Tolerance of Shortnose Sturgeon, *Acipenser brevirostrum*, Juveniles to Different Salinity and Dissolved Oxygen Concentrations[1]

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Abstract: Cultured shortnose sturgeon juveniles, age 11–330 days, were exposed to different salinity (0–35 ppt) and dissolved oxygen concentrations (2.0–5.0 mg/liter) in a series of experiments designed to examine tolerance levels. Tolerance to increased salinity improved with age. Fish 76 days old experienced 100% mortality in a 96-hour test when exposed to salinities ≥15 ppt while 330-day-old fish tolerated salinities as high as 20 ppt for a duration of 18 hours but exhibited 100% mortality at 30 ppt. Younger fish were also more susceptible to low oxygen concentrations than older fish. In a 6-hour test, fish 64 days old exhibited 86% mortality when exposed to DO concentrations of 2.5 mg/liter. However, sturgeon >100 days old were able to tolerate concentrations of 2.5 mg/liter with <20% mortality.

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During the past century, environmental perturbations have been partly responsible for the decline in abundance of anadromous shortnose sturgeon, *Acipenser*

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brevirostrum, which is now listed as an endangered species in the United States (Miller 1972). Although spawning and culture techniques have been developed (Smith and Jenkins 199, Smith et al. 1985, 1986), little is known of the environmental requirements of this species. Information is now being collected on the habitat requirements and movements in southern rivers, particularly the Savannah River (Hall et al. 1991, Smith and Collins 1993). These studies have indicated that adult shortnose sturgeon utilize the entire river system during the January–April spawning migrations and spend a portion of the summer months in the estuarine portion (Hall et al. 1991, Collins and Smith 1993). Juvenile shortnose sturgeon appear to utilize the lower section of the river, especially river kilometer 32, as a nursery area (Smith and Collins 1993). The lower Savannah River is highly industrialized and is impacted by stream modifications such as dredging and point source discharges. This portion of the estuary experiences substantial water quality degradation, especially in the Back River which is influenced by a tide gate (Winn and Knott 1992). In addition, natural resource managers regularly receive requests for permits that may further impact water quality in this river. Due to the lack of information on the environmental tolerances of shortnose sturgeon, these permit requests are often reviewed in absence of sufficient data to assess potential impact on shortnose sturgeon.

This study was conducted as part of a cooperative effort between the South Carolina Wildlife and Marine Resources Department and the U.S. Fish and Wildlife Service. Our objective was to obtain basic information on effects of salinity and dissolved oxygen on shortnose sturgeon of various ages. This information is critical for evaluating the potential impact of current and proposed environmental modifications. Also, such information will be useful in refining and optimizing culture systems for shortnose sturgeon. Due to various constraints including limitations of facilities and test animals, strictly controlled and standardized methods could not be followed in all tests. The findings reported should be considered as preliminary until such time as more rigorous testing can be accomplished. Funding was provided by the U.S. Fish and Wildlife Service AFS-17. We thank Bob Steffen, Robert Van Dolah, and Richard Winn who assisted with the tests and Karen Swanson who provided assistance with graphics.

Methods

Experimental fish were obtained by spawning wild adult fish captured in the Savannah River and rearing the larvae in captivity (Smith et al. 1985). Fish were reared in 2 m diameter × 1 m deep cylindrical tanks which received partially exchanged fresh water. Small juveniles were fed a combination of natural and commercial pelleted feeds (Smith et al. 1993). Test animals were removed directly from nursery systems just prior to initiation of the tolerance tests. All experiments utilized fish which ranged in age from 11–330 days (17–339 mm TL). As fish got older they were exposed to progressively higher salinity and lower oxygen (DO) concentrations. During each oxygen experiment an observer regularly recorded

sturgeon behavior and mortality; during the salinity tests, fish were monitored only during regular work hours. Control groups of fish similarly were monitored in both series of tests. In salinity studies, fish held at 0 ppt served as controls, while in DO trials, fish held at 7–8 mg/liter oxygen concentration served as controls.

Analysis of variance and a Student Newman Keuls test were used to determine if there were significant differences in survival among fish at the test DO concentration or salinities. Arc-sin transformations were used on all percentage data. Experimental groups which were not normally distributed or had non-homogenous variances were analyzed using Krushal-Wallis and Dunn's tests. A computerized statistical analysis system was used for all analyses (Sigma Stat 1992). Significance was examined at the 0.05 level.

Salinity Experiments

The salinity tests were performed using fish ranging in age from 17 to 330 days. Young sturgeon were subjected to lower salinities (range 0–11 ppt) while older sturgeon were subjected to higher levels (range 13–35 ppt). At least 3 replicates were used for each treatment, with size test container varying depending on the size of fish being tested. At the beginning of each salinity test, sturgeon were removed from 0 ppt water in the culture system, weighed, and measured and placed in test chambers containing fresh water. Acclimation to the test salinity was accomplished over a 4-hour period by adding filtered 32 ppt seawater from Charleston Harbor to the test container. In tests where higher salinity was required, salt (Instant Ocean, Aquarium Systems, Mentor, Oh) was added to reach the desired treatment level. Salinity levels were monitored a minimum of every 24 hours with a conductivity meter (YSI Model 33 S-C-T meter, Yellow Springs Instrument Co., Yellow Spring, Oh) and were further verified with a temperature compensated refractometer or a Hydrolab unit. Oxygen and temperature were measured with a Yellow Springs Model 57 meter. Each container was aerated via a central air blower system and dissolved oxygen was maintained at 7–8 mg/liter and temperature averaged 21°–23° C. Other water quality parameters including pH, ammonia, nitrate, and nitrite were monitored with colorimetric test kits manufactured by Lamotte Chemical Co. Satisfactory levels were maintained through water exchange. Mortality was recorded at least every 24 hours.

The salinity tolerance tests were conducted using fish aged 17, 22, 39, 63, 76, and 330 days old. The first series of tests using 17-day-old fish (20 mm TL) was run for 96 hours in 20-cm diameter glass petri dishes. Each salinity treatment (0, 1, 3, and 5 ppt) had 4 replicates with 5 fish/replicate. A second test was conducted on 22-day-old fish (22 mm TL) using the same experimental design except duration was 48 hours and salinity treatments were 5, 7, 9, and 11 ppt. The tests utilizing 39- (54 mm TL) and 76- (81 mm TL) day-old sturgeon utilized 3 replicates of 5 fish. The tests with 63-day-old fish (62 mm TL) were conducted with 3 replicates of 10 fish/replicate. The tests for 39- and 76-day-old fish were conducted in 38-liter aquaria for 96 hours while the 63-day-old fish were tested in 4-liter aquaria for 48 hours. The salinity concentrations tested for each age group were as fol-

lows: 39-day-old fish - 0, 5, 7 and 9 ppt; 63-day-old fish - 0, 5, 15, and 20 ppt; 76-day-old fish - 0, 7, 9, 11, 13, 15, 17, and 19 ppt. The final series of salinity tests were conducted on 330-day-old fish (339 mm TL) at salinities of 15, 20, 25, 30, and 35 ppt for a duration of 18 hours with 4 replicates containing 3 fish/replicate (12 fish/treatment).

Oxygen Experiments

The ability of sturgeons to tolerate hypoxia was examined in a series of trials. Dissolved oxygen levels of 2.0–5.0 mg/liter were tested on fish from 11–310 days old. Oxygen levels were maintained through use of a proportional flowmeter (Aalborg, Inc., Monsey, N.Y.) which mixed pressurized nitrogen and oxygen before it was delivered to each treatment container through air stones. Oxygen tests on fish ≤125 days old were conducted with 5 replicates containing 5 fish/replicate (25/treatment). Two tests on fish 307 and 310 days old were conducted with 3 replicates stocked with 3 fish/replicate. As in salinity trials, size of test chambers varied based on the size of animals. Fish were removed from the main culture system and without acclimation were placed directly into the test chamber containing the desired treatment concentration of oxygen. Control treatment groups were established for each trial. Oxygen in control tanks was maintained at 7.5 mg/liter. All tests were conducted at mean temperature of 22.5° C and were run for 6 hours. Dissolved oxygen levels were monitored every 30 minutes throughout the experiment with an oxygen meter (YSI Model 57) that had been calibrated against a Winkler titration (Am. Public Health Assoc. 1980). Adjustments were made to the gas mixture and flow as needed to keep oxygen concentration at appropriate treatment levels. The majority of the tests were conducted in fresh water. However, fish between 84 and 109 days old which were being reared at 5 ppt were tested at that salinity to avoid any synergistic effect of having to adjust to both a change in DO concentration and salinity at the same time. Other water quality parameters (pH, ammonia, nitrate, nitrite) were monitored as described for the salinity trials. Water quality parameters were monitored every hour during each trial and each parameter remained at satisfactory levels.

Results

In the first salinity trial, 17-day-old fish were able to tolerate salinities up to 5 ppt with no mortalities for 96 hours (Fig. 1). When the test was terminated the fish were behaving and swimming normally in all salinity treatments. Fish 22 days old tested for 48 hours survived significantly better at 5 and 7 ppt than those tested at 11 ppt (Fig. 1). The survival of fish stocked at 9 ppt (40%) was substantially higher than that of fish stocked at 11 ppt (90% mortality) (Fig. 1). The 39-day-old fish also exhibited a survival of 100% when tested at salinities to 7 ppt for 96 hours. However, 80% of fish from this age group survived exposure to 9 ppt (Fig. 1). Fish 63 days old had a statistically similar tolerance to salinity levels of 15 ppt and the lower 0 or 5 ppt treatment levels. Mortality in this treatment (15 ppt) was 47% after

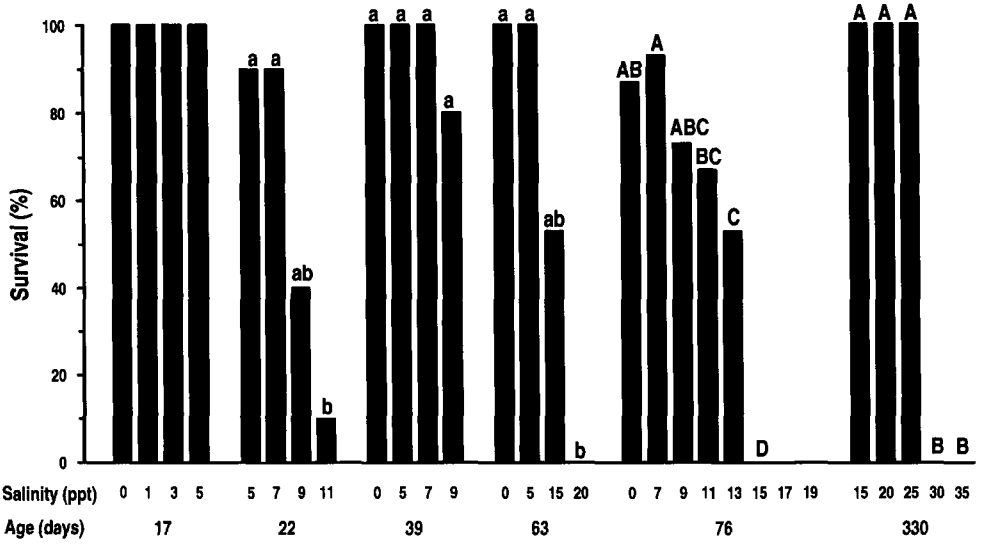

Figure 1. Survival of shortnose sturgeon exposed to different salinity concentrations. Test durations were as follows: 18 hours, 330-day-old fish; 48 hours, fish 22 and 63 days old; 96 hours, fish 17, 39, and 76 days old. Data with the same letter (anova=uppercase; Kruskal-Wallis=lower case) within a particular age group are not significantly different.

48 hours. Fish exposed to 20 ppt showed signs of stress almost immediately after the salinity treatment level was reached and mortality was 100% after 6 hours. The 76-day-old fish were tested over a more gradual salinity gradient for 96 hours. The salinity levels tested ranged from 0–19 ppt with a 2 ppt interval from 7–19 ppt. Survival at 0, 7 and 9 ppt was similar; however, survival of fish at 9 ppt was also similar to those stocked at 11 and 13 ppt which had survival levels of 67% and 53%, respectively, at the end of the test. All fish stocked at salinities ≤ 13 ppt survived at a significantly better than fish stocked at the higher test salinities. All fish stocked at salinities ≥15 ppt experienced 100% mortality during the 96-hour test (Fig. 1). The final series of salinity tolerance test was conducted on 330-day-old fish for 18 hours. Survival for fish stocked at 15, 20, and 25 ppt was 100% when the study was terminated. At that time, the fish in the 15 and 20 ppt treatments were behaving and swimming normally in the test containers; however, the fish stocked at 25 ppt treatment were visibly stressed and would probably have died if the experiment had lasted any longer. Mortality at the higher salinities tested (30–35 ppt) was 100% (Fig. 1). Examination of dead fish indicated that the fish had experienced significant osmoregulatory stress during this trial. The dead fish were dehydrated and had lost weight. Sampling indicated that fish in the 15 and 20 ppt treatments had lost 5% and 6% of their total weight, respectively, during the 18-hour trial. In comparison, fish in the 25 ppt treatment experienced a significantly higher 9% weight loss. The weight losses for the fish in the 30 and 35 ppt salinity treatments were significantly higher than all groups in the test (17% and 20%, respectively).

The hypoxia experiments were conducted using fish which were 11 to 310 days old (17–365 mm TL). Different aged fish were exposed to the same DO concentration for the same duration. In each test survival of the control groups was >95%. Fish ≤13 days old were tested at DO concentrations of 4.0, 4.5, and 5.0 mg/liter. No mortalities were observed at these treatment levels for any group of fish tested (Fig. 2). When treatment concentration was lowered to 3.5 mg/liter, 19-day-old fish (26 mm TL) experienced 22% mortality. However, this was not significantly lower than 90-day-old fish (92 mm TL, 100% survival) exposed to the same DO concentration (Fig. 2). Five groups of fish (20–307 days old) were tested at a concentration of 3.0 mg/liter. Fish <80 days old experienced significantly higher mortality than the 103-day-old (97 mm TL) for which no mortalities were observed (Fig. 2). The 307-day-old fish could not be compared. Six groups of fish (25–310 days old) were exposed to a DO concentration of 2.5 mg/liter. As in the test at 3.0 mg/liter, survival among the younger fish was substantially lower than that recorded for older fish (Fig. 2). There was no statistical difference in survival of 64-, 104-, and 125-day-old fish tested at 2.5 mg/liter. However, there was a significant difference between the survival of 125-day-old control fish and the same age fish in the treatment. There was no difference between the 104- and their 310-day-old fish and their corresponding control groups. Fish 310 days old survived at the same rate (88%) as the 104-day-old group. When the test DO concentration was lowered to 2.0 mg/liter the control group (DO 7.5mg/liter) survived significantly better than all groups held at the test concentration. Within the test group there was no difference between survival of 110- and 125-day-old fish (Fig. 2). The 46-day-old fish experienced significantly higher mortality than either group of older fish (Fig. 2).

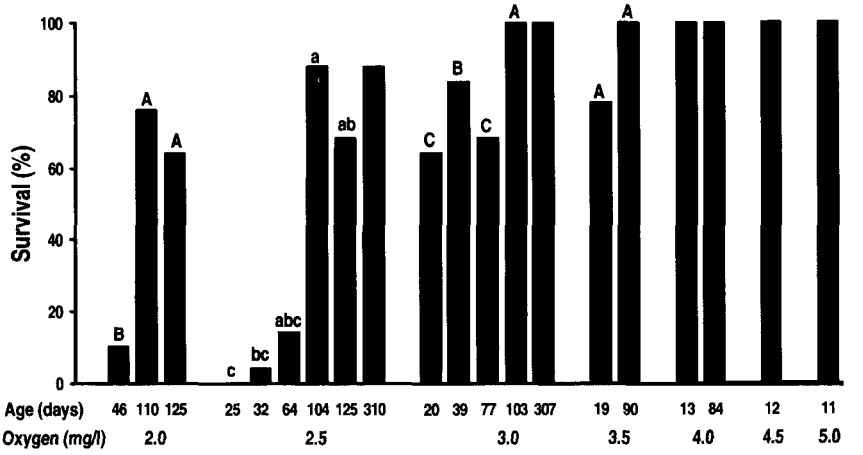

Figure 2. Survival of various aged shortnose sturgeon exposed to different oxygen concentrations. Data with same letter (anova=uppercase; Kruskal-Wallis=lowercase) within a particular test concentration are not significantly different.

Discussion

Researchers have demonstrated that shortnose sturgeon adults utilize both estuarine areas of rivers as well as near shore ocean habitats (Dadswell et al. 1984). Information on salinity tolerance for juveniles is limited. Studies in Russia on 3 species of sturgeon indicate that salinity tolerance increases with age and size (Krayushkina and Dyubin 1974). Although results of the present experiments are difficult to compare directly because of the differences in experiment duration, comparisons within test groups are possible and general trends support the hypothesis of increasing tolerance with increasing size and age. Survival among all groups held at salinities up to 7 ppt was excellent and suggests that small sturgeon could be raised at this salinity. In fact, fish reared in our nursery system are now routinely cultured at salinities of 5 ppt after day 30. A salinity of 9 ppt appeared to be a threshold at which significant mortalities began to occur, especially among the youngest fish. Fish 22 days old experienced 60% mortality after 48 hours at 9 ppt, but the 39-day-old fish experienced only 20% mortality after 96 hours. Survival of each group was significantly lower than that for the same age fish held at 7 ppt. As salinity was increased to 11 ppt, survival among the 22-day-old group declined to 10%. However, 67% of 76-day-old fish survived the 96-hour test at 11 ppt. This survival level was statistically similar to the same age fish held at 0 ppt and indicates an increased tolerance to higher salinities. The 53% survival for 63-day-old fish at a salinity of 15 ppt was higher than that recorded for 76-day-old fish but the shorter duration of the test for 63-day-old fish may be responsible for the differences. The 330-day-old fish were able to tolerate salinities up to 25 ppt for 18 hours but observations indicated that these fish were stressed. Fish in both the 15 and 20 ppt treatment levels appeared to be behaving normally but each group had lost weight during the test. The duration of the test makes it difficult to determine the 330-day-old fish's maximum tolerance level but there is little doubt that they could not tolerate salinities ≥30 ppt with the short acclimation they received in these tests. These data support the importance of estuarine habitat as nursery areas for juvenile shortnose sturgeon.

Hypoxia tests results indicated that older fish were able to tolerate low oxygen levels for short periods. A dissolved oxygen level of 2.5 killed 100% of fish 25 days old, 96% of fish 32 days old, and 86% of fish 64 days old but only 12% of the 104- and 310-day-old fish. Young fish also died at significantly higher rates at oxygen levels of 3.0 mg/liter while this concentration did not appear to adversely affect fish >77 days old. Finally, all age groups tested at 2.0 mg/liter died at significantly higher rates than the control groups. However, the older fish tested survived significantly better than the 46-day-old fish. In each test at a DO concentration of ≤3.0 mg/liter, changes in the fishes behavior were noted. The fish would become immobile and the only movement that could be detected would be the rhythmic movements of the operculum as it pumped water over the fishes' gills. This behavior has been noted and examined in detail by researchers studying white sturgeon, *Acipenser transmontanus*, and appears to be an adaption to living and

feeding on the bottom (Burggren and Randall 1978). Older and larger fish may be able to more efficiently pump water during hypoxic conditions, and this may be why older fish tolerated low DO concentrations better than the young fish in these experiments.

The dissolved oxygen levels tolerated in these short tests should be considered absolute minimum tolerance levels. Chronic levels of ≈5.3 mg/liter have been shown to cause mortality and reduced growth among white sturgeon (Cech et al. 1984).

In situations involving shortnose sturgeon, this data should serve as a preliminary guide until more rigorous tests can be conducted to determine absolute values. In addition, this data should be of use in development of future culture and stocking protocols.

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