomly) for each temperature were fed to satiation (Silvercup pellets) and the rest were fed at 50% of satiation. The 50% ration level was determined by dividing the mean daily satiation rations at each corresponding temperature by two. The different ration levels for this experiment simulate varying amounts of available food in the wild. Fish were fed twice daily, and the amount of food consumed was quantified by collecting and counting the uneaten pellets and subtracting their pre-determined weight from that of the total fed for each feeding. The mean daily food consumption rate (CR, g feed/ g fish/ d) for each tank of fish was calculated using:

$$CR = \frac{\sum C}{0.5\,(W_1 + W_2)t} \qquad (2)$$

where: C = total food consumed (g) for the duration of the experiment; W_1 and W_2 = fish wet weights (g) at the beginning and end of the experiment, respectively; and t = experiment time (34 d).

Growth

The fish were individually weighed and measured at 10-d intervals and were fasted 24 h prior to weighing and measuring to minimize feed weight contributions. Fork length (FL) and total length (TL) were measured to the nearest mm and weights to the nearest 0.1 g (electronic balance), were taken from blot-dried fish. Mean live weights were used to calculate the specific growth rate (SGR, % weight/ day) using:

$$SGR = \frac{(\ln W_2 - \ln W_1)}{t} (100) \qquad (3)$$

Food Conversion Efficiency

Following the experiment, 6 fish from each treatment were dried to a constant weight at 60°C (drying oven) to derive the relationship between dry weight (DW) and wet weight: DW = 0.203W – 3.435. We calculated (gross) food conversion efficiencies (CE, g fish gained/ g feed) for each tank of fish using:

$$CE = \frac{DW_2 - DW_1}{\Sigma C} \qquad (4)$$

where: DW_1 = mean dry weight (g) of fish sampled from the holding tank at experiments' start, DW_2 = mean dry weight of the experimental fish in each tank at the experiment's end, and ΣC = total dry weight of feed consumed during the experiment.

Experiment 2: Metabolic Rate and Activity

Routine metabolic rates were determined by measuring (age-0, mean body weight: 67.9 g) oxygen consumption rates using closed respirometry (Cech, 1990). Fish (n = 39, 33, and 33 for 11, 19 and 24°C, respectively) were randomly taken from their holding tank and transferred to three, indoor experimental tanks also receiving air-equilibrated well water at 19°C. Randomly chosen, two of the three tanks were cooled or warmed at 1°C / day to reach the acclimation temperatures of 11 and 24°C, respectively. A blank respirometer (without fish) was used to account for microbial respiration. A pilot study showed that age-0 fish continued (even after 8 h of respirometer acclimation) to exhibit activity (tail beats), therefore, we quantified tail beats using videotapes from an overhead video camera. Metabolic rates (MO_2, mg O_2/ h) were calculated using:

$$MO_2 = \frac{(C_{O2i} - C_{O2f})\ (V_R)}{t} \qquad (5)$$

where: C_{O2i} and C_{O2f} = the O_2 concentration (mg/ L) at the beginning and end, respectively, of the experiment V_R = the respirometer volume (L); and t = the experiment time (h). The O_2 partial pressures (P_{O2}) were measured with a Radiometer PHM71/E5046/D616 O_2 analyzer system and converted to O_2 concentration using an O_2 solubility nomogram (Green and Carrit 1967).

Resting routine metabolic rates were determined by measuring the (age-1) juvenile green sturgeons' oxygen consumption rates using open respirometry (Cech, 1990). Age-1 fish were transferred and acclimated as described, above, for age-0 fish. Age-1 (mean body weight: 850.74 $\pm$ 30.99g) fish were completely quiescent in their triangular (cross-section) 11-L respirometers after 8 h respirometer acclimation. Respirometer water flow rates were measured using timed collection of water in a calibrated, graduated cylinder, and oxygen contents were calculated from inflow and outflow P_{O2}s, as above. Measurements from a blank respirometer accounted for microbial respiration. Age-1 fish M_{O2}s were calculated using:

$$M_{O2} = (C_{O2in} - C_{O2out})\,(Vw) \qquad (6)$$

where: C_{O2i} and C_{O2out} = the O_2 contents (mg O2/L) of water flowing into and out of the respirometer, and Vw = the water flow rate through the respirometer (L/ h). Between temperature comparisons were facilitated using Q_{10}s, following Schmidt-Neilsen (1999).

Experiment 3: Thermal Preference

Thermal preference experiments on age-0 green sturgeon (n = 20, 20, and 9 for 11, 19 and 24°C respectively) were conducted in an annular, flow-through thermal gradient tank designed to avoid vertical stratification (Fogel et al.). A light-colored shade cloth cover shielded the apparatus and fish from investigators, while observations of the single, juvenile green sturgeon's position were made using a CRT monitor wired to an overhead video camera. Water flows throughout the thermal gradient tank were isothermal at the

individual fish's acclimation temperature for the first hour after each fish was placed in the apparatus. Then, the thermal gradient (11.5-31.0°C) was established. During each 1-h experiment, location of the fish and the corresponding water temperature (YSI 44TD tele-thermometer with 10, calibrated YSI 401 thermistor probes placed at regular intervals around the gradient tank) data were recorded at 10-min intervals.

Experiment 4: Swimming Performance

Age-1 green sturgeon (n = 19, 12, and 9 for 11, 19 and 24°C respectively) critical swimming velocities (Ucrit) were determined with a 200-L recirculating-water flume (Brett 1964) incorporating a variable-speed motor. The flume was partly immersed in a temperature-controlled water bath and the velocities were calibrated with a digital Marsh-McBirney (model 201D) water current meter. Individual fish were placed in the swimming chamber and after a 1-h acclimation at a 10 cm/s water velocity the Ucrit was measured by 10 cm/s step water velocity increases every 20 min, until the fish was fatigued (Beamish 1978). A fish was considered fatigued when it impinged three times at the downstream end of the chamber. Absolute Ucrit ($Ucrit_a$ cm/s) was calculated (Brett 1964) using:

$$Ucrit_a = Ui + (10\ cm/s)(Ti/20min) \qquad (7)$$

where: Ui = highest velocity (cm/s) maintained for 20 min, and Ti = time (min) elapsed at fatigue velocity. Relative Ucrit ($Ucrit_r$ body lengths/s) was calculated by dividing $Ucrit_a$ by FL (cm). Tail beat frequencies (TBF) were measured for each fish by counting the number of tail beats over a 1-min period at each swimming velocity.

Statistical Analyses

A one-way ANOVA (parametric data) was conducted to test for significant effects of experimental factors ($k > 2$; e.g., temperatures), with Tukey's tests used for post hoc pair-wise comparisons. Only the age-0 metabolism/activity data were non-parametric, requiring a Kruskal-Wallis to test for significant effects and Dunn's test for post hoc pair-wise comparisons. Student's t-test were used to compare the two food ration levels data. All of these analyses were facilitated with SIGMASTAT software. Analysis of covariance as used to determine significant interacting effects of body weight and activity on metabolic rate (SAS software). Statistical differences were considered significant at $P < 0.05$.

Results

Food Consumption

Both increases in temperature and ration level influenced juvenile green sturgeon food consumption rates (Figure 1). Temperature increases significantly increased mean food consumption rates for both ration levels between 11 and 15°C ($Q_{10} = 2.99$) but no significant difference was found for either ration level between 15 and 19°C ($Q_{10} = 1.33$) (Figure 1). The fish fed to satiation (100% rations) consumed twice as much (significantly more) food compared to the fish being fed 50% rations (Figure 1).

Growth

Increases in temperature and ration level influenced juvenile green sturgeon growth rates. Temperature increases caused significant increases in specific growth rates between

the 11 and 15°C (Q_{10} = 4.73) groups for both rations, whereas no significant differences were detected between the 15 and 19°C (Q_{10} = 1.50) groups at either ration level (Figure 2). Significant increases in specific growth rates were also observed at the 100% rations versus 50% rations at all temperatures (Figure 2).

Food Conversion Efficiency

Temperature did not affect ($P > 0.05$) green sturgeon food conversion efficiency, although there was a slight increase (Q_{10} = 1.41). Increases in ration level significantly decreased food conversion efficiencies in the 15 and 19°C treatments only (Figure 3).

Metabolism and Activity

Increases in temperature increased mean oxygen consumption rates and activity for juvenile green sturgeon (mean weight = 22.3 g). The temperature increase from 11 to 19° C did not affect MO2s (Q_{10} = 1.37), but the increase from 19 to 24°C resulted in a significant increase of MO2 (Q_{10} = 4.37, Fig. 4). Significant increases in volitional activity (tail beat frequencies) were observed in all temperature treatments (Figure 4).

In age-1 fish (mean weight = 850.7g), temperature increases were associated with significant increases in mean resting routine metabolic rates for all treatments, with more similar Q_{10} values between the 11 & 19°C (1.57) and 19 & 24°C (1.82) treatments, compared with age-0 fish. Ventilatory frequency (VF) showed significant increases for all the treatments (Figure 5).

Thermal Preference

Fish acclimated to 11°C and 19°C did not differ significantly ($P>0.05$) in their thermal preferences (15.9°C and 15.7°C respectively), but fish acclimated to 24°C exhibited significantly higher ($P<0.05$) preferred temperature (20.4°C, Figure 6).

Swimming Performance

There was no significant difference ($P>0.05$) between absolute Ucrit values in 11 to 19°C treatments (69.1 +- 2.6 cm/s and 79.5 +- 4.9 cm/s, respectively). However, the 24°C group's Ucrit (57.5 +- 7.2 cm/s) was significantly lower than the 19°C group (Figure 7). There were no significant differences in relative Ucrit between any of the temperature treatments. Approximately 60% of the 24°C fish died following their transport prior to being tested for swimming performance.

Discussion

Food Consumption

The significant differences in mean food consumption rates among all temperature treatments can be attributed to warmer water temperature-related increases in the energetic demands of the fish. To satisfy these increased energy demands, the fish ingested more food. These higher energetic demands can be attributed to both increased maintenance and activity demands (Figures 4 and 5). The largest food consumption increase was at 15°C, followed by a slight increase at 19°C. Further studies at higher temperatures are needed to show the temperatures at which food consumption would eventually reach a plateau, followed by a decline for age-0 green sturgeon.

Increases in food consumption with increasing water temperatures are not unique to sturgeon species. Larsson and Berglund (1998) found this pattern for age-0 Arctic charr (*Salvelinus alpinus L.*) where food consumption peaked at 16°C over a 5-20°C range, followed by a decline. In contrast, food consumption for green sturgeon never declined but continued at even warmer temperatures. This suggests the ability for these fish to inhabit warmer temperatures (up to 19°C), which is not surprising when comparing these two species which are phylogenetically distinct and are under very different conditions in the wild. The lack of a decline in food consumption with increasing temperature also suggests more green sturgeon food consumption data is needed at higher temperatures to find an eventual decline. Koskela et al. (1997) found food consumption increases in age-1 Baltic salmon (*Salmo salar L.*) over 11-17°C range, eventually decreasing at 23°C. This pattern is similar to our data, suggesting that these two species inhabit similar water temperatures at the ages studied. The decrease in food consumption further illustrates the need for green sturgeon food consumption data at higher water temperatures. Vigg and Burley (1991) found an exponential increase in food consumption for Northern Pikeminnow (0.5-2.0 kg.) at 8.0-21.5°C, indicating a relatively high metabolic sensitivity to temperature brought on by an overall increase in energy demands.

Growth

Temperature is a very important environmental variable controlling growth in fishes. When food is available, growth increases with temperature, followed by a decline or lethal temperatures are approached (Moyle and Cech 2000). Temperature had an affect on growth for all temperature treatments, but only significantly between 11 and 15°C.

Although the highest growth rates were at 19°C, they were not significant increases. This diminishing temperature is probably attributable to increased energy demands for maintenance and/or activity brought on by increases in temperature (Figures 4, 5). Preliminary studies looking at green sturgeon growth from 25-60 days post-hatch have shown a decrease in growth from 19 to 24°C (Allen and Cech, unpublished data). Maximum growth rates may have been limited by the twice-daily, rather than continuous feeding schedule. To measure food consumption rate and conversion efficiency, we counted the uneaten pellets, which would significantly erode after 20 min. Because sturgeon are relatively slow feeders compared to salmonids (Hung et al. 1993), we may have underestimated maximum food consumption and growth rates.

Despite the potentially sub-optimal feeding regime in this study, juvenile green sturgeon showed relatively high growth rates, given their acclimation temperature and ration levels, compared to other Acipenser species of similar body size (Table 1). For example, sterlet sturgeon (*A. ruthenus*) at a higher temperature and ration level showed a slower growth rate. Furthermore, siberian sturgeon (*A. baeri*) at temperatures similar to this study showed a slower growth rate, despite higher ration levels. Conversely, Chinese sturgeon (*A. sinensis*) and Atlantic sturgeon (*A. oxyrinchus*) at similar to higher temperatures grew faster than our green sturgeon, although being fed higher rations (Table 1). However, the smaller body size (< 70g) of the Chinese and Atlantic sturgeon used in these experiments could be a reason for higher specific growth rates (Ricker 1979). Lake sturgeon (*A. fulvescens*) reared at similar temperatures and ration levels exhibited slower growth rates compared to age-0 green sturgeon. It took substantially

higher ration levels for lake sturgeon growth rates to approach that of age-0 green sturgeon (Table 1).

Studies involving sympatric white sturgeon (*A. transmontanus*) under similar experimental conditions to our green sturgeon showed an overall slower growth. It was only at higher water temperature treatments and smaller body size where white sturgeon growth rates were similar to those of our green sturgeon. Current studies in our laboratory (Allen and Cech, unpublished data) indicate that green sturgeon growth rates are lower at 24°C than at 19°C. Thus, white sturgeon should benefit more than green sturgeon from inhabiting warmer (>19°C) water temperatures (Tables 1,2).

Food Conversion Efficiency

Food conversion efficiency in fish typically increases with increasing temperature to some maximum, followed by a decline (Jobling, 1981). Larsson and Berglund (1998) observed an increase in feeding efficiency, peaking at 9.0°C, followed by a decrease for age-0 Arctic charr. A temperature effect was observed for only 50% rations but not for the 100% rations (Figure 3). These data suggest that juvenile green sturgeon fed to satiation remain unaffected in their efficiency in converting food into body mass at the temperature range tested. However, in the wild, fish are rarely satiated with food on a regular basis (Cite).

Green sturgeon appear to have similar food conversion efficiencies, compared with other *Acipenser* species. Using feeding efficiency (FE = wet weight gained per amount of feed consumed during the experiment) as a surrogate for comparative purposes, juvenile

green sturgeon FEs are similar to, or slightly higher than those of juvenile white sturgeon at similar temperatures (Table 2). Hung et al. (1993) and Hung and Lutes (1987) found that the highest feeding efficiency for juvenile white sturgeon was between 20 and 23°C (Table 2), which was beyond that used in our studies. Both white and green sturgeon are found in the Sacramento-San Joaquin Estuary. Green sturgeon are more marine in their life history (Galbreath 1985), going out to sea and traveling far distances along the coast. White sturgeon spend more of their life history in estuaries (Cite). California coastal ocean temperatures are cooler than that in the estuary, which may explain sustainable growth rates of white sturgeon at higher temperatures than for green sturgeon.

Increases in fish food conversion efficiency with decreased rations are also well documented. Fish that are fed less are more efficient in converting ingested food into body mass (Paloheimo and Dickie 1966). This increased efficiency is beneficial in areas where food availability is low.

Metabolism/Activity

Increasing metabolic rates with increasing temperature are common among ectotherms (Schmidt-Nielsen 1999), including fishes (e.g., Atlantic cod *Gadus morhua*, Castonguay 1998). While the high Q_{10} value for age-0 green sturgeon between the 19 and 24°C treatments (4.31) shows high metabolic temperature sensitivity, the lower Q_{10} value (1.47) and lack of significant difference between the 11 and 19°C treatments' MO2s demonstrates thermal flexibility. This thermal flexibility would be advantageous for age-0 green sturgeon, allowing them to forage in thermally diverse habitats. The larger Q_{10} value and significant difference in MO2s between the two higher temperature treatments

suggests that age-0 green sturgeon inhabiting water >19°C would be energetically penalized by expending significantly larger amounts of energy towards maintenance and activity (Figure 4) and comparatively less on growth (Figure 2).

Increases in volitional swimming/activity with temperature typify teleostean behavior (e.g., Wurtsbaugh & Cech 1993). The activity observed in this study increased linearly and was positively correlated with temperature ($r^2 = 0.98$). White sturgeon also increase activity with temperature significantly over a 10- 25°C range (Cech et al. 1984, Crocker and Cech 1997). Such increases presumably assist more widespread foraging to meet warm temperature-associated increased energy requirements for growth (Cech et al. 1984) and metabolism (Crocker and Cech 1997). Although there was an increase in volitional activity with temperature over the 11-19°C range (Figure 4), routine metabolism remained unchanged over the same range (Figure 4). This would leave more energy for growth with temperature increases over the same range (Figure 2, equation 1). Faster growth decreases predation risks by minimizing the time spent at small-sized, more vulnerable prey (Werner and Hall 1988). Although age-1 green sturgeon (Figure 5) significantly increased their MO_2s with each temperature increase (Figure 5), they were quiescent in flow-through respirometers and their Q_{10}s were more consistent across the temperatures, compared to those of age-0 fish (Figures. 4,5).

Despite the fact that larger organisms tend to have relatively lower metabolic rates (when "weight adjusted" by dividing mg O_2 consumed per h by body mass, Schmidt-Neilsen 1999), green sturgeon appear to exhibit relatively high metabolic rates, in comparison to other sturgeon species (Table 3). Burggren & Randall (1978) reported a white sturgeon (950 g) mean MO_2 at 15°C that was lower than the mean for green

sturgeon (850.7g) at 11°C. McKenzie et al. (1997) reported an MO_2 in Adriatic Sturgeon (*A. naccarii*, mean wt.= 198 g) at 23°C that was less than half the value for green sturgeon at 24°C.

Thermal Preference

Whereas juvenile green sturgeon acclimated to 11°C or to 19°C both preferred temperatures of approximately 16°C, the 24°C treatment group preferred a significantly higher temperature (20.1°C, Figure 6). Thus, the 11°C acclimated group preferred a warmer temperature than that to which it was acclimated and the 19 and 24°C acclimated groups preferred somewhat cooler temperatures than those to which they were acclimated. Although both the 11 and 19°C acclimation temperatures fall within the thermal regime of the Klamath River (Chamberlain, C. Pers. Comm), the similar thermal preference values (15.9 and 15.7 respectively) suggest that 15 -16°C is the approximate preferred temperature by age-0 green sturgeon in their natural habitats. Interestingly, 15° C was the temperature where the fish showed the sharpest increase in growth rates and in food conversion efficiency (Figures 2, 3) followed by slight increases beyond 15°C. Fishes commonly select temperatures that promote optimal growth (Jobling 1981) and where physiological functions operate at maximum efficiency (Crawshaw 1977). The less pronounced temperature effect beyond 15°C suggests a lesser benefit with respect to faster growth for age-0 green sturgeon at these temperatures. Conversely, regimes cooler than 15°C would decrease the growth rate, regardless of food availability and can also decrease muscle twitch time (Rome 1990), which could increase vulnerability to predators via slower escape initiation or swimming performance. Kita et al. (1996) looked at

temperature preference and tolerance, and oxygen consumption of juvenile (mean and SD = 10.7 ± 1.4g) marbled rockfish (*Sebastiscus marmoratus*) at different temperatures and found that 21°C was the preferred temperature regardless of acclimation temperature (long-term observations; e.g., >24 hrs.) over a 15 to 25°C range. Furthermore, they also found the temperature where MO2 increases gradually lessened coincided with the observed preferred temperature. This is similar to our study where the preferred temperature (although based on short laboratory observations) was found to be between the temperatures 11 and 19°C where MO2s were not statistically different in age-0 green sturgeon.

Thermal tolerance was originally planned to be part of this study; however the anatomy (large pectoral fins) and demersal nature of green sturgeon made it difficult to determine any loss of equilibrium for the fish which is the endpoint for measuring this variable. Jobling (1981) reported a linear regression relationship between preferred and lethal temperatures in fish as:

$$Y = 0.66X + 16.45 \ (r = 0.880)$$

where Y is the lethal temperature and X denotes the preferred temperature. When applying this equation to the preferred temperature of this study (15.8°C) we obtain a lethal temperature value of approximately 27°C. As mentioned in the earlier, a large percentage of the age-1 fish acclimated to 25°C died after being transported to a holding tank. Identical transportation age-1 green sturgeon acclimated to cooler temperatures never resulted in a mortality during this study. Based on the observation that the only fish that died in the experiment due to transport stress were fish kept at 25°C suggests that this temperature is near the thermal critical maximum for age-1 green sturgeon. The lethal

temperature would likely be found not far beyond this temperature, which would support the value from the regression equation. It would be beneficial in the future to conduct thermal preference studies with more fish per treatment to narrow down its thermal preference. Brett (1971) and Kellogg and Gift (1983) have pointed out that the final preferendum coincides with the optimum temperature for various physiological processes, particularly for growth.

Swimming Performance

The presence or absence of significant differences in absolute and relative Ucrit values appear to be confounded by thermal sensitivity of the fish brought on by exercise. Although all fish were handled identically, the incidence of equilibrium loss among fish during the acclimation period increased with increasing temperature, with mortalities occurring in the 24°C brought on by previous transport stress.

Peake et al. (1997) found that temperature increases significantly increased the swimming performance of lake sturgeon (*Acipenser fulvescens*) acclimated at 14°C swimming at 10-min intervals. Adams et al. (1997) looked at critical swimming velocities of shovelnose sturgeon (*Acipenser platorynchus*, fork length: 57-69 cm) acclimated to 16° C. The swimming intervals were similar to this study (15 min) and the relative Ucrit values were similar to, or slightly higher than in green sturgeon similar at temperatures (1.03- 2.19 body lengths/ sec). We attribute the significant decrease in relative and absolute Ucrit values to thermal stress brought on by relatively high water temperatures.

In conclusion, age-0 green sturgeon increase their food consumption rate with

temperature accompanied by an increase in growth rate. Growth reaches a maximum rate between 15-19°C, suggesting this range to be optimal. Because both food consumption and growth rates increase, food conversion efficiency remains similar within the temperature range used. Increasing the ration level causes a growth increase and a food conversion efficiency decrease. The MO_2 of green sturgeon increases with temperatures in age-1 fish and at temperatures > 19°C in age-0 fish. Green sturgeon juveniles acclimated to 11 and 15°C exhibit similar preferred temperatures, coinciding with their maximal growth temperature range. At temperatures above 19°C, green sturgeon allocate more energy towards maintenance (metabolic rate) and activity, and less for growth. Swimming performance of these fish decreased at higher temperatures, from a peak performance at 19°C.

The physiological and behavioral variables investigated in this study illustrate the importance of temperature as a controlling factor. Dams control the flows of two major rivers where green sturgeon spawn in California (Klamath and Sacramento Rivers). Appropriate dam releases and better watershed management can help to maintain water temperatures in these rivers that favor survival, growth, and recruitment of juvenile green sturgeon.

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Table 1. Summary of studies on growth rates for sturgeons.

Species	Mean Wt. (g)	Temp. (°C)	Growth Rate (% body wt/day)	Feed Rate (% body wt/day)	Reference
A. transmontanus	0.5-5	15, 20, 25	1.6, 2.6, 2.9	Ad lib.	Cech et al. 1984
A. sinensis	3.8	15.6	4.5	6.0	Xiao et al. 1999
A. ruthenus	20.0	23.0	1.1	3.5	Jahnichen et al. 1999
A. transmontanus	26.2	20	2.1	2.0	Hung & Lutes 1987
A. transmontanus	30.3	23, 26	2.2, 1.9	2.0	Hung et al. 1993
A. oxyrhynchus	10-70	19, 26	5.0, 3.6	2.5	Secor et al. 1997
A. baeri	181	11-19	1.4	1.5-2.1	Prokes et al. 1997
A. medirostris	184.0	11, 15, 19	1.1, 2.0, 2.3	1.3, 1.8, 2.1	This study
A. transmontanus	250.0	18	1.5	2.0	Hung et al. 1989
A. fulvescens	10-1322	17.5	-1.5, 0.0, 1.8, 2.6	0.0, 2.2, 9.4, 13.2	Diana et al. 2000

Table 2. Summary of studies on feeding efficiencies for sturgeons.

Species	Wt. (g)	Temp. (°C)	Feeding Efficiency	Feed Rate (%body wt/day)	Reference
A. ruthenus	20.0	23.0	2.0	3.5	Jahnichen et al. 1999
A. transmontanus	30.3	23, 26	1.3, 1.1	2.0	Hung et al. 1993
A. medirostris	184.0	11, 15, 19	1.0, 1.1, 1.1	1.3, 1.8, 2.1	This study
A. baeri	190.7	11-18	1.4	1.5-2.1	Prokes et al. 1997
A. transmontanus	250.0	18	0.92	2.0	Hung et al. 1989

Table 3. Summary of studies on routine metabolism for sturgeon species.

Species	Mean Wt. (g)	Temp. (°C)	Routine Metabolism (mgO2/h/g fish)	Reference
A. tranmontanus	0.2-63	10, 16, 20	0.26, 0.2, 0.18	Crocker & Cech 1997
A. medirostris	22.3	11, 19, 24	0.10, 0.13, 0.27	This study
A. oxyrhynchus	12-69	19, 26	0.2, 0.3	Secor & Gunderson 1997
A. naccarii	198.0	23.0	0.11	McKenzie et al. 1997
A. medirostris	850.7	11, 19, 24	0.13, 0.23, 0.27	This study
A. transmontanus	950	15	0.08	Burggren & Randall 1978
A. baerii	1800	15	0.06	Nonnate et al. 1993
A. transmontanus	2000	18	0.10	Ruer et al. 1987

Figure 1- Mean ± SEM estimated food consumption rate of age-0 green sturgeon acclimated to 11, 15, and 19oC (180 ± 5.7g mean wet weight). Different numbers, letters and asterisks represent significant differences between and within temperature treatments, respectively. Parenthetical numbers are Q10s between temperatures of the 100% Ration groups.

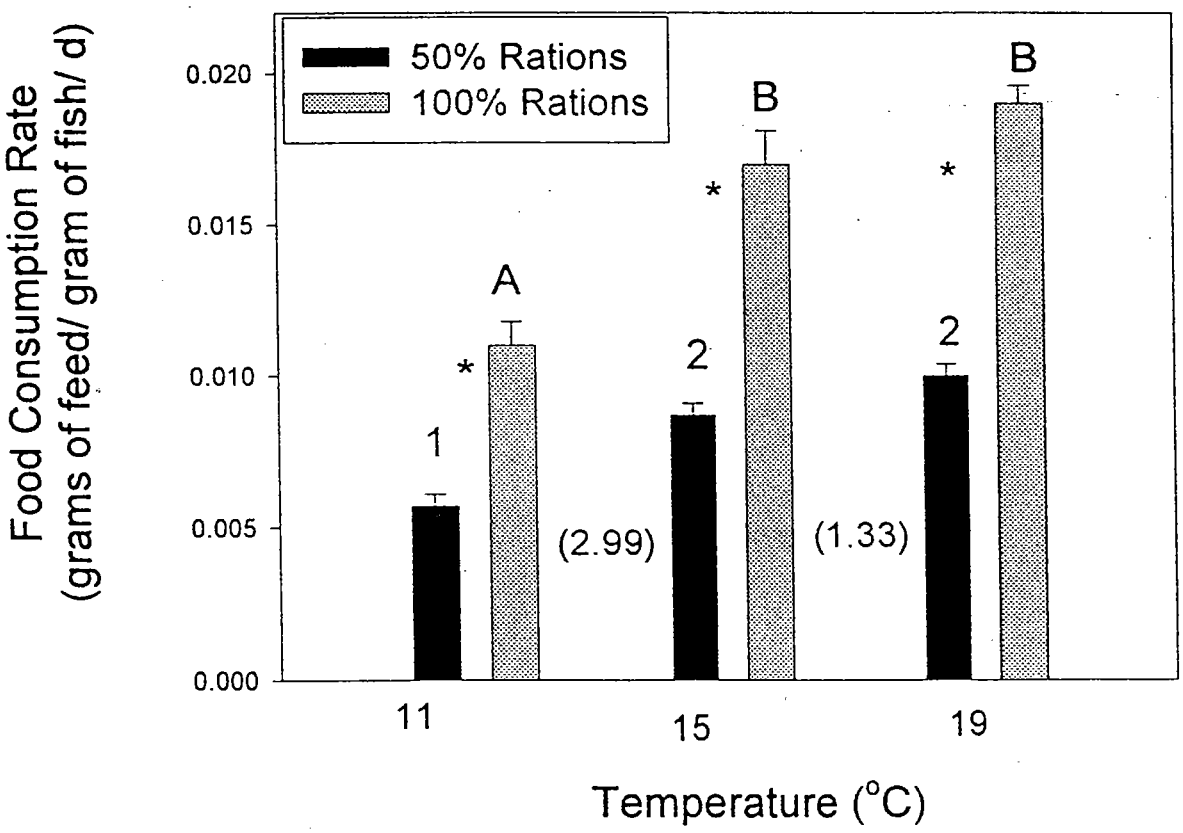

Food Consumption Rate
(grams of feed/ gram of fish/ d)
50% Rations
100% Rations
0.020
0.015
0.010
0.005
0.000
A
B
B
1
2
2
*
*
*
(2.99)
(1.33)
11
15
19
Temperature (°C)

Figure 2. Mean ± SEM estimated specific growth rate of age-0 green sturgeon (mean wet weight: 180 ± 2.5g) acclimated to 11, 15, and 19 °C. Different numbers or letters, and asterisks indicate significant differences between and within temperature treatments, respectively. Parenthetical numbers are Q_{10}s between temperatures of the 100% Ration groups.

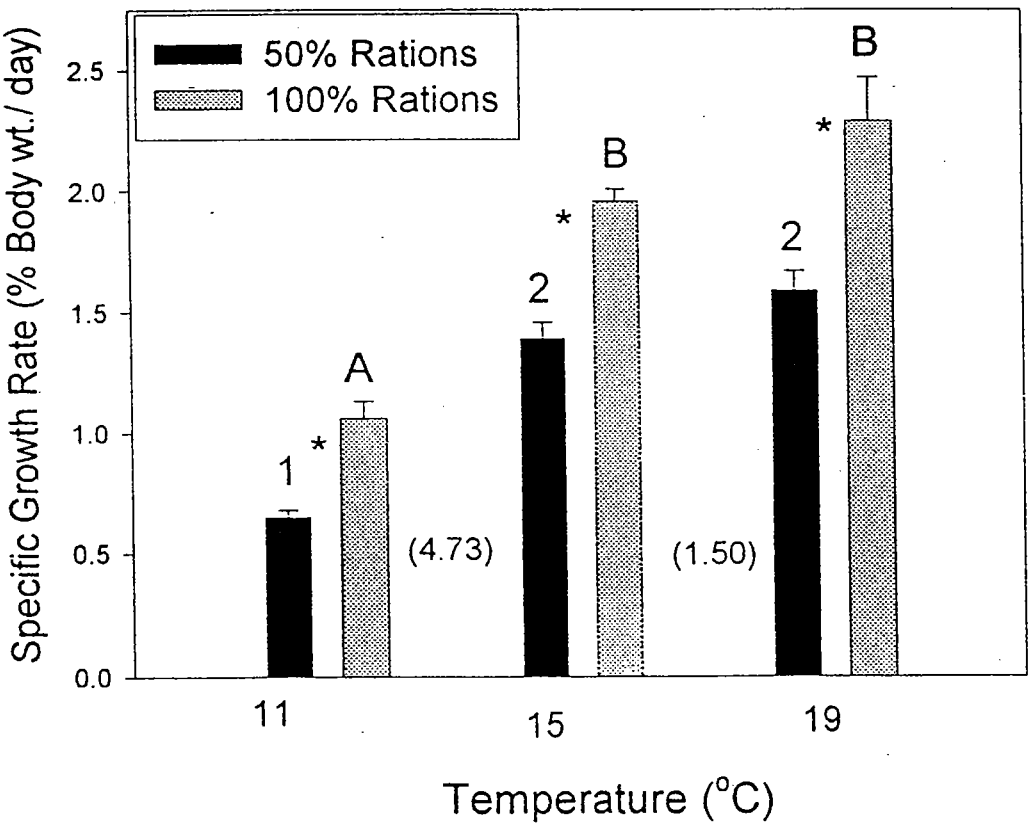

50% Rations
100% Rations
Specific Growth Rate (% Body wt./ day)
2.5
2.0
1.5
1.0
0.5
0.0
1
*
A
2
*
B
2
*
B
(4.73)
(1.50)
11
15
19
Temperature (°C)

Figure 3. Mean ± SEM estimated food conversion efficiency of age-0 green sturgeon (mean wet weight: 180 ± 5.7g) acclimated to 11, 15, and 19 °C and fed 100% rations or 50% rations. Different numbers and asterisks represent significant differences between and within temperature treatments. Parenthetical numbers are Q_{10}s between temperatures of the 100% Ration groups.

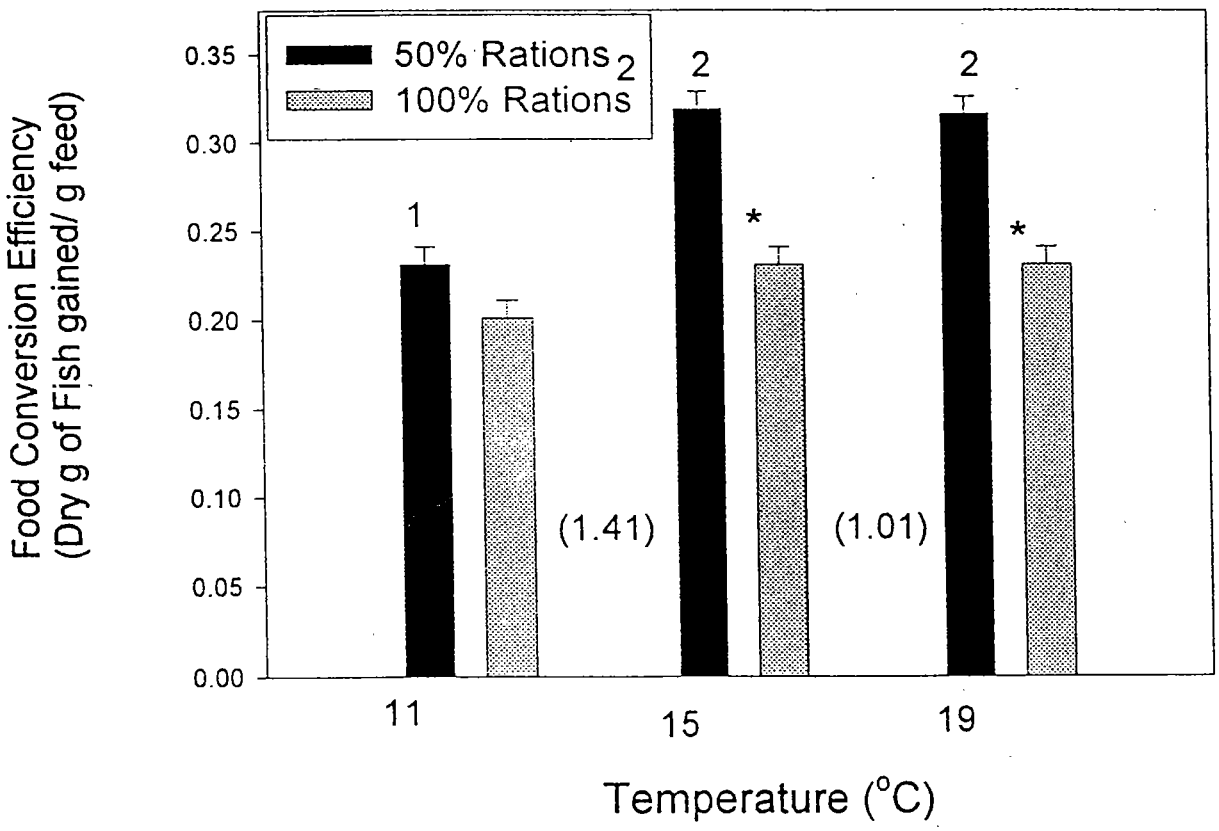

Food Conversion Efficiency
(Dry g of Fish gained/ g feed)
50% Rations
100% Rations
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1
2
2
*
*
(1.41)
(1.01)
11
15
19
Temperature (°C)

Figure 4- Mean ± SEM estimated oxygen consumption rate (MO2) and activity (tailbeats) of age-0 green sturgeon (mean wet weight: 180 ± 2.5g) acclimated to 11, 19, and 24°C. Different numbers and letters indicate significant temperature-associated differences between MO_2s and tailbeats, respectively. Parenthetical numbers are Q_{10}s between temperature treatments.

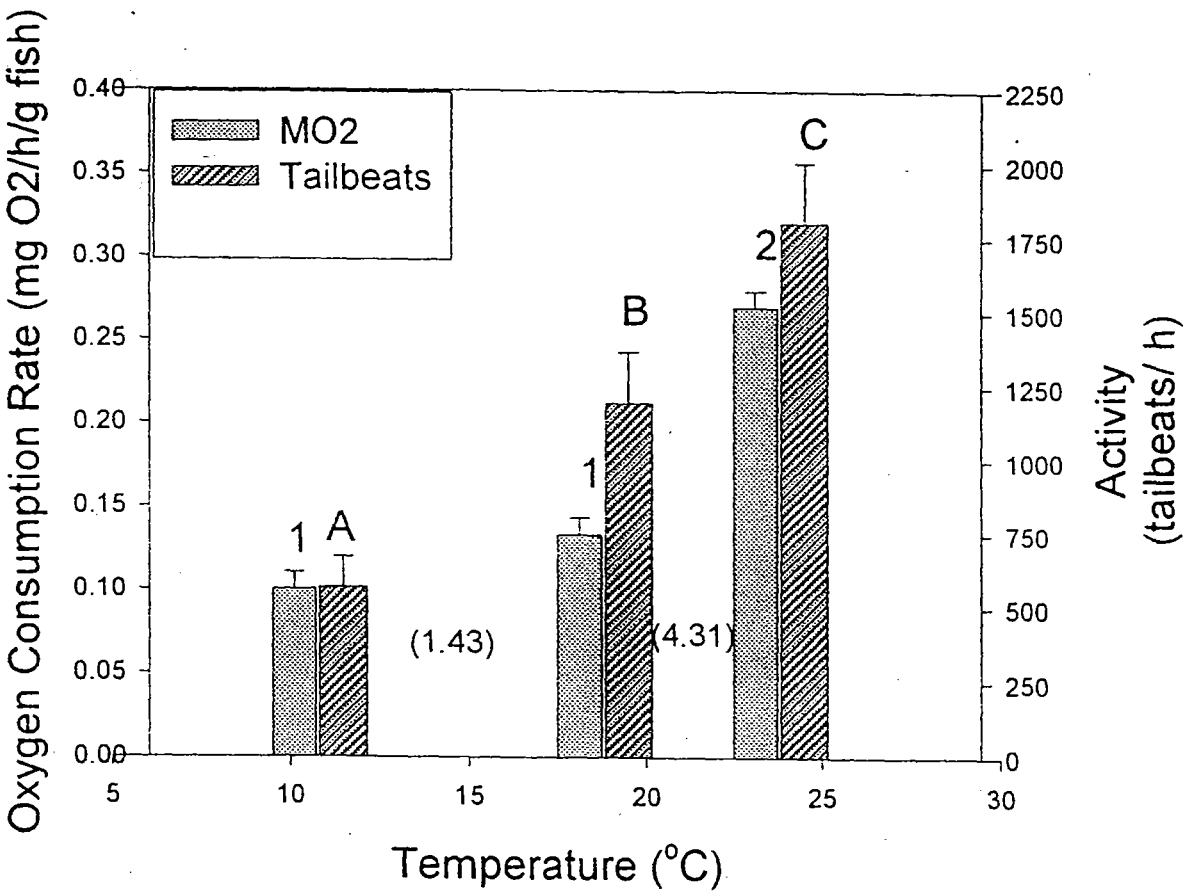

Oxygen Consumption Rate (mg O2/h/g fish)
Activity (tailbeats/ h)
Temperature (°C)
MO2
Tailbeats
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
2250
2000
1750
1500
1250
1000
750
500
250
0
5
10
15
20
25
30
1 A
(1.43)
1 B
(4.31)
2 C

Figure 5. Mean ± SEM estimated oxygen consumption rate (MO2) and ventilatory frequency (VF) of age-1 green sturgeon (mean wet weight: 880.7 ± 25.4g) acclimated to 11, 19, and 24°C. Different numbers and letters indicate significant temperature-associated differences between MO_2s and VFs, respectively. Parenthetical numbers are Q_{10}s between temperature treatments.

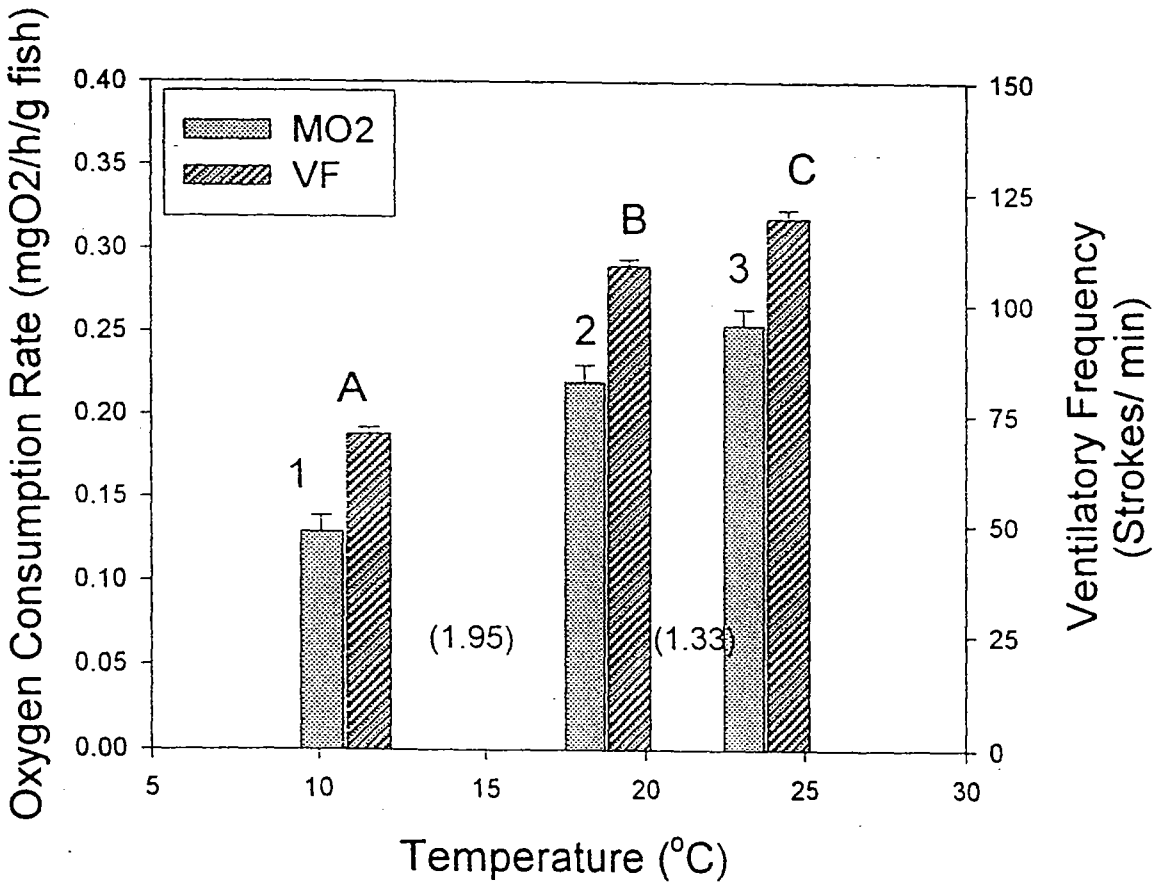

Oxygen Consumption Rate (mgO2/h/g fish)
Ventilatory Frequency (Strokes/ min)
Temperature (°C)
MO2
VF
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
150
125
100
75
50
25
0
5
10
15
20
25
30
1
2
3
A
B
C
(1.95)
(1.33)

Figure 6. Mean ± SEM estimated preferred temperature of age-0 green sturgeon (65.0 ± 2.5g mean wet weight) acclimated to 11, 19, and 24°C. The asterisk indicates a significant difference ($p < 0.05$) in mean preferred temperature.

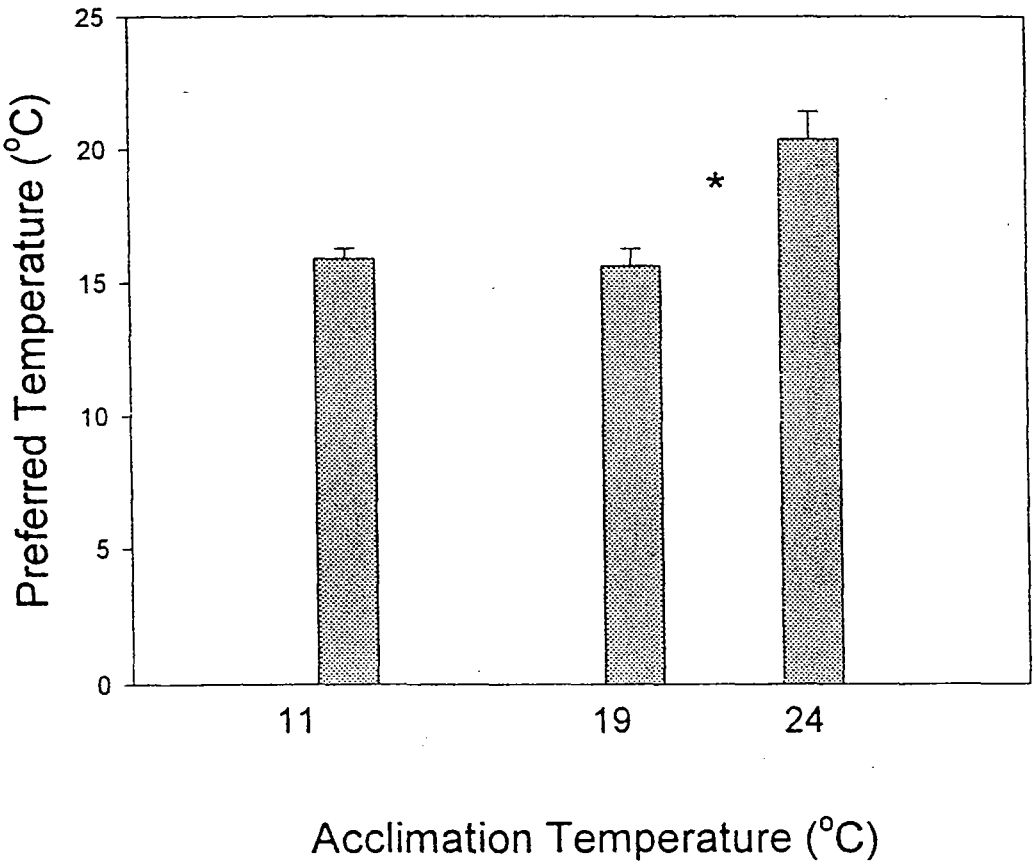
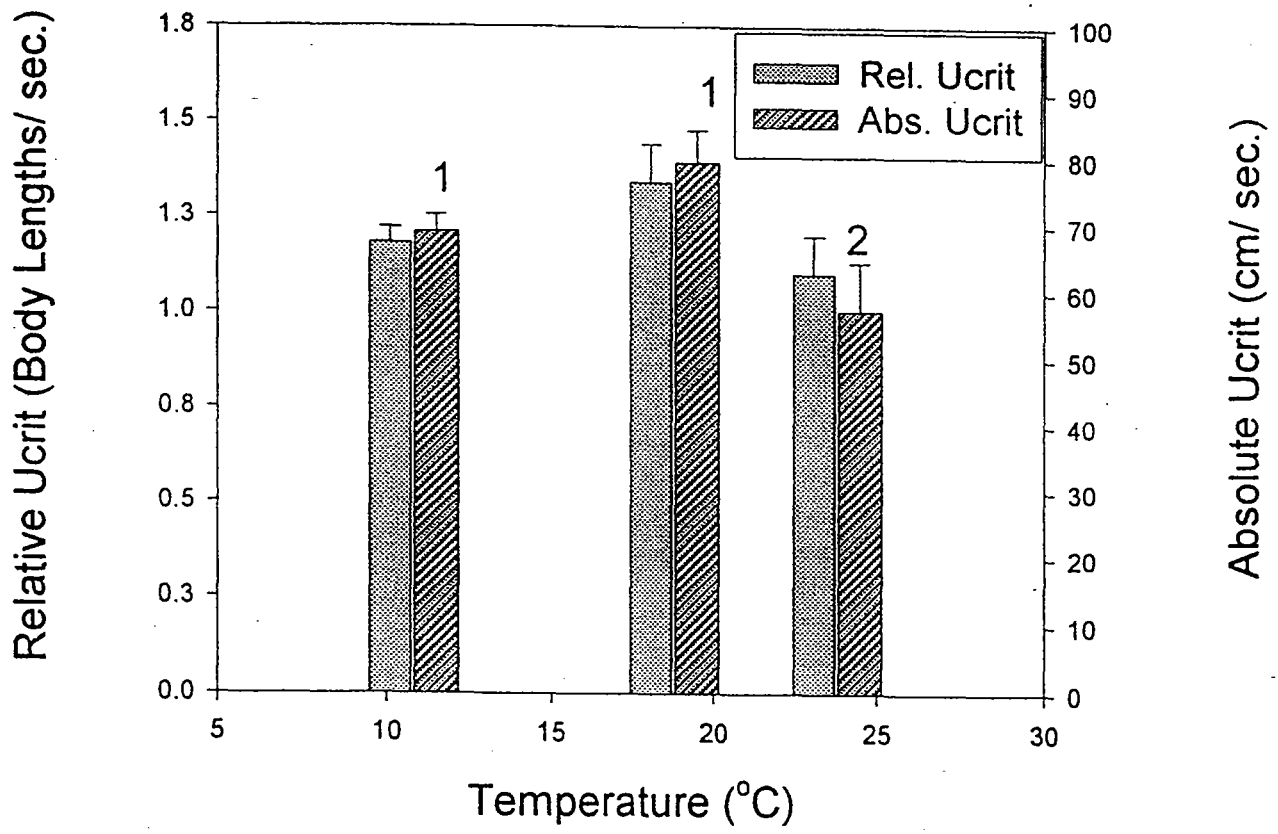

25
20
15
10
5
0
Preferred Temperature (°C)
*
11
19
24
Acclimation Temperature (°C)

Figure 7. Mean ± SEM of relative and absolute swimming performances of age-1 green sturgeon (mean wet weight: 1125.7± 68.2 g) acclimated to 11, 15, and 19 °C. Different numbers on bars represent significant differences between absolute swimming performances between temperature treatments.


Relative Ucrit (Body Lengths/ sec.)
Absolute Ucrit (cm/ sec.)
Rel. Ucrit
Abs. Ucrit
1
1
2
1.8
1.5
1.3
1.0
0.8
0.5
0.3
0.0
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
Temperature (°C)

Phase 2, Task 2: Reproductive and Developmental Characteristics of Green Sturgeon

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Biological Assessment of Green Sturgeon (*Acipenser medirostris*) Task 2: Reproduction and Culture

CALFED Bay-Delta Program
Phase 2, Project # 98-C15, 2002

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INTRODUCTION

Anadromous green sturgeon is considered a vulnerable species in the United States and Canada (Moyle et al., 1994; Campbell, 1997) and an endangered species in Russia (Artyukhin and Andronov, 1990). The American green sturgeon is less abundant than white sturgeon but supports small commercial and tribal fisheries. The abundance of green sturgeon stocks may depend on their reproduction occurring in a few rivers. The only known spawning populations of the green sturgeon in North America are in the Klamath, Rogue and Sacramento Rivers, all of which have flow regimes affected by water projects (Moyle et. al., 1994).

Knowledge of green sturgeon reproductive biology is necessary for the development of an effective stock management program. The goal of our studies is to elucidate the reproductive biology of green sturgeon, including gonadal development, spawning, and early life history. The specific objectives are: (1) to investigate reproductive characteristics of green sturgeon broodstock; (2) to develop captive breeding and culture methods; (3) to characterize development and environmental requirements of embryos and larvae.

We focus on the green sturgeon stock of the Klamath River, where the studies on reproduction are more feasible due to the presence of significant spawning run and collaboration with the Yurok Tribe's Fishery Program. The results of Phase 1 studies on artificial reproduction and early development of green sturgeon are described in Van Eenennaam et al. (2001) and Deng et al. (2002), and detailed information on early development was presented by Deng (2000). This report includes our observations on the reproductive characteristics of broodstock, captive breeding, and laboratory experiments with embryos and larvae of green sturgeon conducted during 2000-2002.

REPRODUCTIVE CHARACTERISTICS OF BROODSTOCK

Methods

Seventy-four adults (30 females and 44 males) were sampled during the spawning migration in April-June (1999-2000), in collaboration with the Yurok Tribe's Fishery Program. Fish were caught by the fishermen (using gill nets with mesh size 17-19 cm) in an area between the mouth of river and confluence of the Klamath and Trinity Rivers (RK 70). Tribal biologists measured total and fork length (L and Lf), fish weight (W), and the gonad weight, with an accuracy 0.1 cm, 500 g, and 25 g, respectively. Ovarian subsamples were collected to estimate fecundity (F), oocyte diameter (OD), and polarization index (PI), and a pectoral fin ray was removed for ageing. Fecundity was estimated by density of eggs in five subsamples multiplied by gonad weight, the OD (n=20 for each fish) and PI (n=15 for each fish) were measured by digital image-analysis (±0.01 mm). The oocyte PI is the distance of cell nucleus from the animal pole divided by oocyte diameter, measured on bisected eggs (formalin fixed). The low PI-values indicate advanced stages of prespawning nuclear migration in the oocytes, with a threshold for spawning readiness at PI 0.100. Gonadosomatic index (GSI) and age of fish were determined as described for Atlantic sturgeon (Van Eenennaam et al. 1996). The statistical analysis employed distribution parameters, t-test, and regression analysis performed on SAS software. The accepted significance level was $P<0.05$.

Results

The spawning run of green sturgeon in the Klamath river extends from April through June, with the majority of fish captured in the month of May (Figures 1 and 2). The spawning run coincides with an increase of river temperature, which may rise above the normal spawning range of sturgeons in June or even in May (Figure 1, for average water year of 1999). The increases of river temperature vary from year to year, depending on river flow.

All captured females had oocytes in an advanced stage of nuclear migration, with mean oocyte PI = 0.041 (Table 1) and all individual PI's below 0.100 (Figure 2) indicating spawning readiness upon entering the river. Frequent captures of milting males and 100% response of broodfish to hormonal injections (see "Breeding and culture") support this observation. Female broodfish were larger and older, compared to males, with significant differences in mean values (Table 1) and a distinct modal class in length (Figure 3). Females ranged in body weight 34-60 kg and males ranged 21-55 kg. Both sexes had fully grown gonads and high GSI (ranges 9-16% in females and 2-7% in males).

Green stugeon had large eggs, compared to other species of Acipenseriformes. The mean diameter of fully grown oocytes was 4.35 mm (Table 1) and ranged from 4.11 to 4.52 mm. There was no significant correlation between oocyte diameter and female length. The mean fecundity of green sturgeon was 144,000 eggs (range 81-205 th), at mean Lf = 182 cm. The relationship between fecundity and fork length fits an allometric function $F= 0.008* Lf^{3.213}$ ($r^2 = 0.52$,

n=22), with low predictive power. Compared with white sturgeon (DeVore et al., 1995), the individual fecundity of green sturgeon is about 60% of that in white sturgeon of similar length (Figure 4).

Table 1. Characteristics of sturgeon broodstock (mean ± SD (n)). Means differ between sexes (P<0.05).

Observations	Females	Males
L (cm)	197.7 ± 12.2 (30)	175.6 ± 15.0 (44)
Lf (cm)	181.3 ± 12.0 (30)	161.0 ± 13.7 (43)
W (kg)	46.3 ± 7.6 (26)	30.4 ± 8.4 (37)
Age (yr)	27 ± 4 (30)	20 ± 4 (44)
GSI (%)	12.9 ± 2.3 (24)	5.0 ± 1.3 (33)
F (th)	144 ± 28 (22)	-
OD (mm)	4.35 ± 0.14 (28)	-
PI (ratio)	0.041 ± 0.14 (28)	-

CAPTIVE BREEDING AND CULTURE

Methods

Broodfish were captured as described in the previous section and held in 1.2 x 2.1 x 1.2 m submerged cages. Seven females and 12 males were used for spawning during the four-year study (Table 2). Spawning was conducted on site (Klamath River near Weitchpec) or at UC Davis, after transport (7 h) of the broodfish in an insulated tank with oxygenated water. Spawning and culture procedures followed initially the protocol for white sturgeon (Van Eenennaam et al., 2001) but were modified later. Fish were weighed (± 0.1 kg), measured (± 0.5 cm), and fifty fully-grown follicles were collected by catheter to determine responsive stage of ovarian maturity (PI<0.100). To induce spermiation and ovulation, males received a single injection of 10 μg/kg GnRHa [D-Ala^6, Des-Gly^{10}] –LH-RH Ethylamide (Peninsula Laboratories), while females received priming (2 μg/kg GnRHa + 1 mg/kg of domperidone) and

resolving (18 μg/kg GnRHa + 3 mg/kg domperidone) injections, with 12-h interval. Female treatment was modified in 2001-2002, using GnRHa alone (0.5 μg/kg) for the first injection, and GnRHa (20 μg/kg) with domperidone (1-2 mg/kg) for the second injection. River temperatures during broodstock capture and spawning varied from 13 to 20°C. Gamete collection followed Conte et al. (1988) and sperm was evaluated for motility (5 μL of sperm : 200 μL of river water) under a compound microscope. The eggs were fertilized in vitro by semen diluted 1:200 with river water and gently mixed in a suspension of Fuller's Earth (Sigma Chemical Co., 80 min) to prevent adhesion. The number of collected ova was estimated volumetrically. Fertilization technique was modified in 2001-2002, by rinsing ova with water before fertilization (two rinses, 15-20 s each) and by using a higher semen density (1:100 dilution) and longer time for fertilization (4-5 min). Fertilized eggs were incubated in MacDonald jars, glass dishes submerged in the flow-through tanks and, more recently, in the commercial upwelling incubators (Eagar, Inc. Utah). Samples of 200-300 eggs were collected at cleavage (stages 5-6, Dettlaff et al., 1993) to determine fertilization rates. Hatching rate was estimated by volumetric count of larvae.

Larvae were reared to metamorphosis (35-45 d post hatch) in circular 1.2 m diameter, 355 L capacity tanks at temperatures 16-19° C and density 2000/tank, with spray bars at the water inlet to create a circular current (Conte et al. 1988). Each tank had three automatic feeders (The Fish Sitter) placed on the tank wall at equal distance apart. Fish were fed a semi-moist fry feed (Nelson & Sons, Inc.) distributed every hour over a 24-h period (15 to 150 g per tank throughout rearing period). Feeding started with small amount of food on day 8 post hatch to ensure the availability of food at first feeding, which occurs at 12-16 d post hatch in population. Larval survival to metamorphosis ranged 70-95 % and mean weight 1.6 – 2.5 g in different trials.

Results

With the availability of a limited number of green sturgeon broodstock and the remote location of the capture site from UC Davis, the improvement of breeding was achieved empirically, by modifying methods established for white sturgeon. The results of spawning trials with green sturgeon conducted to date are shown in Table 2, and some procedures are illustrated in Plate 1.

All broodfish responded with spermiation and ovulation to hormonal treatment, but fertilization and hatching rates were low and variable during 1999-2001 (Table 2). Factors responsible for these spawning results could be the condition of fish (stress and injuries at capture), condition of river (rising temperature in May), and the unsuitability of MacDonald jars for the incubation of green sturgeon eggs. Reproductive performance improved in 2002, when broodfish were captured early in the season (late April, during low river water temperatures 12.7-13.7°C), and the methods of artificial fertilization and incubation were modified. Fertilized eggs

of all seven females spawned to date had low adhesivity and a weak attachment to substrate, as we reported early for two females spawned in 1999 (Van Eenennaam et al., 2001).

Table 2. Results of green sturgeon spawning, 1999-2002. Spermiation was induced by 10μg kg^{-1} GnRHa, ovulation by 20 μg kg^{-1} GnRHa + 1-3 mg kg^{-1} domperidone. Eggs were incubated in McDonald jars, glass dishes, and upwelling incubators (see Plate 1).

Female ID	1999-1	1999-2	2000-3	2000-4	2001-5	2002-6	2002-7
Date of spawn	May 20	May 20	May 12	May 24	May 24	Apr 28	Apr 29
Site of spawn	River	River	UCD	River	River	UCD	UCD
River T° C	13-14	13-14	13	16–17	18-20	13	13
Female W, kg	38	48	38	41	35	50	56
Fertilization, %	26	41	40	40	78	95	80
Hatching, %							
McDonald jars	1	28	0	0	0.3	0	0
Dishes	-	-	38	12	-	-	-
Upwell. incub.	-	-	-	-	-	70	70
Fry produced, th	1	23	4	1	2	26	39

Based on the reproductive conditions of green sturgeon broodstock (previous section) and our experience with spawning, we have developed the following preliminary guidelines for best culture practices with green sturgeon.

Broodstock capture and handling. For best spawning results, broodfish must be captured in April, when the river temperatures do not exceed 14° C. Fish can be held in river cages for a maximum 3 days before hormonal injection, or they can be transported in an insulated tank with oxygenation to campus (7-h transport time). While mature green sturgeon are present in the Klamath River from April to June, changes of flow and temperature in May-June (Figure 1) increase susceptibility of brood fish to stress, which results in poor gamete quality. Since green sturgeon enter the Klamath River ready to spawn (Table 1: oocyte PI < 0.100), the ovarian biopsy for measuring oocyte PI and GVBD bioassay (Van Eenennaam et al., 2001) do not appear to be needed before hormonal treatment. The hormonal treatment should be applied within 3 days of capture.

Spawning induction, gametes handling, and fertilization. Best hormonal treatment protocols to date were the single injection of males with 10 μg/kg GnRHa and two injections for females (first with 0.5 μg/kg GnRHa and a second injection with 19 μg/kg GnRHa + 1 mg/kg domperidone, 12 h later). Spermiation and ovulation occur in 24 and 26 h after the first injection. Ovulation latency (time between 2nd injection and ovulation) is shorter in green sturgeon (14 h), compared to white sturgeon (20-21 h) at similar temperatures. Gametes are collected by catheter (semen) and cesarean section (ova), as for white sturgeon (Conte et al., 1988). Collection of ova by stripping, although not systematically attempted, is more problematic in green sturgeon due to the large size of eggs. Typical sperm motility in green sturgeon is 3-4 min, longer than in white sturgeon (Van Eenennaam et al., 2001). Rinsing ovulated eggs, higher sperm density (1:100 dilution, 4 L of diluted semen per 1 L of eggs), and a longer fertilization time significantly improved fertilization success.

Egg incubation. Green sturgeon eggs have a thinner and softer chorion, compared to white sturgeon, resulting in high mortality during the incubation in McDonald jars used for white sturgeon (Table 2). Small batches (300-500 eggs) can be incubated in glass dishes kept in tanks with flowing water, but this procedure is impractical for incubation in mass because it requires daily manual removal of unfertilized eggs and dead or abnormal embryos. We had our best results with the upwelling incubators (Eagar Inc., North Salt Lake, UT), successfully tested in 2002 (Table 2 and Figure 7). The optimal environmental conditions for green sturgeon eggs incubation appear to be similar to those for white sturgeon (Conte et al., 1988). The rate of development to hatching is also similar to white sturgeon (Deng et al., 2002). In contrast to white sturgeon, hatched green sturgeon do not swim up and must be siphoned from the incubators.

Larval rearing. Larval rearing, including transition to exogenous feeding, has been very successful in green sturgeon culture, due to the large size (length 15 mm at hatch) and robustness of larvae. Larvae are reared in circular tanks (Figure 7) at temperatures 15-18 °C. They exhibit negative phototaxis, strong clumping behavior, and do not have a post-hatching pelagic phase characteristic of white sturgeon (Figure 7). Green sturgeon larvae also have a longer period of endogenous feeding (ca.15 d post hatch at 15-18 °C) due to the larger yolk sac, compared to white sturgeon. They readily accept commercial diets, but survival can be improved by supplementing with life food (chopped Tubifex worm) during the first two weeks of feeding (Van Eenennaam et al., 2001). The survival from hatching to metamorphosis ranged 70-95 % in different trials. Metamorphosis is completed at age 45 d and weight 2.5 g (Deng et al., 2002).

EARLY LIFE STAGES

Methods

Effect of temperature on embryo survival. The ova of two females spawned in April 2002 (Table 2) were fertilized by sperm from three males (female 6 by pooled semen of two males,

female 7 by semen of one male). Fertilization was conducted at 15.5° C. Fertilized eggs were incubated to hatching at six different constant (± 0.1° C) temperatures: 16, 17.5, 19, 20.5, 22, 23.5° C, in five replicate tanks for each treatment level and each female. Following the artificial fertilization and 20-min silt treatment, 15 ml (about 270) of eggs were placed into individual 190 mm crystallizing dishes with 1.5 L of 15.5° C well water, and dishes were allowed to float 20 min in tanks at each temperature step for acclimation. After the acclimation, the dishes were submerged. Flow rate through each tank was ca. 0.5 gpm. Unfertilized eggs, dead and unhatched embryos were removed daily and counted at early cleavage, neurulation, and hatching. Larvae hatched in each tank were euthanized (MS-222) and fixed in buffered formalin. We are currently processing samples preserved to determine percent of hatching, rates and types of abnormalities at hatching, and length of hatched larvae in each temperature. After completion of sample processing, we will analyze these data using one-way analysis of variance, with six temperature treatments, two replications (females), and five subsamples (tanks).

Resting metabolism of green sturgeon larvae. This experiment was conducted in collaboration with the laboratory of Professor Joseph Cech. The larvae were obtained from a single spawning in 2001 (Table 2). One thousand hatched larvae, divided in two equal groups, were held in circular tanks at constant temperature 16° C and artificial photoperiod 12L:12D. Food (commercial diet + chopped Tubifex worm) was presented to one group from 12 d post hatch, while the other group was food-deprived for entire duration of the experiment (31d post hatch). Larvae (n=10) from each treatment were sampled daily to determine oxygen consumption (per larva and mass-specific) rates, using static-type respirometers (60 ml glass syringes) as described by Marty et al. (1990). The PO_2 was measured using the oxygen analyzer (PHM71/MK2 Radiometer, Copenhagen). Sampled larvae were weighed on a microbalance (accuracy 0.01 mg) and preserved for histological study. Oxygen consumption rates of fed and starved larvae were compared using t-test. The relationships between oxygen consumption and body weight were determined using allometric function (Rombough, 1988).

Histological development of digestive system in feeding and starving larvae. Formalin-fixed larvae from the above were utilized to examine development of digestive system from hatching to 31d post hatch and the effects of food deprivation. Larvae were dehydrated in ethanol, embedded in paraffin, sectioned at 4-6 m, and stained by hematoxylin and eosin for topographic observations. In addition, slides were stained by periodic acid-Schiff reaction to reveal neutral mucosubstances, and by Alcian Blue at pH 2.5, 1.0, and 0.5 to reveal glycoconjugates and sialic acid (with HCl hydrolysis). Tissue differentiation of digestive organs (gut, liver, and pancreas) and the degenerative changes caused by starvation were examined using light microscopy and by measuring height of the epithelial cells from the different regions of digestive tract (Bisbal and Bengtson, 1995).

Results

Effect of temperature on embryo survival. The experiment with the offspring of two females has been completed in May 2002 and the samples are being processed. Incubation temperature 23.5° C was lethal for the embryos from both females (all died during gastrulation).

At temperature 22° C the embryos from both females exhibited 90-100% abnormalities at neurulation, with 0% hatching in one female and ca. 35% hatch of abnormal embryos in the other female. Hatching occurred at all other temperatures, with very few deformities at 16 and 17.5° C, and with moderate deformities at 19 and 20.5° C. The estimates of incubation mortalities are similar to those in a pilot experiment reported in Phase 1 (Doroshov et al., 2000), suggesting that the river temperatures at or above 20° C are not suited for normal development of green sturgeon embryos. We will present the final analysis of this experiment in a manuscript for publication.

Resting metabolism of feeding and starving larvae. Food deprivation resulted in a decrease of larval body mass, presumably due to tissue resorption (Figure 5). During the endogenous feeding stage (1-15 d post hatch), oxygen consumption by larvae ($\mu g\ O_2 larva^{-1} h^{-1}$) increased 5-fold before the yolk sac reserves became limiting and metabolic rates declined. The oxygen consumption rates decreased by 3-fold in food-deprived larvae from 15 to 31 d post hatch, when most larvae died (Figure 6). In the allometric functions expressing relationships between the oxygen consumption rate and body mass (aBW^b), the mass exponent was greater than 1.0 (b = 1.64) during the endogenous feeding phase and equal to unity (b = 1.04) during the exogenous feeding phase. The magnitude and changes of MO_2 in green sturgeon larvae reflected developmental events in early ontogeny, especially during the yolk utilization phase when the allometric increase in metabolic rate was associated with organogenesis, acquisition of organ functions, and with conversion of yolk material into new larval biomass. Similar observations were reported by Khakimullin (1984) for Siberian sturgeon, with mass exponents 1.31 and 0.85 for the endogenous and exogenous feeding phases, respectively. Compared with other species of sturgeon (Gershanovich et al., 1987), oxygen consumption rates of green sturgeon larvae were slightly higher, but such comparisons may be affected by variations of fish activity and environmental factors in experimental designs.

Digestive system of feeding and starving larvae. At hatching, the larval digestive system in green sturgeon consists of two rudiments: the large endodermal yolk sac and a primordial hindgut. During the endogenous feeding phase, the wall of the yolk sac differentiates into a stomach (glandular and non-glandular regions) and the anterior and intermediate intestine, while the hind-gut primordium differentiates into the spiral valve and rectum. At the onset feeding (15 d post hatch, at 16°C), the histological organization and a cytoarchitecture of the digestive tract are similar to those in juveniles, as in white sturgeon (Buddington and Doroshov, 1986; Gawlicka et al. 1995). Food deprivation caused progressive deterioration of larval digestive system after 5 days of starvation at temperature 16° C (Plate 2). Histopathological changes included shrinkage of digestive epithelia (statistically significant changes in cell height) followed by the severe atrophic changes in the tissues of digestive tract, liver, and pancreas. Histological analysis of digestive system provides sensitive indicators of the nutritional state in green sturgeon larvae and can be used for field observations in larval nursery habitat, where the food availability for larval stage depends on river flow.

CONCLUSIONS

Green sturgeon broodstock are generally similar in size, age, and sex composition to two other anadromous sturgeon species of North America, white (Kohlhorst et al. 1980; Chapman et al. 1996) and Atlantic sturgeon (Van Eenennaam and Doroshov, 1998). The GSI of green sturgeon females is also similar to the other species, which indicates that low fecundity of green sturgeon is a function of larger egg size and greater investment of maternal yolk in embryo-larval development (Van Eenennaam and Doroshov, 2001; Deng et al., 2002). Under natural river conditions, the larger egg size would compensate for lower fecundity by increased survival of juveniles.

The spawning run of green sturgeon in the Klamath River extends from April to June, with an apparent spawning peak during the month of May, as indicated by broodfish capture and stage of maturity. The low oocyte PI in all females and the presence of milting males during the collection of broodfish for artificial spawning suggest that green sturgeon spawning sites are located in the middle reach of the lower river. Identification and characterization of green sturgeon spawning sites (our CalFed proposal, 2002) will provide the information needed for stock management in the Klamath River and will facilitate identification of spawning sites in the Sacramento River.

Unstable river flow during the spawning season appears to play significant role in reproduction of green sturgeon. The reduced flow and elevated temperatures may hinder the spawning run and survival of embryos and larvae, as indicated by our spawning trials and laboratory experiments. The studies with cultured white sturgeon clearly showed detrimental effects of temperatures 15-20° C on the final ovarian maturation and spawning (Webb et al., 1999; Linares-Casenave, 2000; Webb et al, 2001). Our recent (2002) laboratory observations confirm that temperatures above 20° C are lethal for green sturgeon embryos (similar to white sturgeon, Wang et al., 1985).

In conclusion, our studies in Phase 2 provide additional information on reproductive conditions of broodstock, captive breeding, and early life stages of green sturgeon. We plan to continue investigations of reproductive biology and early life history of this species, including gonadal development and temperature effect on larval stage. We will continue development of captive breeding techniques, useful for research and conservation hatchery.

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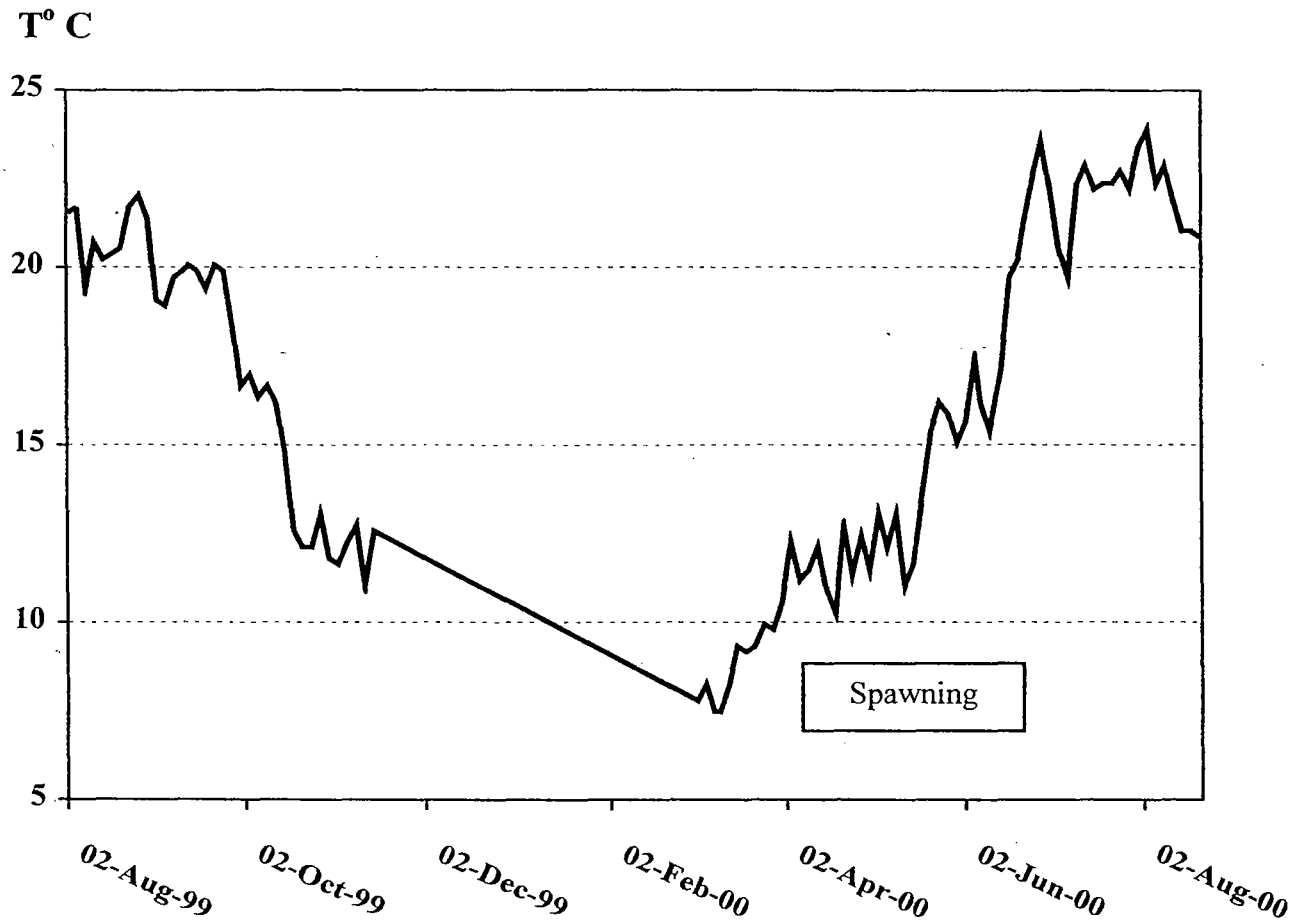

Figure 1. Lower Klamath River temperatures and time-interval of green sturgeon spawning run at Weitchpec (data from Yurok Fisheries Program)

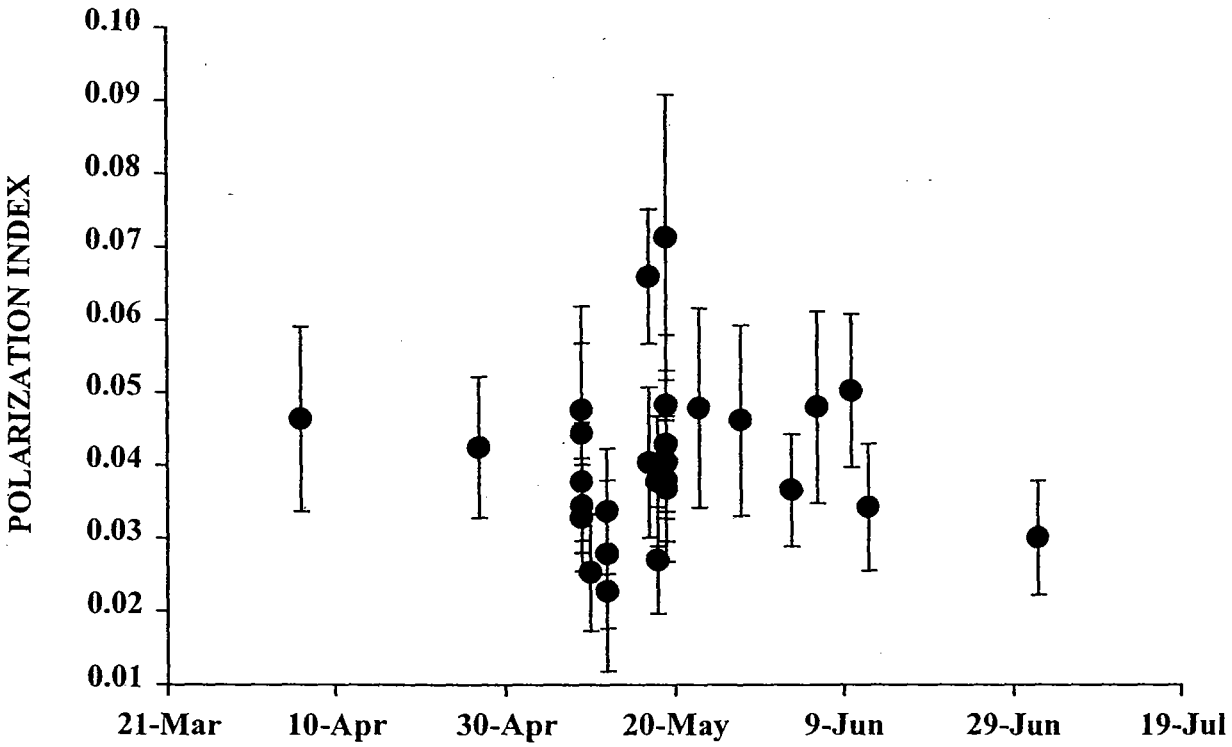

Figure 2. Oocyte polarization index (mean ± SD) in green sturgeon females during the spawning migrations 1999-2000, in the lower Klamath River (samples collected by the Yurok Fisheries Program)

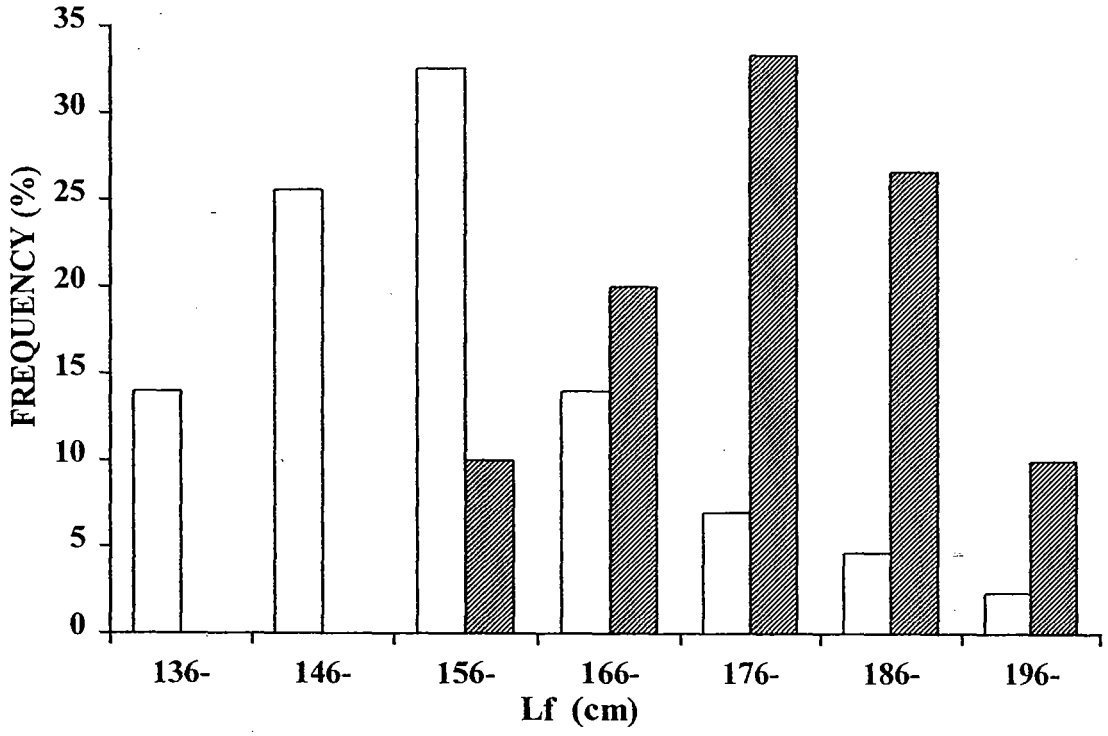

Figure 3. Frequency distribution in fork length for male and female green sturgeon, in the lower Klamath River (1999 - 2000)

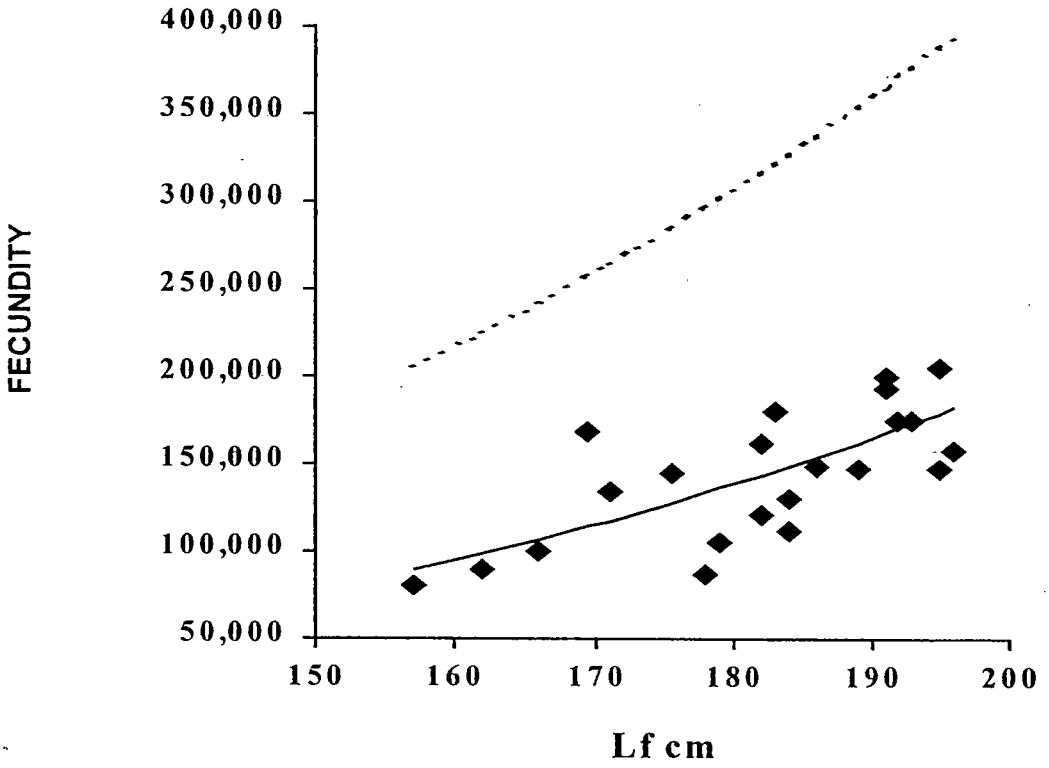

Figure 4. Relationship between fecundity and fork length in green sturgeon, Klamath River (line and scatter), compared to white sturgeon from Columbia River (dotted line). Equation for white sturgeon from DeVore et al., 1995: $F = 0.072 * Lf^{2.94}$.

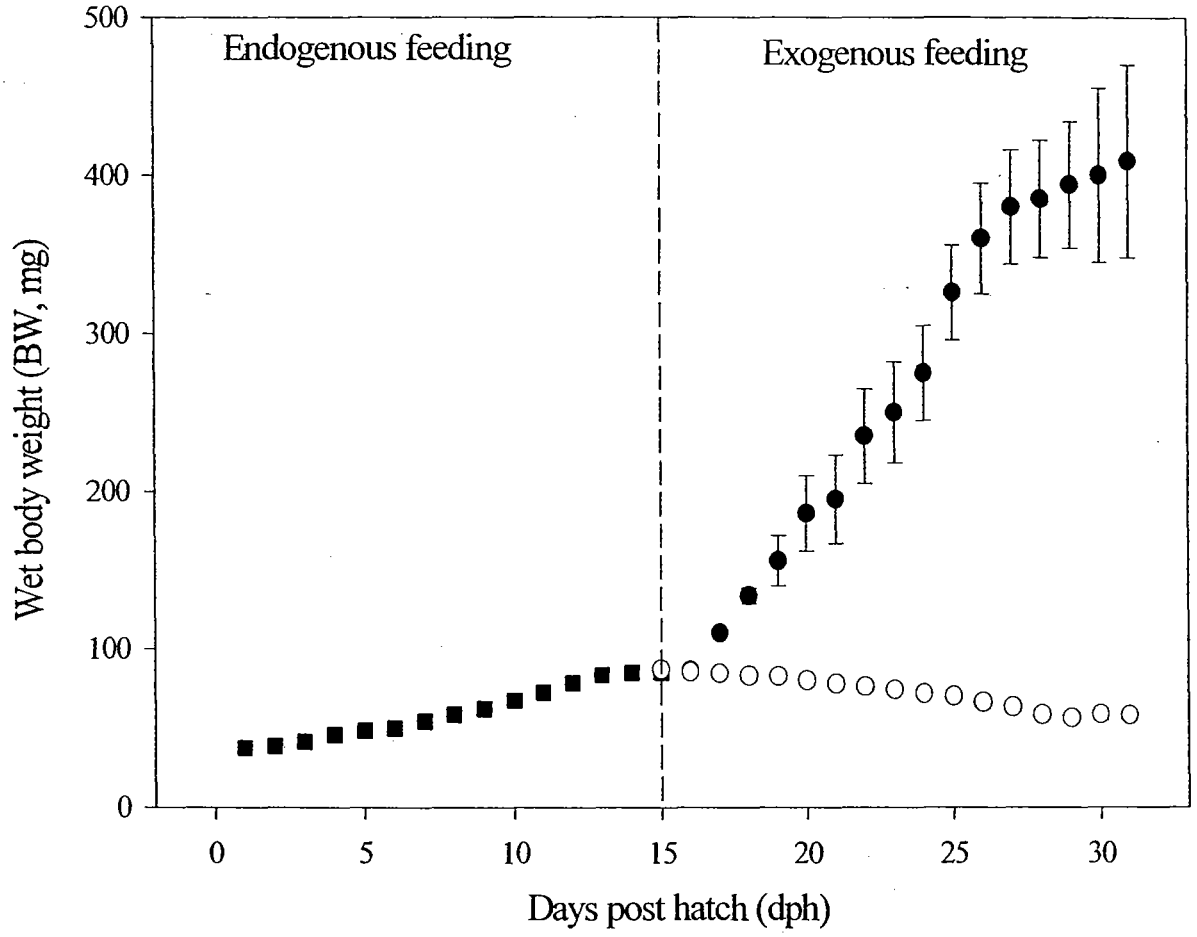

Figure 5. Wet weight of green sturgeon larvae (mean ± SD, n=10). Open symbols represent food-deprived larvae

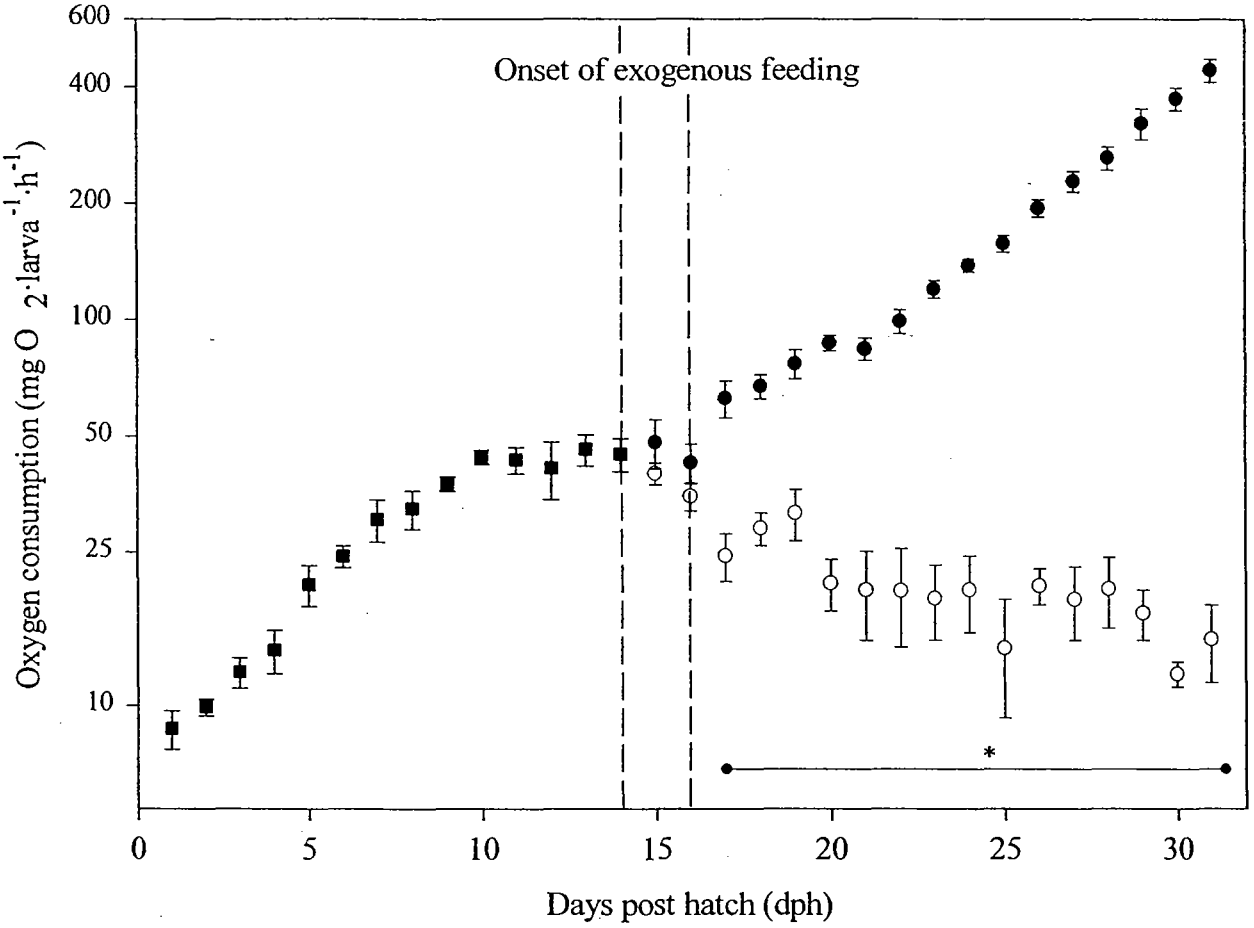

Figure 6. Oxygen consumption by feeding and food-deprived (open symbols) green sturgeon larvae (mean ± SD, n=10). Asterisk and horizontal bar denote significantly different means ($P<0.05$)

PLATE 1 (3 pages)

1. Klamath river near Weitchpec, at the confluence with Trinity river
2. The Ishy Pishy Fall on the Klamath river (upstream from Weitchpec) believed to be an impassable barrier for upstream migration of green sturgeon
3. Adult green sturgeon captured by the gill net, near Weitchpec.
4. Ventral side of adult green sturgeon: fish has an elongated snout with numerous receptors, interrupted lower lip of mouth, and wide mid-ventral green stripe.
5. Mature female green sturgeon: the ovaries are longer but not as wide as in white sturgeon, the ovarian folds with fully grown eggs fill abdominal cavity.
6. Mature male green sturgeon: testes are not as large as the ovaries and have lobular structure.
7. River cage for holding and spawning green sturgeon
8. Transfer of brood fish (using tube net), from the cage to river bank for spawning
9. Collection of semen
10. Collection of ovulated eggs
11. Bathing fertilized eggs in silt suspension
12. Upwelling egg incubator (Eagar Inc.): water is supplied from the bottom through through the flat screen to create relatively uniform upwelling flow
13. McDonald jar: water is supplied from the top by central pipe, flow is reversed upward by the sphere at the bottom; the eggs are rotated in suspension and are exposed to significant mechanical forces at the bottom of jar
14. Circular tank for larval green sturgeon: note the spray bar (water inlet), authomatic feeders, and central standpipe with screen
15. Green sturgeon larvae exhibit prominent clumping behavior in tank, from hatching through the onset of feeding
16. White sturgeon larvae exhibit pelagic behavior lasting 4-5 days after hatching
17. Hatched green sturgeon larva (TL ~15 mm), at low and high magnifications: note the large ovoid yolk sac and position of heart in the concavity of antero-ventral yolk sac
18. Green sturgeon larva at age 5 d after hatching (TL ~ 20 mm), at low and high magnifications: note differentiated intestine (ventral to stomach rudiment filled with yolk), liver (anterior to stomach), and gills (not fully covered by the operculum).


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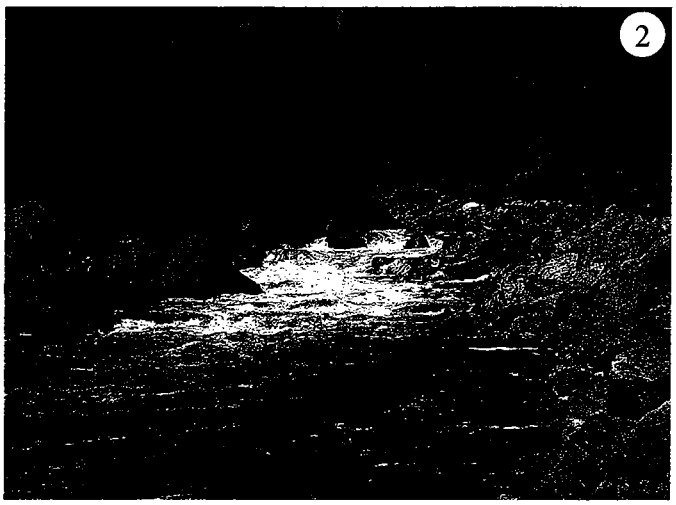
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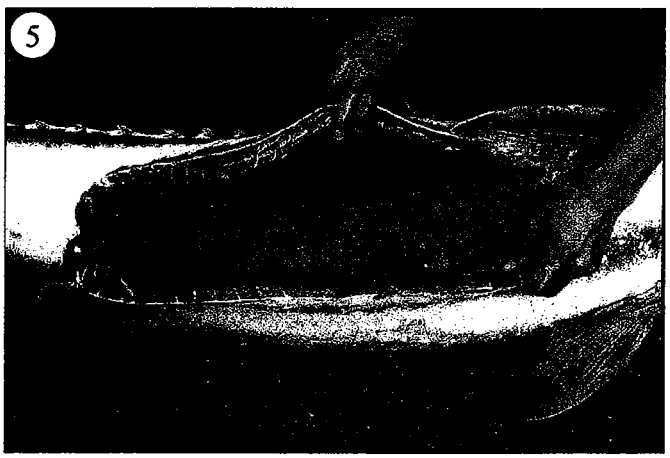
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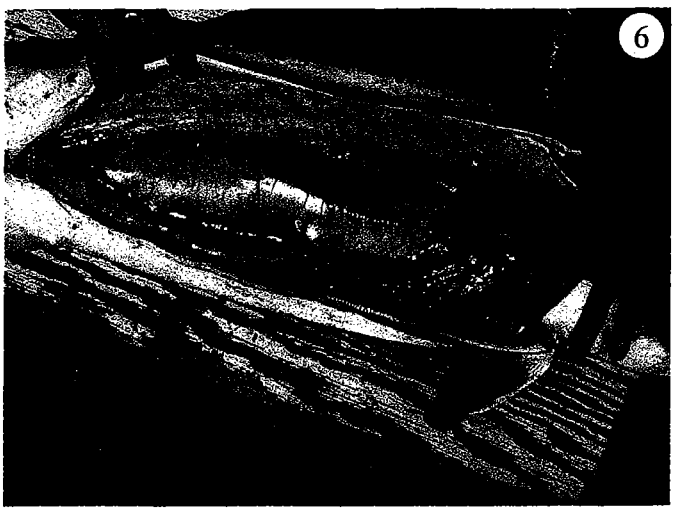
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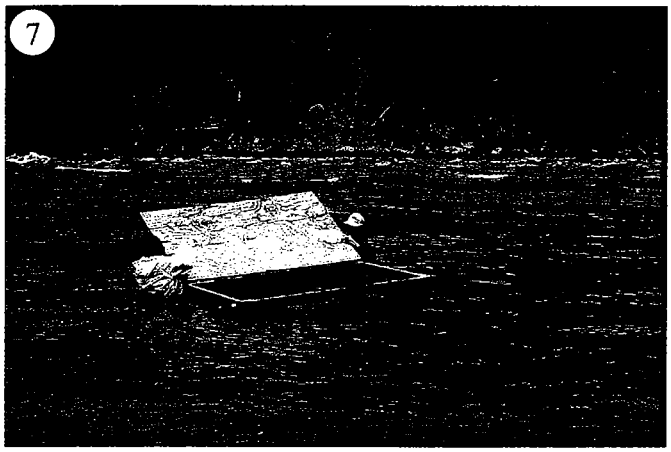
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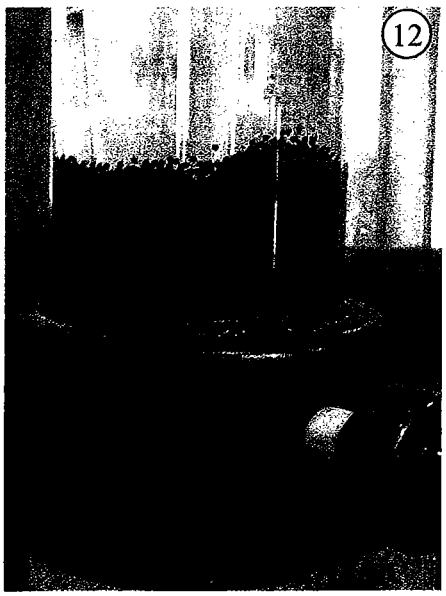
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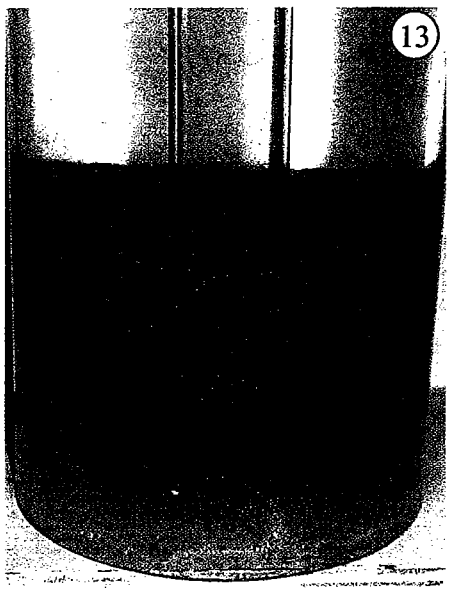
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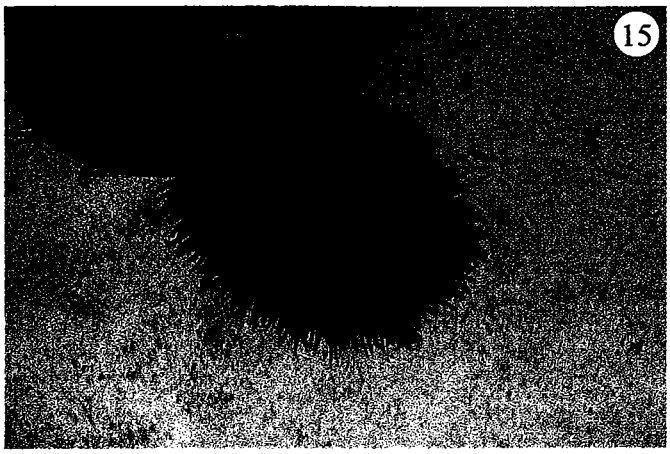
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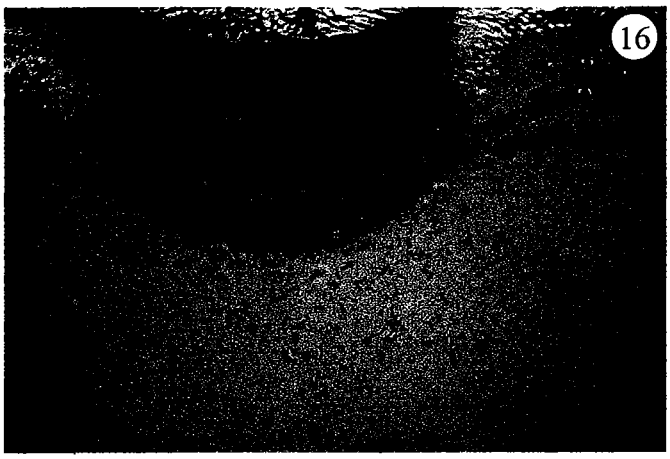
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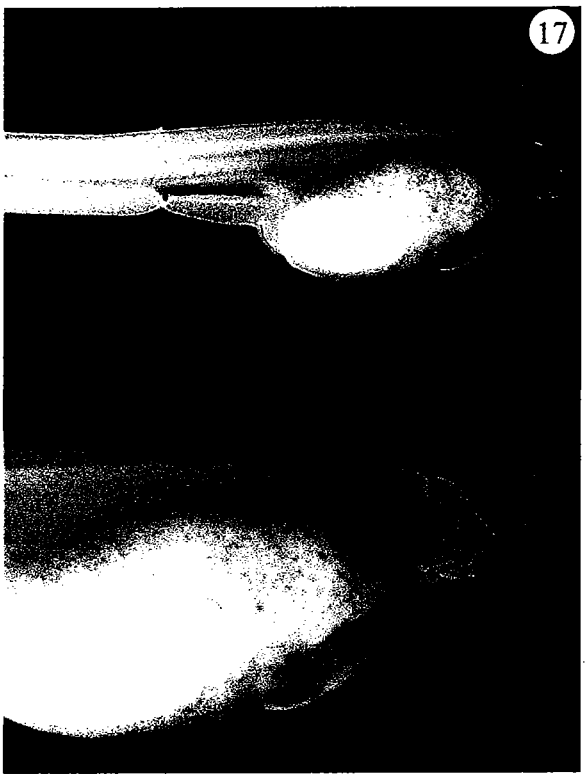
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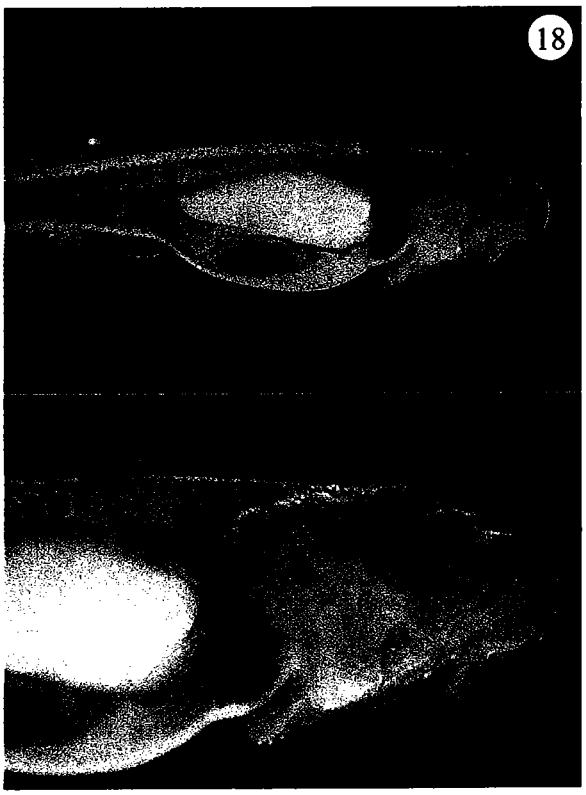
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PLATE 2

Photomicrographs of digestive organs (histological slides) in feeding and food-deprived green sturgeon larvae:

a) liver, feeding larva at 25 d post hatch: normal tissue structure with polygonal hepatocytes around the sinusoids

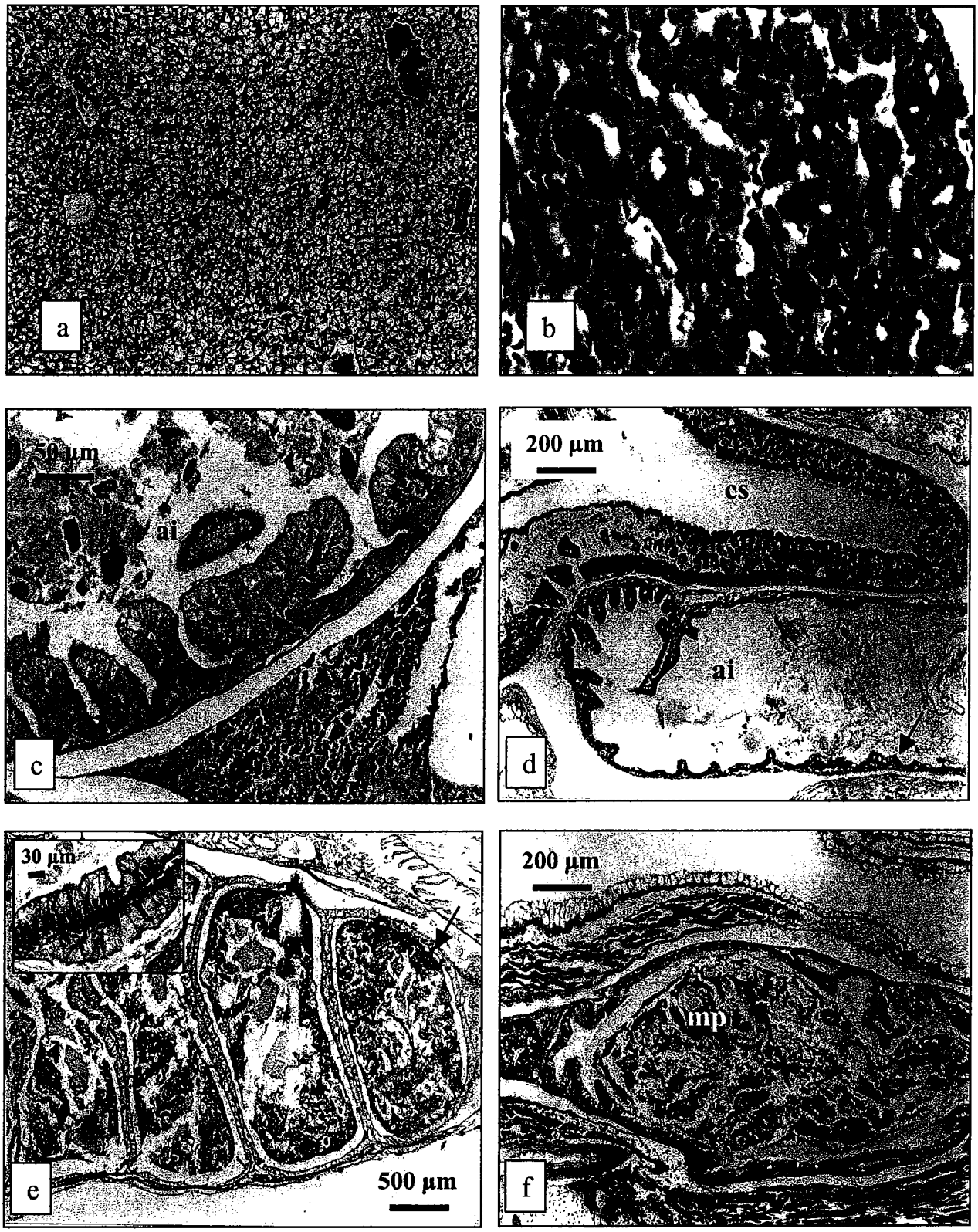
b) liver, starving larva at 27 d post hatch: abnormal structure, with large intercellular spaces, collapsed cytoplasm and pycnotic nuclei in hepatocytes

c) anterior intestine (ai) and pancreas (pa), feeding larva at 20 d post hatch: normal structure, with intestinal folds

d) cardiac stomach (cs) and anterior intestine (ai), starving larva at 28 d post hatch: reduction of folding, thinning of mucosa (arrow) and collapsed enterocytes

e) spiral intestine, feeding larva at 25 d posthatch: normal structure, spiral valve walls with darkly stained mucous cells (arrow). Inset: normal arrangement of the enterocytes in a simple layer

f) spiral intestine, starving larva at 27 d post hatch: abnormal structure, with thin mucosa, reduction in folding, and presence of the "melanin plug" with a dark pigment (normally discharged at the onset of feeding)


a
b
50 µm
ai
c
200 µm
cs
ai
d
30 µm
e
500 µm
200 µm
mp
f

Publications and manuscripts (Task 2)

Deng, X. 2000. Artificial reproduction and early life stages of the green sturgeon (*Acipenser medirostris*). M. S. Thesis, University of California, Davis.

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Presentations (Task 2)

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Artificial Spawning and Larval Rearing of Klamath River Green Sturgeon

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Abstract.—Two female and five male prespawning green sturgeon *Acipenser medirostris* that were caught and held in cages in the Klamath River, California, were induced to spawn by injections of gonadotropin releasing hormone analog (GnRHa) and domperidone. All broodfish produced mature gametes for artificial fertilization and were sampled for age, body size, sperm motility, hatchery fecundity, and oocyte diameter. The females were estimated to be 25 and 32 years old, respectively; they weighed 38 and 48 kg and produced 52,000 and 82,000 ova. The mean diameters of fully grown oocytes in the two females were 4.52 and 4.24 mm. The males ranged from 18 to 30 years in age and from 23 to 55 kg in weight. Their sperm exhibited 100% motility in river water for up to 5 min. Ovulated eggs were fertilized with milt pooled from all five males, and the eggs were transported to university facilities in oxygenated bags and incubated in MacDonald jars. Fertilization rates were 26% and 41% for the two females' eggs. In all, 23,000 (28%) normal embryos hatched from the female with the higher fertilization rate; the eggs from the other female were discarded at 4 d owing to the low number of viable embryos (<5%). Five-d post hatch larvae were reared in circular flow-through tanks and fed a commercial semimoist diet, either alone or in combination with live *Tubifex* worms. The survival to metamorphosis (age 35 d, length 66.4 mm, and weight 1.78 g) was significantly higher for fish in the treatment with the combined commercial and live diet (74.2% versus 85.5%, $P < 0.05$), but there was no difference in the length and weight of juveniles.

The green sturgeon *Acipenser medirostris* is an anadromous species inhabiting Asian and American shorelines of the northern Pacific Ocean. Despite its wide geographic distribution and the occasionally large landings in the commercial fisheries of the West Coast (Houston 1988), the green sturgeon is considered a rare or vulnerable species in the United States and Canada (Birstein 1993; Moyle et al. 1994; Campbell 1997) and an endangered species in Russia (Artyukhin and Andronov 1990). Unfortunately, there is a scarcity of literature on green sturgeon life history and population biology (Houston 1988; Moyle et al. 1994). The only known spawning populations in North America are in the Klamath, Rogue, and Sacramento rivers, California, all of which have flow regimes that are affected by water projects (Moyle et al. 1994). There is no information on the reproductive characteristics, spawning migrations, spawning and nursery habitats, and early life stages of the green sturgeon. Of the eight members of North American Acipenseriformes, the green sturgeon is the only species that has never reproduced in captivity. The Asian stock appears to be less abundant and is known to spawn in only one river. Artyukhin and Andronov (1990) described the spawning run of the green sturgeon in the Tumnin River and succeeded in artificially spawning two females.

The green sturgeon supports a minor commercial fishery on the Pacific coast, with the majority of fish being caught in the lower Columbia River. During the period 1995–1998, annual commercial catches of green sturgeon ranged from 400 to 1,600 fish (Washington Department of Fish and Wildlife and Oregon Department of Fish and Wildlife 1999), while the fishery of the sympatric white sturgeon *Acipenser transmontanus* harvests about 46,000 annually from the same portion of the river (DeVore et al. 1995). However, this minor fishery is important to Native Americans. The Klamath River population of green sturgeon has been central to the life of the Yurok tribe for more than a thousand years, and its fishery remains an integral

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part of the tribe's subsistence, culture, and economy. Their gill-net fishery has had an annual catch of about 250 fish for the past 15 years (Hillemeier, unpublished data). In addition to using the meat of the sturgeon for food, the Yurok make traditional "sturgeon bread" from the eggs and process other parts to make a glue for manufacturing regalia and tools such as sinew back bows, arrow shafts, and wood plugs for canoes. According to Yurok tradition, everything used to make regalia is sacred.

A research project was initiated to investigate the reproductive and environmental physiology of green sturgeon, with one objective being to develop artificial spawning and culture techniques. This paper provides the details on our artificial spawning of wild green sturgeon and presents data on hormone-induced ovulation and spermiation, egg size, fertilization and hatching rates, and larval survival and growth to metamorphosis.

Methods

Wild broodfish for this study were obtained from the Yurok tribe's gill-net fishery on the lower Klamath River and spawned on site; the fertilized eggs were transported for incubation and larval rearing to the Center for Aquatic Biology and Aquaculture (CABA) at the University of California, Davis. Migratory broodfish were caught from May 8 to May 14, 1999 in the Weitchpec area between river kilometers 56 and 66, which is downstream from the confluence of the Klamath and Trinity rivers. The fishers used anchored gill nets that were 10–15 m long and 7–9 m deep, with a stretch-bar mesh of 17–19 cm. Nets were typically set along the shoreline in back eddies that were 3–6 m deep. The five males and two females that were caught were placed into two wood frame cages measuring 1.2 × 2.1 × 1.2 m that were covered with a plastic-coated, 16-gauge wire mesh (2.5 cm square) and a hinged plywood cover 1.3 cm thick. Cages were submerged in the river and anchored with 14-kg cement weights and river anchors. The maximum holding time in a cage was 10 d.

On May 18, 1999, all seven fish were removed from the cages, weighed (±0.1 kg), measured (±0.5 cm), and tagged for identification during spawning procedures. At this time, 50 fully grown ovarian follicles were collected by catheter (Conte et al. 1988) and placed in a Ringer solution (Dettlaff et al. 1993) containing 0.03 g/L of penicillin and 0.05 g/L of streptomycin sulfate. Thirty follicles were boiled, chilled on ice for 20 min, and stored in a 10% solution of phosphate-buffered formalin for measuring the polarization index (PI, a ratio of the distance of the germinal vesicle from the animal pole to the oocyte animal-vegetal diameter), and 20 were fixed directly in formalin for measuring oocyte diameter. Ten boiled eggs were bisected on site before spawning to estimate the stage of germinal vesicle migration under a dissecting microscope. The remainder were measured (±0.01 mm) in the laboratory using a dissecting scope with camera lucida and a digital image-analyzing tablet (Nikon Microplan II) with microcomputer interface. The condition factor of the broodstock was calculated as 100 × [body weight (g)/fork length^3 (cm)].

The spawning protocol followed Conte et al. (1988) and Webb et al. (1999), with the following modifications: The males received a single injection of 10 μg/kg body weight of mammalian gonadotropin releasing hormone analog (GnRHa) [D-Ala^6, Des-Gly^{10}]–LH-RH Ethylamide (Peninsula Laboratories), and the females received a priming injection of 2 μg/kg GnRHa plus 1 mg/kg of domperidone (a dopamine antagonist; Research Diagnostics, Inc.) followed in 12 h with a resolving injection of 18 μg/kg GnRHa plus 3 mg/kg domperidone. All injections were intramuscular and given underwater to minimize handling stress. River temperature during the injection period ranged from 12.9°C to 13.6°C. Spawning mats, made of weighted cotton rope and placed in the female cage, were checked for released eggs beginning 20 h after the second injection. Ovulated eggs were removed surgically from each fish, which was placed into a hooded stretcher (Conte et al. 1988). Because of the human health risk associated with the release and potential capture of fish containing residues of injected drugs, broodfish were euthanatized during surgery by an overdose of anesthetic (500 mg/L tricaine methanesulfonate), which was pumped across the gills with a submersible pump with a vinyl tube 5 cm in diameter and a 100-L ice chest as a sump. Age was estimated from the cross sections of the dried base portions of pectoral fin rays, as described in Van Eenennaam et al. (1996b).

Milt was collected from each male with a 60-mL plastic syringe and a 4-cm-long vinyl catheter inserted into the urogenital pore (Conte et al. 1988). Sperm was evaluated for percent initial motility and the time to less than 50% motility under a compound microscope, using 5 μL of semen diluted immediately with 200 μL of river water. Eggs were fertilized with 20 mL of pooled milt (4 mL

American Fisheries Society Symposium 00:000–000, 2001

Comparison of Early Life Stages and Growth of Green and White Sturgeon

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Abstract.—Gametes of green sturgeon *Acipenser medirostris* (caught in the Klamath River, California) and farm-reared white sturgeon *A. transmontanus* were obtained using hormonal induction of ovulation and spermiation. The offspring of one female in each species were reared in the laboratory, to compare their development and growth. Green and white sturgeon embryos had similar rates of development and hatched after 169 h and 176 h, respectively, at incubation temperature 15.7 ± 0.2°C. Embryos of both species exhibited similar holoblastic development and passed through 36 stages characteristic of acipenserids. Green sturgeon fertilization and hatching rates were 41.2% and 28.0%, compared with 95.4% and 82.1% for the white sturgeon. Larval survival to 45 d (metamorphosis) was 93.3% in green and 92.1% in white sturgeon. Newly hatched green sturgeon (length 13.7 ± 0.4 mm, mean ± SD) were larger and less pigmented, compared with white sturgeon. They had large ovoid yolk sacs and did not exhibit pelagic behavior that was observed in white sturgeon. The onset of exogenous feeding in green sturgeon occurred at age 10–15 d and length 24.0 ± 0.5 mm, and metamorphosis was completed at age 45 d and length 74.4 ± 5.9 mm (rearing temperature 18.5 ± 0.2°C). Weight and length of green sturgeon larvae and juveniles were considerably greater than in white sturgeon at each sampling time, but the relative growth rate and weight-length relationship were similar in both species. This suggests an effect of larger egg size and maternal yolk supply on the growth of green sturgeon. We conclude that green sturgeon differs from the white sturgeon in their reproductive strategy and, potentially, reproductive habitat.

Green sturgeon *Acipenser medirostris* are widely distributed in the coastal waters along the north Pacific Ocean, having been recorded from at least six countries: the United States, Canada, Mexico, Russia, Japan, and Korea. However, because of low abundance, the green sturgeon are considered a threatened or vulnerable species in Canada and the United States and an endangered species in Russia (Houston 1988; Artyukhin and Andronov 1990; Moyle et al. 1994). Spawning populations of the anadromous green sturgeon have been identified in only a few rivers. In Asia, spawning has been found in the Tumnin River, and a successful artificial spawning of two females was reported (Artyukhin and Andronov 1990). In North America, green sturgeon spawning occurs in the Sacramento and Klamath Rivers, California, and in the Rogue River, Oregon (Moyle et al. 1994). Despite the green sturgeon's wide geographic distribution, there is no information on spawning migrations, spawning and nursery habitats, or the early life stages of this species.

We recently initiated studies on hatchery spawning and reproduction of green sturgeon and conducted the first artificial spawning in 1999 on the Klamath River (Van Eenennaam et al. 2001). As part of a larger project to study the biology of green sturgeon, the objective of this study was to examine the development and growth of green sturgeon in comparison with the sympatric white sturgeon *A. transmontanus*. Although the fertilized eggs of only one green and white sturgeon female were used in this study, we describe the important characteristics of the embryonic development and early life stages, as well as the larval growth in two species.

Methods

The artificial spawning of green sturgeon was conducted on the lower Klamath River at Weitchpec, during 18–20 May 1999. One female (weight 48 kg) and five males, captured by gill net, were induced to ovulate and spermiate by administration of GnRHa and domperidone (Van Eenennaam et al. 2001). Fertilized with pooled milt, eggs were transported (6 h) to facilities of the Center for Aquatic Biology and Aquaculture (CABA) at the University of California, Davis. Fertilized eggs from one female white sturgeon (weight 63 kg)

were obtained from Stolt Sea Farm California, LLC (Wilton, California), in July 1999, and transported (1 h) to CABA. Gametes were obtained using the same spawning induction protocol as for the green sturgeon but without domperidone. All husbandry procedures and sampling methods were identical for both species, as described below.

Upon arrival to CABA, fertilized eggs were acclimated to the incubation temperature for 1 h and placed in McDonald jars at a density of 1–1.5 L of eggs per jar (Conte et al. 1988). A 14 L:10 D photoperiod was maintained by artificial illumination throughout the incubation period. The water flow in the incubators was carefully adjusted, to allow the eggs to roll gently during the early stages (cleavage and gastrulation), and was increased at the later stages of development, to reduce the incidence of fungal infection. Dead eggs were periodically removed by siphon and counted as mortality. Incubation was conducted in a semirecirculation system, where the water was filtered and chilled and temperature was maintained at 15.7 ± 0.2°C (range 15.5–15.8°C).

Hatched larvae were transferred into the circular larval receiving tank (1.2 m-diameter) connected to the semirecirculation system, and during the next 5 d the water temperature, was increased to 18.5°C, at a rate of 1°C per day. One-thousand five-hundred 5 d old larvae were stocked into a circular flow-through tank of 1.2 m-diameter and 355 L-volume, in a separate building with skylight windows. Water was supplied by a spray bar, at a rate of 9–10 L/min, creating a circular current in the tank. Fish were fed a commercial semimoist fry feed (Silver Cup, Nelson & Sons, Inc., Murray, Utah), provided continuously over 24 h by 3 automatic feeders placed at equal distances around the wall of the tank. The fish were also hand fed 5 g of chopped *Tubifex* worms twice a day. The tank was exposed to natural photoperiod, but the light intensity was partially reduced by black plastic shade cloth extending over 2/3 of the tank. The tank was cleaned daily, and mortalities were removed and recorded. Larval behavior was observed during the day and at night, with a white dim flashlight with which no obvious disturbance occurred. Water temperature was recorded daily and averaged 18.5 ± 0.2°C (range 18.2–18.7°C). The dissolved oxygen exceeded 8.6 mg/L during the entire 45 d rearing period.

Staging of embryonic development followed the classification of Dettlaff et al. (1993) that includes 36 stages to hatching. More than 300 eggs were randomly sampled at cleavage (stages 5–6) to determine the rates of fertilization. The embryonic development was closely monitored in McDonald jars, and the published developmental rates of white (Wang et al. 1985) and Russian (Dettlaff et al. 1993) sturgeon *A. gueldenstaedti*, at similar temperatures, were used to estimate sampling time for specific stages of the green sturgeon. To estimate the rate of development, the post fertilization time was recorded when more than 50% of the eggs reached a defined stage. All embryos were fixed in 10% phosphate-buffered formaldehyde solution for further observations and measurements. Thirty preserved ova and stage 5 (second cleavage, fully formed perivitelline space) eggs were weighed (±0.1 mg) and measured (±0.01 mm) for maximum diameters under a dissecting microscope with a digital image-analyzing tablet. The chorions of the additional five eggs at each stage were removed under a dissecting microscope before fixation, to facilitate photomicrography.

Samples of 30 larvae and juveniles were collected at days 0 (hatching), 1, 3, 6, 10, 15, 21, 28, 36, and 45 post hatch. Animals were euthanatized by overdose of MS-222 and preserved in buffered formalin for further observations and measurements. All larvae were individually weighed and measured for length under a dissecting microscope or by using a micrometer caliper. Measurements reflect minor shrinkage due to fixation in formalin.

Mean weight and length of green and white sturgeon were compared by Student's *t*-test. A few larvae (with bent bodies or other defects) were deleted from the samples before calculation of means and standard deviations. The specific growth rates were calculated as $100 \times (\ln W_t - \ln W_0)/t$, where W_t and W_0 are mean body weight at each of the two samplings, and t is the number of days between the two samplings. Weight–length relationships were evaluated by linear regression analysis using $\log_{10}$-transformed data. The regressions were tested for the lack of fit and their slopes compared using a *t*-test. The JMP Statistical Software (Version 3, SAS Institute, Cary, NC) was used for data analysis. The accepted significance level was $P < 0.05$.

Photomicrographs of embryos and larvae were scanned by SprintScan 35 plus (Polariod Cooperation, Cambridge, Massachusetts) and edited using Adobe Photoshop Software (Version 5.0) to remove the dark background and to adjust contrast for clearer pictures.

Results

Survival Rate

Fertilization (Stage 5) and hatching (Stage 36) rates for eggs from the wild green sturgeon were 41.2% and 28.0%, respectively (Van Eenennaam et al. 2001), whereas the domestically reared white sturgeon exhibited higher fertilization (95.4%) and hatching (82.1%) rates. Larval survival from hatching to metamorphosis (age 45 d) was high in both species, 93.3% in green and 92.1% in white sturgeon. No mortalities occurred after 32 d posthatch in green sturgeon, while cumulative mortality of white sturgeon exhibited small but steady increases throughout the 45 d experiment.

Embryonic Development

Early development of green sturgeon followed the holoblastic style of Acipenseriformes described in detail by Dettlaff et al. (1993). We refer to their descriptions and briefly characterize selected stages with the distinguishing characteristics of green sturgeon (Figure 1 and 2). For comparative purposes, similar stages of white sturgeon are given (for more detailed illustrations see Beer, 1981). Enumeration of stages corresponds to the classification of Dettlaff et al. (1993).

Stage 5 (second cleavage, Figure 1). —The eggs of green sturgeon, although varying slightly in pigmentation, had a flattened white animal region with a small dark pigmented spot in the center, which was unevenly divided by the two cleavage furrows. Their vegetal hemisphere was brown-olive green. A narrow pigmented ring (with lighter pigmentation on the other side), which was not apparent at this stage in white sturgeon eggs, appeared along the boundary between animal and vegetal regions. The weight of green sturgeon eggs that completed hydration and hardening increased from 35.6 ± 0.9 mg (ova) to 38.6 ± 1.2 mg (stage 5) and their maximum diameter from 4.17 ± 0.12–4.44 ± 0.15 mm, respectively (N = 30). Smaller white sturgeon eggs were more darkly pigmented and exhibited similar relative increases in weight (from 17.9 ± 1.1–21.6 ± 0.8 mg) and diameter (from 3.40 ± 0.09–3.57 ± 0.11 mm) at stage 5 (N = 30).

Stage 14 (early gastrula, Figure 1).—The horizontal blastopore in green sturgeon embryos appeared as a short shallow groove and was darkly pigmented. Blastomeres positioned along the border between animal and vegetal regions (marginal zone) were intermediate in size and darker than those at either pole, forming a wide speckled zone that appeared at the late cleavage stage. A ratio of the distance from blastopore to animal pole to that from blastopore to vegetal pole varied within a range of 0.60–0.95, indicating the variable location of the blastopore above the egg equator. The horizontal blastopore of white sturgeon embryos appeared on the equator (Beer 1981; Bolker 1993b).

Stage 22 (late neurula, Figure 1).—The neural plate (in a process of folding) slightly protruded above the surface of the green sturgeon egg with a diamond-shaped opening in the anterior part and incomplete closure of neural fold in the trunk region. The region of the neural plate was grayish, and the rest of the embryo was yellowish. White sturgeon eggs had a similar neurulation pattern with a darker pigmented neural plate. Epiboly was completed in both species before the onset of neurulation.

Stage 35 (embryo before hatch, Figure 1).—The egg chorion was softened and became fragile after secretion of a hatching enzyme (Dettlaff et al. 1993). The hatching gland was evident in front of the mouth cleft. With an actively twisting tail and trunk, the tail of an embryo was able to break through the chorion and stretch out. The chorion surrounding the head and ovoid shaped "yolk sac" (endoderm in sturgeon) was discarded by further movement of the trunk.

Rates of Embryo Development.—The chronology of embryonic development of the green and white sturgeon was similar (Table 1). Despite the great difference in egg size, the green and white sturgeon embryos exhibited an overall similar rate of development at 15.7°C. Cleavage and morphogenetic movement appeared to proceed at slower rates in green sturgeon. However, the developmental pace was accelerated before hatching, resulting in an 8 h earlier mass hatching than in the white sturgeon. Hatching occurred over two days in both species, from 144 to 192 h after fertilization in green sturgeon and from 152 to 200 h in white sturgeon. The earlier hatching of the green sturgeon could also be associated with its thin and fragile chorion.

Larval Development

Larvae at hatch (Figure 2A). —Newly hatched green sturgeon (L = 12.6 - 14.5 mm, range) were grayish in the trunk and had a large ovoid yolk

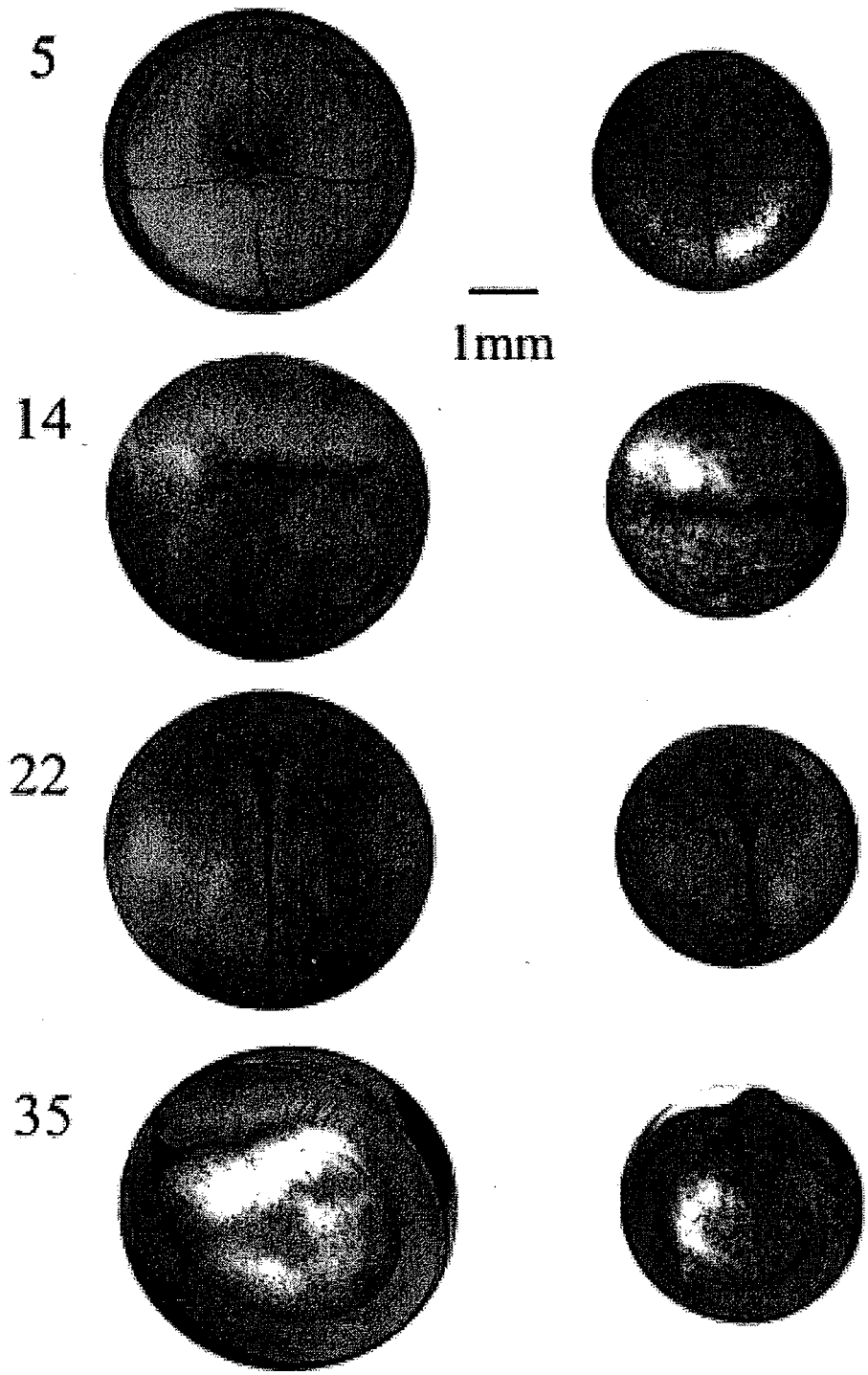

Figure 1. Stages of embryonic development of green sturgeon (left) and white sturgeon (right). Stage 5–second cleavage; Stage 14-early gastrula; Stage 22–late neurula; Stage 35–prehatch embryo (stages by Dettlaff et al. 1993).

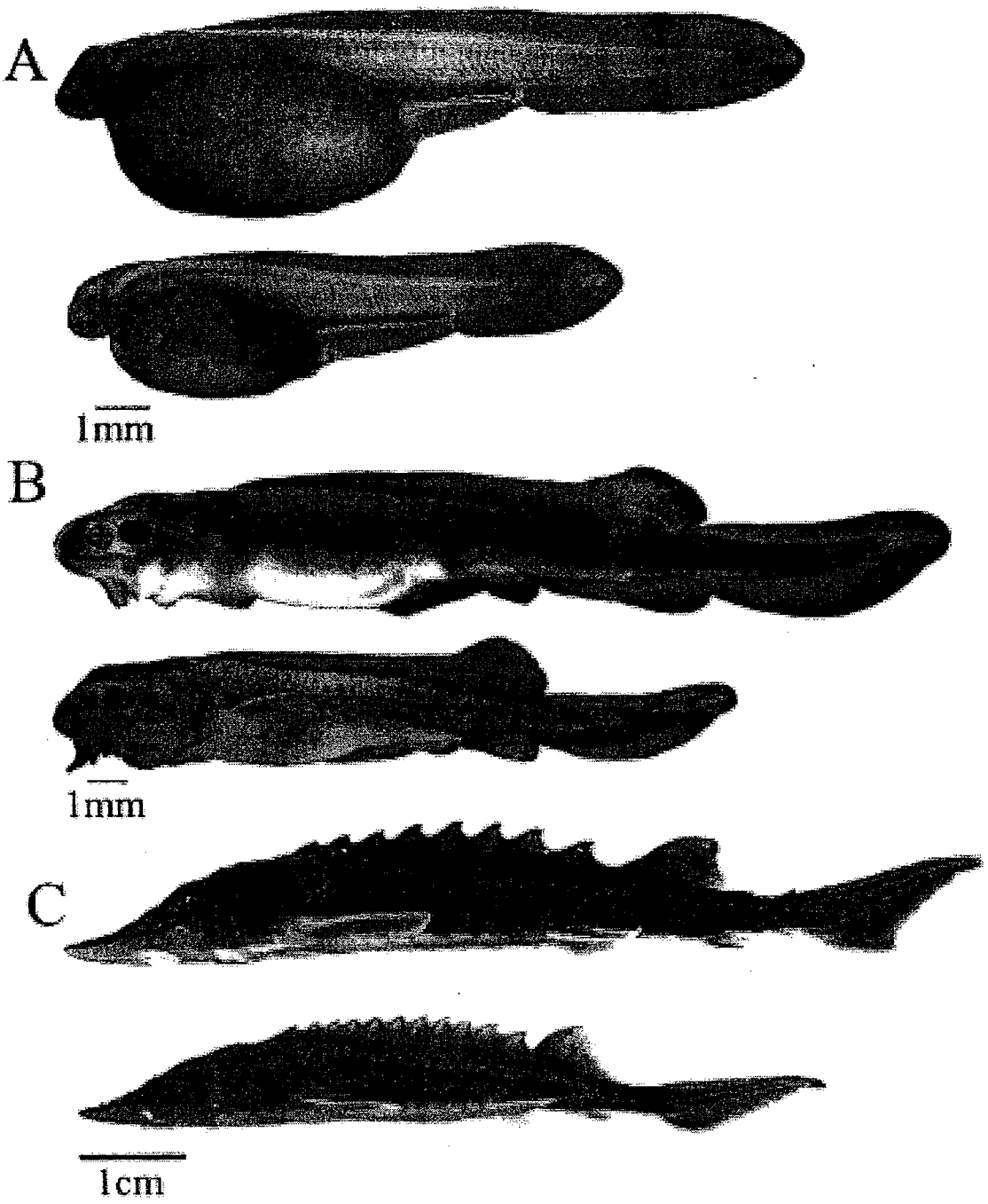

Figure 2. Larvae and juveniles of green and white sturgeon at the same age. Green sturgeon on the top and white sturgeon at the bottom in A, B, and C, respectively. A–posthatch larvae (stage 36); B–larvae at the onset of feeding (10 d posthatch); C–juveniles (45 d posthatch).

Table 1. Chronology of embryonic development of green and white sturgeon at 15.7°C. Time of respective stages (Dettlaff et al. 1993) is in h:min after fertilization.

Stages	Green sturgeon	White sturgeon
1	0	0
4	6:10	4:10
5	7:40	4:55
6	9:40	5:50
7	10:30	7:00
8	10:57	7:30
9	–	9:10
10	–	10:10
11	17:55	13:00
12	23:58	–
13	27:15	23:40
14	31:35	–
15	–	31:20
16	41:45	35:00
17	45:25	39:50
18	47:45	–
19	48:30	–
20	49:25	46:10
21	52:50	49:00
22	55:00	52:50
23	57:30	–
24	65:40	–
25	68:52	70:00
26	–	–
27	80:40	77:20
28	89:50	83:00
29	97:35	–
30	–	95:00
31	–	104:00
32	145:35	120:00
33	154:05	128:30
34	161:50	144:00
35	–	159:40
36	169:00	176:40

sac with yellowish coloration. They were considerably less pigmented than the white sturgeon larvae. The larval body had 63–71 myotomes, of which 36–41 are anterior to the cloaca and 27–31 were posterior to the cloaca. The eyes were well developed, with differentiated lenses and a dark pigmented spots. Olfactory and auditory vesicles were present. Mouth and gill cover differentiation began as a shallow cleft. The posterior intestine contained a dark pigment in the spiral valve. The paired Cuvier's ducts and yolk veins with red blood were highly developed. The continuous fin fold was interrupted at the cloaca and had a slightly wrinkled area in the preanal region posterior to the yolk sac. The rudiments of pectoral fins appeared as small buds on the dorsal part of the yolk sac behind the pronephros. White sturgeon hatchlings (L = 10.0 - 11.0 mm) were darker, with dense melanin pigmentation of the trunk and yolk sac. White sturgeon larvae appeared to be less developed at hatching, lacking eye lenses and pectoral fin buds. Their fin folds were smooth and not wrinkled at the preanal regions as it was in green sturgeon.

Larvae at the Onset of Feeding (Figure 2B). —At 10 d of age, pigmentation of green sturgeon (L = 23.0 - 25.2 mm) greatly increased on the head and along the trunk, except the ventral region. The larvae became dark gray. Myotomes extended to the ventral side of the yolk sac, which was greatly diminished in size, resulting in a streamlined body shape. The barbels had been elongated. Rays started to form in all fin rudiments, including the lower lobe of the caudal fin. The pectoral fins moved down to the ventral region and acquired a horizontal position. The fin fold discontinued posterior to both the dorsal and anal fins. The lateral lines extended over the mid-body. The third pair of branchial arches had formed. Larval teeth were visible on the upper and lower jaws. The spleen was present as a bright red spot. In white sturgeon of the same age (L = 17.3 - 19 mm), larvae were darkly pigmented in the tail region. Their yolk was practically absorbed, and the lateral lines were completely developed. Some of the larvae started releasing their melanin plugs. White sturgeon larvae started exogenous feeding at this stage.

Juveniles at Metamorphosis (Figure 2C) —At 45 d of age, the green sturgeon (L = 62.5 - 94.4 mm) had completed metamorphosis, which was characterized by development of dorsal, lateral, and ventral scutes, elongation of barbels, rostrum, and caudal peduncle, full resorption of caudal and ventral fin folds, and development of fin rays. Juveniles were similar to adults in body shape and olive-green coloration, with a dark midventral stripe. Development of lateral scutes started at the anterior portion of the trunk immediately posterior to the gill cover, progressing toward the caudal region, while dorsal and ventral scutes started differentiation in the mid-body region anterior to the dorsal and ventral fins. The ventral scutes appeared at 28 d after the dorsal and lateral scutes had been differentiated. At age 45 d, green sturgeon juveniles had 25.7 (24–28) lateral scutes, com-

pared with 37.8 (34–40) in the white sturgeon (mean and range, $N = 5$). A few small bony grains and platelets started to develop at age 28 d between the dorsal and lateral scutes, but they were not abundant at metamorphosis and gave the green sturgeon skin a smooth appearance, compared with the white sturgeon. In white sturgeon (L = 31.0 - 78.2 mm at 45 d), the bony grains and platelets began to appear on the dorsal portion of the head as early as 15 d post hatch. They continued to develop and became abundant on the head, operculum and the whole trunk (except the ventral portion), giving the skin of white sturgeon juveniles a rugged appearance. White sturgeon juveniles had the uniform gray coloration of their bodies.

Growth and Weight-Length Relationship

Changes in length and weight of green and white sturgeon larvae and juveniles are shown in Figure 3. Mean (±SD) weight and length of newly hatched larvae were 36.3 ± 2.4 mg and 13.7 ± 0.4 mm ($N = 29$), for green sturgeon, and 15.8 ± 0.9 mg and 10.6 ± 0.3 mm ($N = 28$), for white sturgeon. Based on the biochemical study with white sturgeon larvae (Wang et al. 1987), the increase in wet weight during the endogenous feeding (days 0–10) was caused by an increase of moisture content, since the dry matter decreases during the endogenous feeding phase. At the feeding stage (age 10 d), mean weight and length were 88.3 ± 4.3 mg and 24.0 ± 0.5 mm ($N = 27$), for green sturgeon, and 41.9 ± 2.4 mg and 18.4 ± 0.5 mm ($N = 27$), for white sturgeon. Mean weights increased rapidly in both species during exogenous feeding (days 15–45), and green sturgeon were larger at each sampling time (Figure 3). At age 45 d, the weight and length of green sturgeon juveniles were 2500 ± 525 mg and 74.4 ± 5.9 mm ($N = 27$), while the weight and length of the white sturgeon were 1471 ± 864 mg and 60.9 ± 15.3 mm ($N = 29$). However, the specific growth rates during the exogenous feeding phase (15–45 d) were similar in both species, 10.4% d^{-1} and 10.2% d^{-1} for green and white sturgeon, respectively.

The analysis of weight-length relationship revealed two developmental periods of allometric growth: the yolk absorption phase, with low regression slopes, and the exogenous feeding phase, with higher slopes (Figure 4). The linear equations for log-transformed variables are given below (W, L, and R^2 are body weight, total length and coefficient of determination):

Green sturgeon: age 0–6 d $\log W = 0.118 + 1.267 \log L$, $R^2 = 0.93$ ($N = 115$)

21–45 d $\log W = -1.764 + 2.764 \log L$, $R^2 = 0.99$ ($N = 111$)

White sturgeon: age 0–6 d $\log W = -0.207 + 1.384 \log L$, $R^2 = 0.93$ ($N = 117$)

15–45 d $\log W = -1.869 + 2.795 \log L$, $R^2 = 0.99$ ($N = 146$)

There was no significant difference in the regression line slopes for each developmental period between the two species, indicating similar patterns of allometric growth. Fish sampled at the onset of exogenous feeding (10 d in white and 10–15 d in green sturgeon) had a large variation in weight and length and did not fit either regression line (Figure 4, shown by larger symbols).

Larval Behavior

Green sturgeon larvae did not exhibit the pelagic swim-up behavior seen in other acipenserids. During the first 5 d post hatch, the green sturgeon larvae exhibited a strong tendency to clump together in large numbers at the bottom, around the edges of stones, polyvinyl chloride (PVC) pipes, the central drain pipe, or along the wall of the tank. They remained in clumps with limited movement during the night. Larvae began to display a nocturnal swim-up behavior at 6 d post hatch, when the rudiments of the pectoral and ventral fins were developed, dorsal and anal fin rays became apparent, yolk of the mid-intestine was depleted, and the mandible started rhythmic movement. Larvae clumped under the shade cloth during the day but swam actively during the night. These nocturnal behavior patterns persisted in green sturgeon from the onset of exogenous feeding to metamorphosis.

Unlike the green sturgeon, white sturgeon exhibited pelagic behavior during the first 5 d after hatching. The white sturgeon larvae swam up and out of the incubation jars upon hatching, dispersed throughout the water column in the rearing tank, and swam constantly during the day and night. They began to display nocturnal behavior, similar to green sturgeon, at 6 d post hatch, with the transition from the pelagic to a demersal swimming during the day and dispersal into the water column during the night. The white sturgeon swam at the bottom of the tank, aggregating in small groups, but never clumped as strongly as the green sturgeon. At the onset of exogenous feeding, the clumping behavior disappeared, and larvae dispersed along the tank bottom.

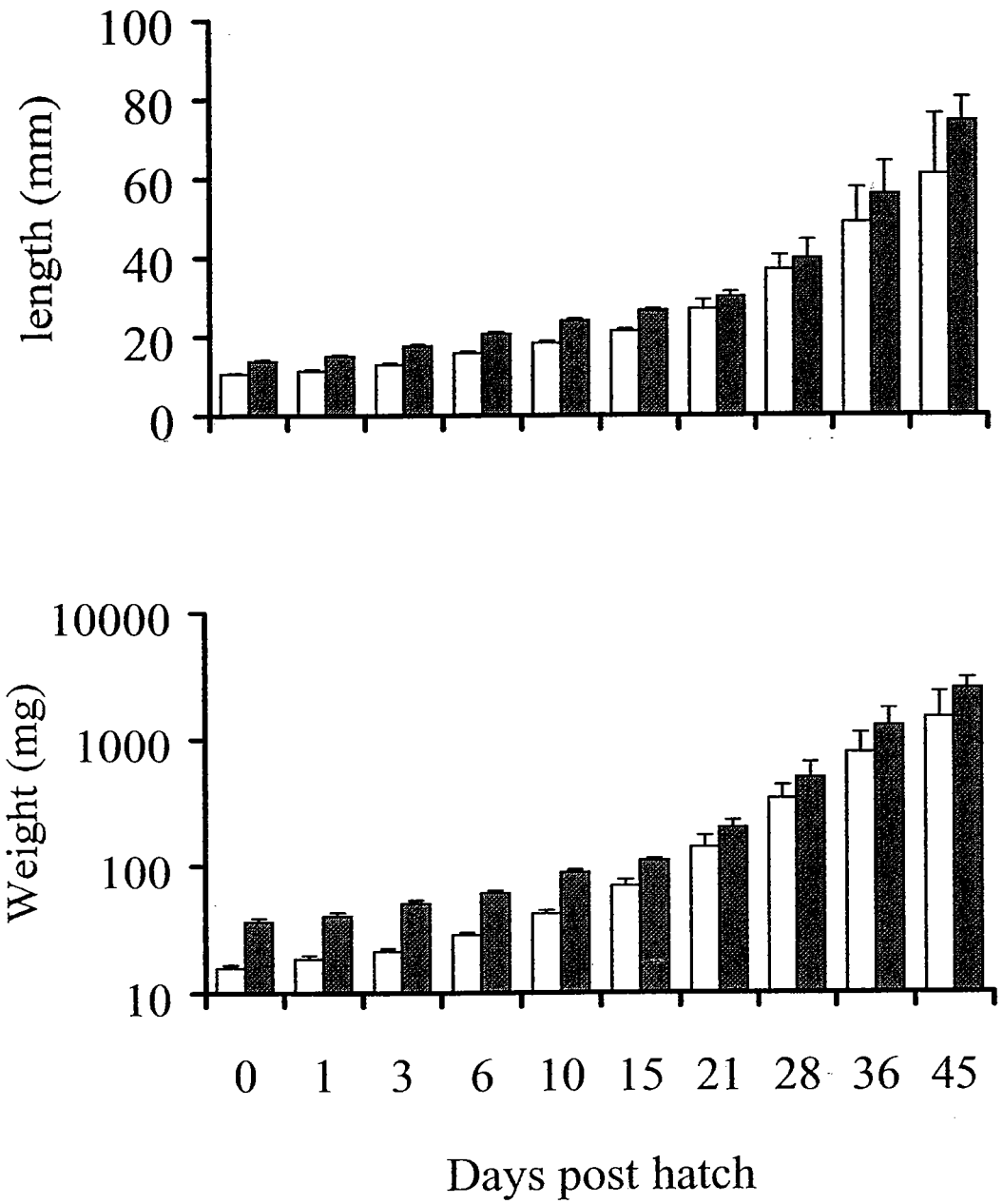

Figure 3. Length and weight (mean ± SD) of white (open bars) and green (shaded bars) sturgeon from hatching to metamorphosis. Weight is shown in log-scale.

Discussion

Embryos of green sturgeon exhibit the holoblastic pattern of development, similar to white sturgeon (Beer 1981; Bolker 1993a, 1993b) and other acipenserids (Dettlaff et al. 1993). The rates of embryonic development and hatching time, at temperature15.7°C, are similar in green and white sturgeon. Newly hatched larvae of green sturgeon are longer and heavier than larvae of white sturgeon, and they possess large reserves of endodermal yolk. They can be distinguished from white sturgeon by their light pigmentation and the

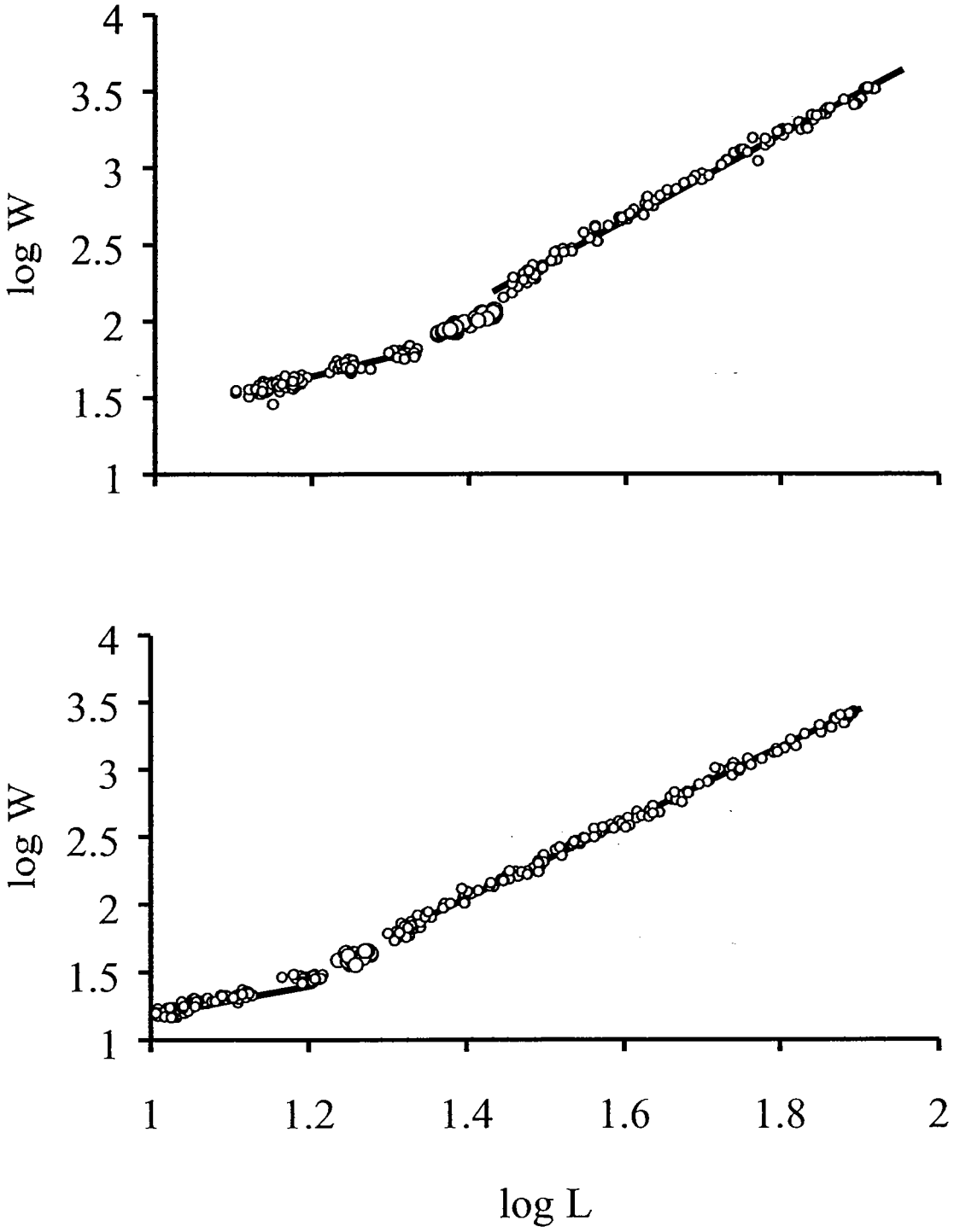

Figure 4. Weight–length relationships of green (top) and white (bottom) sturgeon (scatters and regression lines). The enlarged symbols (circle) show samples taken at the onset of exogenous feeding (not included in regression).

shape and size of the yolk sac. Juveniles of the two species are clearly distinguished by the number of lateral scutes, coloration, and the smooth skin in green sturgeon (versus rugged skin of white sturgeon).

While the fertilization and hatching rates were lower in green sturgeon (likely due to stress from capture and handling or delayed removal of eggs), the survival of larvae to metamorphosis was very high (93.3%). The large size and robustness of green sturgeon larvae contributed to their high survival rate in our previously reported hatchery trials (Van Eenennaam et al. 2001). Since the specific growth rates of green and white sturgeon, from the onset of exogenous feeding to metamorphosis, were similar, it appears that green sturgeon juveniles are larger due to the greater reserve of maternal yolk and, consequently, larger size at the onset of exogenous feeding.

Based on one progeny of each species, we present the first information on green sturgeon development and growth, which are comparable to those of white sturgeon under our specific culture conditions. While our study does not account for individual variation of egg size in the two species, the literature currently available does support significantly larger egg size in green sturgeon (Van Eenennaam et al. 2001), compared with white sturgeon (Lutes et al. 1987). However, egg size and color are known to vary greatly in sturgeon (Dettlaff et al. 1993); therefore, the egg pigmentation pattern needs to be verified, and the effects of egg size and maternal yolk reserves on growth of juveniles should also be further investigated.

Egg adhesiveness in sturgeon is another characteristic considered to be species-specific and associated with reproductive behavior, including selection of spawning substrate and hydrological environment. Based on studies of seven sturgeon species with different structure and adhesiveness of egg chorions, Vorobyeva and Markov (1999) concluded that the anadromous species, spawning under conditions of variable and generally slower river currents, had less adhesive eggs than the resident species spawning in strong water current. This appears to contradict our first (Van Eenennaam et al. 2001) and more recent (unpublished) observations on the poor adhesion of eggs in green sturgeon spawning in the fast flowing Klamath River. Our experience with the artificial spawning of wild green sturgeon indicates a consistent weak adhesiveness of the fertilized eggs, and preliminary histological observations of the egg membranes revealed that the outer layer of chorion was approximately half the thickness of that in the white sturgeon. Vorobyeva and Markov (1999) also noted that the thickness of the chorion varied among the species with strongly or weakly adhesive eggs. The adhesiveness of eggs in sturgeon probably depends on the adhesive material secreted upon activation, the specific molecular structure of the outer chorion membrane, and the mode of egg attachment to a substrate. It is possible that green sturgeon eggs may not be attaching at all to the open substrate in the fast flowing Klamath River; instead, they may be trapped in the crevices of river bedrock or under gravel where the early development occurs. The pale coloration, limited mobility, and photophobic behavior of newly emerged green sturgeon larvae support this explanation.

We observed substantial differences in larval behavior between the two species. Unlike white sturgeon larvae, green sturgeon larvae do not swim up after hatching, similar to the observations of Artyukhin and Andronov (1990) on the Asian green sturgeon. It appears that green sturgeon larvae do not have an early pelagic phase, which facilitates larval dispersal and downstream migration to nursery grounds, as in white sturgeon of the Sacramento and Columbia rivers.

In conclusion, our study provides the first comparative information on the early development of North American green and white sturgeon. Sharing a holoblastic style of development, green sturgeon differ from white sturgeon by having larger eggs and larvae, weaker adhesiveness of fertilized eggs, and demersal larval behavior, suggesting a reproductive strategy different from that of white sturgeon, with regards to conditions of spawning rivers and larval nursery habitat. Unfortunately, neither spawning nor rearing habitats of green sturgeon are known at present time, and our laboratory results are not verified by field observations. Characterization of the reproductive habitat of green sturgeon is a priority for stock management and preservation of this species.

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Histology of developing digestive system and the effect of food deprivation in larval green sturgeon (*Acipenser medirostris* Ayres)

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Abstract

Histological development of the digestive tract in hatchery-reared larvae of green sturgeon (*Acipenser medirostris*) and the effects of food deprivation on the digestive system were studied from hatching until 31 days post hatch (dph). At hatching, larval digestive system consists of two rudiments: large endodermal yolk sac and primordial hindgut. During the endogenous feeding phase, the wall of yolk sac differentiates into a stomach (glandular and non-glandular regions) and the anterior and intermediate intestine, while the hind-gut primordium differentiates into the spiral valve and rectum. At the onset feeding (15 dph at 16°C), the organization and cytoarchitecture of digestive system in green sturgeon larvae are generally similar to those in juveniles and adults. As a result of food deprivation from the onset of feeding, the digestive system of green sturgeon larvae exhibited progressive deterioration, with the subtle pathological changes observed after 5-day starvation: shrinkage of digestive epithelia and tissue degeneration, and necrosis observed at 10-15 days of starvation (30 dph). No changes were observed in mucous secretion in different regions of the digestive tract of food-deprived larvae. The histological analysis of larval digestive system can be used to evaluate the nutritional condition of larval green sturgeon in their nursery habitat in spawning rivers, which are affected by dams and flow diversions.

Key words: Acipenser medirostris, green sturgeon, larvae, digestive system, histology, histopathology, starvation.

INTRODUCTION

Biological and physical factors interactively affect development, growth, and survival of juvenile fish, determining rate of recruitment and year-class strength in populations (Bailey & Houde, 1989; Fogarty *et al.*,1991; Kamler, 1992). Predation is considered to be the main cause of mortality during the embryo and yolk-sac larval stage, whereas starvation plays an important role during transition to exogenous feeding increasing vulnerability of larvae to predation (Bailey & Houde, 1989). Temperature may influence the time-interval for larvae to establish successful feeding, by changing the rate of metabolism and the pace at which the yolk reserves are consumed and first feeding occurs (Bisbal & Bengtson, 1995). Various, temperature-dependent, periods of food deprivation may result in abnormal behaviour and development, including degeneration of the alimentary tract and trunk musculature, reduced food utilization efficiency and feeding activity (Heming *et al.*, 1982).

The effects of experimental food deprivation on larval conditions have been described using morphometric and gravimetric (e.g. Elrich *et al.*, 1976; Mookerji & Rao, 1999), biochemical (e.g. Robinson & Ware, 1988; Clemmesen, 1993, Suneetha *et al.*, 1999), and histological (e.g. Oozeki *et al.*, 1989; Theilacker & Watanabe, 1989; Green & McCormick, 1999) criteria, or a combination of those (Watanabe, 1986; Bisbal & Bengtson, 1995). The detection of starvation conditions is important for studies of both natural and cultured populations. However, such studies must be preceded by an experiment wherein the starvation indicators are validated for fish of known nutritional history (Bisbal & Bengtson, 1995).

Green sturgeon is the least studied species among North American acipenserids, and is considered a rare or vulnerable in the United States and Canada (Birstein, 1993) and an

endangered species in Russia (Artyukhin & Andronov, 1990). In North America, the only known spawning populations of green sturgeon are in the Klamath, Rogue and Sacramento Rivers, all of which are affected by water projects (Moyle *et al.,* 1994). These rivers' flow is largely controlled by dams and water diversion projects, and the significant reduction of flow (especially during the dry years) results in changes of water quality (temperature and dissolved gases) and primary and secondary production, affecting the availability of food resources for larval and juvenile green sturgeon.

Acipenseriformes (sturgeon and paddlefish) differ from the modern teleosts by holoblastic cleavage, intracellular platelet yolk in the embryo, and by differentiation of the digestive system from the yolk sac anlagen (Dettlaff *et al.,* 1993). Development of digestive system has been studied in Russian sturgeon *Acipenser gueldenstaedti* Brandt (Dettlaff *et al.*, 1993), white sturgeon *A. transmontanus* Richardson (Buddington & Doroshov, 1986a, Gawlicka *et al.,* 1995), Adriatic sturgeon *A. nacarii* Bonaparte (Boglione *et al.,* 1999), and Siberian sturgeon *A. baerii* Brandt (Gisbert *et al.,* 1998, 1999). Deng *et al.* (2001) recently described early development in green sturgeon, however there has been no comprehensive study on the digestive system in larval stages of this species.

Using the artificial spawning of green sturgeon (Van Eenennaam *et al.,* 2001), we initiated the studies on cultured fish to better understand the early life history and environmental physiology of this species. The objectives of this study is to characterize, using histology and light microscopy, the development of digestive system in green sturgeon larvae and to evaluate the effects of food deprivation from onset of larval feeding on a histopathology of digestive tract and accessory digestive organs.

MATERIAL AND METHODS

Green sturgeon gametes were obtained from one female (35 kg) and male (25kg), caught in May of 2001 by the gillnets and held in cages in the Klamath River (Weitchpec, California). Ovulation was induced by two intramuscular injections (0.6 and 20 $\mu g\ kg^{-1}$, with 8-h interval) of GnRHa ([D-Ala^6, Des-Gly^{10}]-LH-RH Ethylamide). Domperidone ($2mgkg^{-1}$) was administered with second injection. Spermiation was induced by a single injection of GnRHa ($10\ \mu gkg^{-1}$). The ovulation occurred in 15 h after second injection at river temperature 18.4 - 19.8°C. Gamete collection, artificial fertilization, and the silt treatment of fertilized eggs were conducted as described in Van Eenennaam *et al.* (2001). The eggs (fertilization rate 80% at second-third cleavage) were transported to UC Davis in the oxygenated and cooled (13-14°C) water and incubated at 14-15°C for seven days before hatching. One thousand newly emerged larvae were held in two circular tanks (120 l, ca. 500 larvae per tank) of the indoor semi-recirculation system supplied with well water (dissolved oxygen at saturation, pH 7.8-8.2, constant 16°C temperature, and 12L:12D artificial photoperiod). Small amount of food (semi-moist Silver Cup, Nelson & Sons, Utah, and live chopped Tubifex) was presented to one group (tank) at 12 days post hatch (dph), while the other group was deprived of food during the entire experiment (31 dph). Once first feeding was detected at 14-16 dph, larvae were fed *ad libitum* and the excess of uneaten feed, faeces, and mortalities were removed daily.

Larvae were sampled (n = 10) daily from hatching to 20 dph and with 3-d interval to 31 dph, euthanized (overdose of tricaine methanesulphonate), and fixed in phosphate-buffered formalin. Large sampling mortality did not allow survival analysis. After one month of storage in formaline, larvae were measured (TL, ± 0.01 mm, digital image-analyser), weighed (BW, ± 0.01 mg, digital micro-balance), and five specimens from each

sample were dehydrated in graded ethanol, embedded in paraffin, and sectioned at 4-6 μm (LKB Historange Microtome). Slides were stained by haematoxylin and eosin (HE) for topographic observations, periodic acid-Schiff (PAS) for neutral mucosubstances, and Alcian Blue (AB) at pH 2.5, 1.0 and 0.5 for carboxyl-rich and sulphated (weakly and strongly ionised) glycoconjugates and sialic acid (HCl hydrolysis + AB, pH 2.5) (Gisbert *et al.*, 1999). Melanin pigment granules were identified as previously reported (Gisbert & Sarasquete, 2000). Height of the epithelial cells from different regions of the digestive tract was measured under a microscope with an ocular micrometer (Bisbal & Bengtson, 1995) and compared among feeding and starving larvae, using a Student's *t*-test ($P< 0.05$).

RESULTS

Food deprivation during 15-31 dph resulted in loss of weight and no increase of length in the food-deprived group (Fig. 1). Unfed larvae continued swimming along the bottom and walls of the holding tank, exhibited food searching behaviour, and were reactive to external stimuli. However, most food-deprived larvae died between 28 and 31 dph, whereas no mortality was observed in green sturgeon feeding larvae.

HISTODIFFERENTIATIONOF THE DIGESTIVE SYSTEM IN FED LARVAE

At hatching, larval digestive system is represented by two rudiments, a large endodermal yolk sac and primordial hind-gut, neither is open to the exterior (mouth and anus are not differentiated). The endodermal yolk sac (volume 16.9 ± 2.9 mm^3, $n = 20$) is filled with yolk

platelets and lined with a simple, squamous epithelium, which will differentiate into the walls of stomach and intestine (Fig. 2a). The hind-gut, containing small amount of yolk, appears as an undifferentiated straight and narrow rudiment, which will differentiate into the spiral valve and rectum. The accessory digestive organs (liver and pancreas) are absent at hatching. Histological differentiation of digestive system after hatching is described below.

Buccopharynx

At hatching, the buccopharynx is closed and its lumen filled with small yolk platelets (Fig. 2b). Clusters of basophilic cells (future gill arches) are seen in a circular position in the posteroventral region of the buccopharynx. Between 1 and 2 dph, the mouth opens with two differentiated oral valves composed of a squamous stratified epithelium with numerous cells in mitosis. The buccopharyngeal mucosa consists of a squamous stratified epithelium with connective tissue fibers (Fig. 2c). Epithelial cells contain supranuclear eosinophilic (HE) and PAS-positive yolk platelets and some melanin granules (bleachable with hydrogen peroxide), which disappear at age 4-5 dph. Ciliated cells (scattered through buccopharyngeal epithelium) are present from hatching until 12-14 dph (Fig. 2d). First goblet cells (unreactive to stain) appear at 6 dph. At age 7-8 dph, they are stained by the AB (pH 2.5, 1.0, 0.5) and PAS stains, indicating the presence of neutral and acidic (carboxylated and sulphated) glycoconjugates. The number and size of goblet cells in the buccopharyngeal epithelium increased as larvae developed. Taste buds cells (basophilic) differentiate between 8 and 9 dph, and the taste buds are fully developed at 10-11 dph. Differentiation of canine teeth proceeds from the base of the buccopharyngeal epithelium at 6-7 dph, with the teeth protruding into the oral valves and pharyngeal lumen at age 11-12 dph (larval dentition is present to 31 dph). Between 11 and 12 dph, the ventral and dorsal fungiform and filiform papillae develop in the anterior and central

part of the buccopharynx, respectively. Numerous mucous cells and taste buds develop in the surface of the papillae.

Oesophagus

The oesophagus is not differentiated to 6 dph, and the posterior region of buccopharyngeal cavity remains filled with the residual yolk. At 7 dph, the primordial oesophagus wall consists of pseudostratified columnar epithelium with the numerous cells containing yolk inclusions and melanin granules, which disappear between 8 and 9 dph. Differentiation of the oesophagus proceeds from the posterior region to buccopharynx. At 10 dph, the oesophagus wall is composed of a mucosa with a *lamina propria* (loose connective tissue and a layer of musculature), a submucosa (connective tissue fibres with some blood vessels), and a serosa lined by a thin layer of squamous epithelium. Two regions of the oesophagus can be distinguished by histological characteristics of the epithelia. The anterior region is lined with two cell layers: the inner layer of a simple, cuboidal epithelium and the outer layer of a simple, columnar epithelium with abundant goblet cells staining for neutral mucosubstances (PAS), carboxylated and sulphated acidic mucins (AB pH 2.5 & AB pH 1.0, 0.5), and for sialic acid (HCl hydrolysis + AB pH 2.5). The posterior region is lined with a ciliated columnar epithelium with a very few goblet cells. Some mucosal folds were detected in the region connecting of the oesophagus and glandular stomach (esogaster).

Stomach

At hatching, the large endodermal yolk sac of green sturgeon is surrounded by a thin squamous basophilic epithelium. Two oblique furrows (invaginating epithelium) appear at 2 dph in dorsal and ventral parts of the posterior yolk sac, merging and dividing the yolk sac into

two compartments between 3 and 4 dph (Fig. 3a). The anterior wall of the furrow lined with a squamous epithelium will become the lower wall of the stomach, while its posterior wall lined with a columnar epithelium will become the upper wall of intestine.

Pyloric (non-glandular) stomach starts differentiation at 6 dph in the anterior ventral yolk sac from a fold of stratified squamous epithelium (Fig. 3b) and is well differentiated at 11-12 dph, with mucosal folds surrounded by a prominent tunica muscularis. The epithelial lining of pyloric lumen consists of columnar ciliated cells with supranuclear vacuoles containing eosinophilic (HE) and neutral (PAS-positive, AB pH 2.5, 1.0 and 0.5-negative) mucosubstances. A wall of pyloric stomach is composed of submucosa with connective fibres (AB pH 2.5 and 1.0 -positive), some blood vessels, circular muscle fibres, and a thin serosa with basophilic squamous cells (Fig. 4c). The organ is separated from the anterior intestine by pyloric sphincter. Platelet yolk is present in the lumen of pyloric stomach until the age 14 dph.

Cardiac (glandular) stomach starts differentiation at the age 8 dph, with cytoarchitectural changes (squamous to columnar cells) in the epithelium of the yolk-sac. The gastric glands in cardiac stomach wall are not detectable by the PAS staining at 10 dph, but they are prominent at 12 dph as the multicellular tubular glands composed of a single-type secretory cells with eosinophilic and PAS-positive apical borders and the secretory products containing neutral (PAS-positive) mucosubstances. The glands are surrounded by the compact layers of connective tissue stained for acidic mucins (AB pH 2.5, 1.0 and 0.5), smooth circular musculature, and a thin serosa. The number of gastric glands and thickness of mucosa layers increase during larval feeding phase (15-31 dph), while their histochemical properties remain the same. The platelet yolk is present in the glandular stomach until 14 dph.

Anterior and intermediate intestine

Differentiation of intestinal wall starts at 2-3 dph, progressing in postero-anterior direction. However, the anterior region of the intestine is filled with yolk and does not differentiate until 7 dph. The differentiation of the intestinal mucosa is concomitant with a disappearance of yolk in the supranuclear vacuoles of epithelial cells. The mucosa has generally similar histological structure along the length of intestine, with the exception of number and size of intestinal folds which are less abundant and smaller in the posterior region (Fig. 5a, c). During the yolk resorption, supranuclear lipidic vacuoles in the cells of intestinal epithelium increase in size and number, and they are present until 16-17 dph (Fig. 3d).

First goblet cells appear at age 6 and 10 dph, in the posterior and anterior regions of intestine, respectively. The number of goblet cells increases with differentiation of mucosa, and they are more abundant in the posterior region. Goblet cells contained carboxylated and sulphated glycoconjugates (AB-positive at pH 2.5, 1.0, 0.5) and sialic acid (HCl hydrolysis + AB pH 2.5). Most goblet cells exhibited dark-blue staining (AB and PAS) but some exhibited magenta or purple staining, suggesting the presence of acid mucosubstances in the majority of cells and the neutral (magenta) or neutral and acid (purple) glycoconjugates in some of the cells.

Spiral valve and rectum

Differentiation of spiral valve (posterior intestine) occurs soon after hatching (1-2 dph). The lumen of spiral valve is lined with a simple, columnar ciliated epithelium containing yolk inclusions in the supranuclear vacuoles (Fig. 3c). First goblet cells appear at 2 dph, and they are similar in histochemical properties to those in the anterior and intermediate intestine. The lumen of spiral valve is initially filled with yolk but, as the larvae develop, it becomes devoid

of yolk and accumulates dark pigment known as a "melanin plug" in sturgeon larvae (Dettlaff *et al.*,1993). The posterior region of a hind-gut primordium differentiates into the rectum at 5 dph. The rectal mucosa is lined with a ciliated columnar epithelium containing few goblet cells. The anus opens at 9-10 dph, after complete resorption of the residual yolk in a spiral valve.

Liver and pancreas

Liver rudiment appears at 2 dph in a ventral portion of endodermal yolk sac, and the rudiment of exocrine pancreas develops dorsally to the furrow dividing yolk sac into the stomach and intestine. At 4 dph, the polygonal hepatocytes containing large supranuclear lipidic vacuoles (not stained by HE, PAS and AB) and eosinophilic and PAS-positive glycogen inclusions, are arranged along the sinusoids (Fig. 4a). The lipidic vacuolisation and density of glycogen granules in hepatocytes increase with larval development, especially in feeding larvae. The exocrine pancreatic cells are arranged in acini at age 4-5 dph, around the small intercellular lumina. The acinar cells have eccentric (basal) nuclei and strongly basophilic cytoplasm. The zymogen granules were detected (HE and PAS) in acinar cells before the onset of first feeding (at 14 dph).

EFFECT OF FOOD DEPRIVATION ON DIGESTIVE SYSTEM

The digestive system of food-deprived larvae exhibits progressive deterioration, starting after 5 days of starvation. Starvation-induced changes in tissues and cytoarchitecture of the digestive system are summarized in Table 1 and Figure 6.

In the buccopharynx, filiform and fungiform papilla decrease in size and number between two (17 dph) and sixteen (31 dph) days of starvation, while the ventral papillae are

gradually resorbed and disappear entirely at sixteen days of starvation (31dph). The epithelium becomes thinner and the taste buds and larval teeth strongly protrude into the lumen. The cytoplasm of epithelial cells becomes hyaline after 5-day starvation (20 dph) and the pycnotic nuclei are seen at 14 days (29 dph). The histochemical properties of goblet cells in buccopharynx do not change throughout the starvation period.

Similar to buccopharyngeal epithelium, the epithelial cells of oesophagus shrink at 5-10 days of starvation (Fig. 6). From day 5 of fasting (20 dph) to the end of the study, the oesophageal mucosa and submucosa decrease in thickness during 5-15 days of starvation due to a shrinkage of smooth muscle and connective tissue fibres. Scattered pycnotic nuclei in the oesophageal epithelium are observed at 10 days of starvation, and they are abundant after 16-day starvation (31 dph). The histochemical properties of the goblet cells (secretion of neutral and acidic mucins with sialic acid) did not change throughout the starvation period.

Epithelial cells of the cardiac and pyloric stomachs shrink after 5-10 days of starvation (Fig. 6), and the brush borders lose their smooth appearance as starvation progresses. Scattered pycnotic nuclei are seen in the pyloric stomach after 10-12 days of starvation (25-27 dph). The mucosal folds of pyloric stomach flatten and disappear after 15-16 days (30-31 dph) of starvation (Fig. 4d). At the end of food deprivation period, the smooth muscle fibres separate to form intercellular spaces. The fibroblasts become atrophic or necrotic, resulting in shrinkage of stomach submucosa. In a cardiac stomach, the gastric glands collapse and their lumen almost disappear. The pyknotic nuclei are seen in the gastric glands after 13-day starvation (28 dph). No changes in histochemical properties of the glycoconjugates of the different regions of the stomach were observed throughout the 16-day fasting period.

Changes in the intestinal mucosa are seen after 8-10-day starvation (23-25 dph), including flattening folds of the intestine and spiral valve and shrinking epithelial cells (Fig.

5b, 6). The enterocytes exhibit collapsed hyaline cytoplasm and darkly pigmented elongated nuclei at 10-12 days of starvation (25-27 dph). After 14-day starvation (29 dph), the brush borders of then enterocytes are not smooth and are detached from their apical borders. Pyknotic nuclei are apparent in the intestinal mucosa after 13 days of starvation (28 dph), and autolytic processes and desquamation of intestinal mucosa are observed after 16 days (31 dph). In contrast to fed larvae, the intestinal lumen of food-deprived larvae contains a large amount of mucosubstances, mainly acidic mucins (AB pH 2.5, 1.0 and 2.5). Food-deprived larvae had melanin plug in the spiral valve in advanced stage of starvation (Fig. 5d).

Lipidic vacuoles and glycogen granules in hepatocytes decrease after only 2 days of starvation (17 dph) and disappear after 5 days of starvation (20 dph), but the cells retain their normal appearance (prominent eosinophilic cytoplasm, polygonal shape, and organization along hepatic sinusoids). The hepatocytes with pycnotic nuclei appear after 10 days of starvation (25 dph) and increase in number thereafter. The cytoplasm of hepatocytes collapse and the intercellular spaces increase in size, giving a disordered appearance to hepatic tissue (Fig. 4b). Food deprivation results in disarray of the acinar structure of pancreas. The acinar cells shrink, develop darkly pigmented nuclei, condensed cytoplasm, and the apical zymogen granules (PAS-positive). At the end of starvation period, the exocrine pancreas lost its acinar organization.

DISCUSSION

Anatomically and histologically, the development of digestive system in larval green sturgeon is similar to other acipenserids (Dettlaff *et al.*, 1993; Gawlicka *et al.*, 1995;

Gisbert *et al.,* 1998; Boglione *et al.,* 1999), except for the greater amount of yolk and slightly slower rate of development in green sturgeon (Deng *et al.*, 2001). At hatching, the digestive tract of green sturgeon larvae is closed, and the mouth and anus open at 1-2 and 9-10 dph, respectively. Larvae possess a large endodermal yolk sac lined with an epithelium that differentiates into the walls of stomach and intestine. Primordial hind-gut (spiral valve) is lined with a simple, columnar ciliated epithelium with yolk inclusions in supranuclear vacuoles. In the endogenous feeding phase (from hatching to 15 dph), the accumulation of melanin pigment in a lumen of the posterior digestive tract is likely occurring due to consumption of yolk, which contains proteins rich in tyrosine and lipidic compounds (Gisbert *et al.*, 1999). The epithelial cells surrounding the yolk sac and lining the buccopharyngeal, oesophageal, and spiral valve cavities are filled with yolk inclusions and seem to be the main regions involved in yolk resorption, tyrosine oxidation, and melanin pigment formation (Gisbert & Sarasquete, 2000). The presence of yolk inclusions in the epithelium of different regions of the digestive system reflects holoblastic style of development in acipenserids and participation of yolk-rich endodermal cells in the formation of the alimentary canal (Dettlaff *et al.,* 1993).

Histological differentiation of the alimentary canal in green sturgeon proceeds from the posterior to anterior, with the spiral valve differentiating at 2 dph and the gastric stomach at 14 dph, just before first feeding. At the onset of exogenous feeding, general anatomy and histology of larval digestive system is similar to that in juvenile or adult sturgeon (Buddington & Doroshov, 1986a; Dettlaff *et al.*, 1993; Gisbert *et al.,* 1998, 1999). The buccopharynx is lined with a squamous stratified epithelium with numerous fungiform and filiform papillae, epidermal teeth and taste buds. The lumen of the oesophagus is lined with ciliated and mucous cells, with abundant goblet cells secreting neutral and acidic

mucosubstances in the anterior region, and the ciliated epithelium performing food transport function in the posterior region. The epithelium of cardiac stomach is composed of cuboidal cells, with numerous simple and tubular gastric glands. The neutral secretory products present in this region of the digestive tract may serve to protect the epithelium of the stomach from auto-digestion processes caused by hydrochloric acid and enzymes produced in gastric glands (Domeneghini *et al.,* 1998). The pyloric stomach is lined with a simple, columnar ciliated epithelium containing supranuclear vacuoles filled with neutral mucosubstances and organized in folds surrounded by a prominent *tunica muscularis*. The secretion of neutral mucosubstances, in conjunction with thick mucosa of pyloric region, may serve to protect the underlying layers from chemical and physical damage during trituration processes (Buddington & Doroshov, 1986a). The histological organization of the intestine is generally similar in different regions, with a columnar ciliated epithelium and numerous goblet cells secreting neutral and acidic mucosubstances. At the onset of feeding, the cells of intestinal mucosa are filled with large lipid vacuoles that gradually disappear after onset of feeding (Gisbert *et al.*, 1998). The accumulation of lipids in the intestinal mucosa occurring during endogenous feeding phase may explain the ability of sturgeon larvae to survive long periods of food deprivation in the laboratory experiments (Gisbert & Williot 1997; Gisbert *et al.,* 1998).

While green sturgeon larvae stayed alive during prolonged period of food deprivation, starvation had a marked effect on histological organization of digestive system. In the endogenous feeding phase of sturgeon larval development, the proteins and carbohydrates of yolk are utilized for growth and metabolic energy, while the lipids stored in the liver and intestinal epithelium serve as an energy source for up to 16-17 dph (Wang *et al.,* 1987; Gisbert *et al.,* 1999). When lipid reserves are exhausted, the body tissues of

food-deprived larvae are catabolized, resulting in progressive degeneration of the digestive tract and accessory organs. Histopathological changes in the digestive system of food-deprived green sturgeon larvae are similar to those in larval teleosts, including: (a) changes in the liver organization, decrease in glycogen and lipids stored in hepatocytes (Margulies, 1993; Green & McCormick, 1999; Crespo *et al.,* 2001); (b) reduction in height of enterocytes (Margulies, 1993; Bisbal & Bengtson, 1995; Theilacker & Porter, 1995; Green & McCormick, 1999; Gwak *et al.,* 1999), and (c) degeneration of the exocrine pancreatic tissue (Yúfera *et al.,* 1993; Gwak *et al.,* 1999; Crespo *et al.,* 2001).

Liver glycogen and lipids is the first energy source mobilized by fasted larvae (O'Connell & Paloma, 1981). The mobilization of these nutrients under the conditions of continuing fasting results in reduction of energy available to larvae (Green & McCormick, 1999), and is associated with a moderate to severe deterioration of hepatocytes in green sturgeon larvae starved for 10-15 days. Proteolysis of the intestinal mucosa is important response to starvation. For this reason, the enterocyte height has been used as a reliable indicator of starvation or sub-optimal feeding in teleosts (Theilacker & Watanabe, 1989; Theilacker & Porter, 1995; Bisbal & Bengtson, 1995; Theilacker *et al.*, 1996; Green & McCormick, 1999). The degeneration of enterocytes implies a reduction of the absorption surface area, particularly in the spiral valve which is a main site of nutrient absorption in sturgeons (Gawlicka *et al.*, 1995; Gisbert *et al.*, 1999). The intestine is not the only region where proteolytic processes take place during starvation. Degeneration and separation of muscle fibres in the oesophagus and stomach indicate catabolic processes to provide the energy for starving larvae when the food supply is limited (Green & McCormick, 1999). Histopathological changes in digestive mucosa, pancreas and liver, caused by starvation, can affect food digestion in green sturgeon larvae resumed feeding. The pancreatic

enzymes appear to be particularly sensitive to food deprivation in larval fish. Gwak *et al.* (1999) reported decline of trypsin and amylase activities in starving *Paralichthys olivaceus* Temminck & Schlegel to very low levels, which was associated with a reduction of pancreatic volume and partial necrosis of the exocrine pancreas. In our study, we observed progressive degeneration of exocrine pancreas due to starvation but zymogen granules were still present in larvae starved for 10-15 days, as it has been described in teleost species (Yúfera *et al.,* 1993). While the stomach tissues deteriorated progressively with starvation in green sturgeon larvae, the presence of mucosubstances in pyloric region and intestinal lumen seems to suggest that gastric glands are still secreting pepsinogen and hydrochloric acid (Buddington & Doroshov, 1986b), hence the secretion of mucous protects digestive mucosa from auto-digestion.

In conclusion, histological differentiation of digestive system in green sturgeon followed patterns reported for other acipenserids. Some chronological differences in development could be related to rearing temperature and greater reserve of yolk in green sturgeon larvae. Food deprivation resulted in progressive deterioration of the digestive system, with the first pathological signs after 5 days of starvation followed with severe atrophic changes in the digestive organs after 10-15 days of starvation. The histological analysis of larval digestive system may provide sensitive indicators of the nutritional condition of green sturgeon larvae, and can be used in stock management and studies on nursery habitat affected by water projects.

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