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# Acute Sensitivity of Juvenile Shortnose Sturgeon to Low Dissolved Oxygen Concentrations

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*Abstract.*—There is considerable concern that factors such as eutrophication, industrial pollution, and dredging are adversely affecting the habitats of the endangered shortnose sturgeon *Acipenser brevirostrum*. Specific knowledge of the environmental requirements of this species is required if environmental managers are to adequately protect it. We conducted experiments to obtain information on the acute sensitivity of young-of-year shortnose sturgeon to a low dissolved oxygen (DO) concentration. Flow-through tests were conducted with hatchery-produced fish exposed to the ranges of DO, salinity, and temperature expected in the southeastern United States coastal river–estuary interfaces during spring and summer. The estimated concentration lethal to 50% of the test organisms (the LC50 value) after 24 h that we derived for approximately 77-d-old fish tested at 2‰ salinity and a nominal temperature of 25°C was 2.7 mg/L (32% saturation). An estimated LC50 of 2.2 mg/L (26% saturation) was obtained for approximately 104-d-old fish tested at 4‰ and 22°C. The 24-h, 48-h, and 72-h LC50 values for approximately 134-d-old fish tested at 4.5‰ and 26°C were also 2.2 mg/L (28% saturation). However, the test with approximately 100-d-old fish at 2‰ and a nominal temperature of 30°C yielded a 24-h LC50 of 3.1 mg/L (42% saturation). These data should be of value in derivating DO-protective values for shortnose sturgeon inhabiting estuaries along the Atlantic coast.

The anadromous shortnose sturgeon *Acipenser brevirostrum* occurs on the East Coast of North America from the St. John River, New Brunswick south to the St. Johns River, Florida (Vladykov and Greeley 1963). Historically, it was abundant enough that it was commercially harvested as part of the fishery for Atlantic sturgeon *A. oxyrinchus* (Murawski and Pacheco 1977), but by 1967 the populations had declined to the point that the species was listed as endangered (Hall et al. 1991). In the Savannah River estuarine system, juvenile shortnose sturgeon appear to occur primarily in low-salinity estuarine and tidal freshwater habitats (Collins and Smith 1997). Under normal flow conditions, the Savannah River becomes brackish between 33 and 38 km upstream of the jetties, and tidal influences extend to river kilometer (rkm; measured from the river's mouth) 83 (Hall et al. 1991). Adult shortnose sturgeon are reported to occur primarily in the same areas as juveniles except during the January–April spawning season (Collins and Smith 1997), when some adults have migrated as far inland as rkm 275–278 (Hall et al. 1991). In their review of the distribution of shortnose sturgeon in South Carolina, Collins and Smith (1997) reported that records for this species from the Atlantic Ocean were primarily from river plume areas and that their occurrence in the ocean itself is apparently rare. Kynard (1997) reported that most adult shortnose sturgeon stay within their natal river or estuary.

A number of anthropogenic impacts, such as fishing mortality (bycatch and poaching), water quality deterioration, dredging, and loss of essential habitat have been identified as factors affecting shortnose sturgeon populations (Collins et al. 1996, 2000; Kynard 1997). Collins et al. (2000) reported that the deterioration of dissolved oxygen (DO) in summer refugia utilized by shortnose and Atlantic sturgeon may be creating a recruitment bottleneck in some southeastern U.S. estuarine areas. In their review of the critical effects of hypoxia and temperature on sturgeon, Secor and Niklitschek (2001) concluded that, in terms of their metabolic and behavioral responses, sturgeon in general are unusually sensitive to hypoxia.

In studies of the salinity and DO tolerance of juvenile shortnose sturgeon, Jenkins et al. (1995) determined that younger fish were more susceptible to low DO than older fish. In their 6-h tests at 22.5°C, 86% of the 64-d-old fish exposed to 2.5 mg DO/L in freshwater died, whereas fish more than 100 d old that were tested under nearly the same conditions (5‰ salinity) experienced less than 20% mortality. In preliminary studies with young-of-year shortnose and Atlantic sturgeon, Niklitschek (2001) observed poor survival of both species at DO concentrations less than 40% saturation. Niklitschek (2001) also determined that

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the proportion of energy allocated to growth by juvenile Atlantic and shortnose sturgeon decreased as the DO concentration decreased.

Our studies were performed with hatchery-reared fish to obtain conventional estimates of the concentrations lethal to 50% of the test organisms (the LC50 values) for juvenile shortnose sturgeon exposed to low DO concentrations using the ranges of DO, salinity, and temperature that might be expected at the mouths of southeastern U.S. coastal rivers during spring and summer. These data should be of value to environmental managers with responsibility for protecting shortnose sturgeon and their habitat.

## Methods

The exposure water used in these experiments was derived by diluting salt water to achieve the lower salinity desired for testing. Salt water was pumped from Santa Rosa Sound, Florida, through sand filters and a 20-μm cartridge filter, after which it was pumped into large, aerated fiberglass tanks. Freshwater was obtained from a municipal water supply and carbon filtered to remove possible contaminants, including chlorine, and then passed through 20-μm cartridge filters. Salt water and freshwater were then mixed to achieve the desired salinity and delivered to two 40,000-L fiberglass holding tanks. Both holding tanks were aerated to remove any residual chlorine in the water. Once each tank was filled, chlorine measurements were taken to ensure that the concentrations of chlorine-produced oxidants were within acceptable limits. Chlorine analyses were performed with a Wallace and Tiernan (Belleville, New Jersey) Series A-790 amperometric titrator with a sensitivity exceeding 0.0005 mg/L.

The dissolved oxygen concentrations used in testing were obtained by means of a computer-controlled exposure apparatus. The water was pumped through three sets of filter banks and filtered to 0.45 μm before entering a saturation column. The water in the saturation column cascaded over polypropylene packing with a large surface area as air was blown up through the column, effectively saturating the water with DO. The DO-saturated water was transferred to a 900-L holding tank, and the water temperature was brought to test conditions. Hydrolab minisonde units (Hydrolab Corporation, Austin, Texas) were used to measure and transmit the DO content, temperature, pH, and salinity of the water to the controlling computer. Once the saturation column was filled and the oxygen and temperature requirements of the experiment were met, the water was pumped into the desaturation column. A microlayer of water flowed through the desaturation column, which was also filled with polypropylene packing. This column was under vacuum pressure and removed the dissolved gases from the water. The water was then transferred into a 450-L holding tank. Degassed water in the desaturation column was continuously cycled to maintain a low DO concentration. The temperature in the degassed tank was also controlled.

Once the appropriate DO concentrations, water levels, and temperature levels were met, the water was delivered to the dosing aquaria. Computer-controlled metering pumps proportionally mixed flows of DO-saturated and deoxygenated water to obtain the desired flow rates and oxygen concentrations.

Fish were exposed to selected DO concentrations in glass exposure aquaria (24 × 41 × 23 cm) with a capacity of 21 L. The water filled the aquaria and seeped out between the silicone seal and the glass lid, effectively preventing air spaces in the aquaria. Two replicate aquaria were used for each of the five DO concentrations. The aquaria were partially immersed in a temperature-controlled water bath. Flows to all aquaria were measured and adjusted before the beginning of each test.

Test animals were young-of-year shortnose sturgeon obtained from the U.S. Fish and Wildlife Service's Bears Bluff National Fish Hatchery in South Carolina. According to hatchery personnel, fish were captured from the Savannah River drainage and artificially spawned during the mid-1980s to produce a captive broodstock at the Bears Bluff Hatchery. The fish provided for our experiments were from artificial spawns of the captive broodstock. All fish tested during 1999 were the progeny of one female and one male; similarly, fish tested during 2000 were the progeny of one pair of fish. The hatch date for the fish that we obtained on June 30, 1999, was about April 15, 1999; the fish obtained on June 15, 2000, hatched about April 18, 2000. Upon arrival at our laboratory, the fish were distributed among aquaria receiving flowing filtered seawater of the same salinity as used in testing. The acclimation aquaria were located in a temperature-controlled water bath and continuously aerated. The photoperiod during both acclimation and testing was 14 h light : 10 h dark. Fish were usually fed two or more times per day during acclimation and holding. The foods provided were frozen bloodworms that were purchased commercially and a pelletized food (Rangen, Inc., Frank-

TABLE 1.—Exposure conditions in tests to determine the acute sensitivity of juvenile shortnose sturgeon to low dissolved oxygen (DO) concentrations.

| Variable | Fish age (d) | | | |
|---|---|---|---|---|
| | ~77 | ~100 | ~104 | ~134 |
| Acclimation period (d)[a] | 12 | 12 | 13 | 15 |
| DO concentration (mg/L; range)[b] | 2.2–8.0 | 2.2–7.4 | 1.9–8.4 | 1.6–7.8 |
| pH (range) | 8.2–8.3 | 8.3–8.4 | 7.9–8.1 | 7.9–8.2 |
| Salinity (‰) | 2 | 2 | 4 | 4.5 |
| Nominal temperature (°C) | 25 | 30 | 22 | 26 |
| Mean wet weight ± SD (g)[c] | 2.6 ± 0.5 (20) | 2.6 ± 0.5 (20) | 2.4 ± 0.8 (10) | 2.3 ± 0.8 (25) |
| Year tested | 2000 | 2000 | 1999 | 1999 |

[a] Number of days within 1°C of nominal test temperature prior to testing.
[b] Range in mean measured exposure concentrations.
[c] Sample sizes are in parentheses.

linton, Louisiana). Fish were acclimated to the nominal test temperature (±1°C) for 12–15 d prior to testing (Table 1).

Once the dosing aquaria were filled and the desired oxygen concentrations achieved, fish were randomly transferred to the exposure aquaria without acclimation to low DO. Ten fish were placed in each of two replicate aquaria for each of the five exposure concentrations. Because of concerns about loading in our test system, the fish selected for the initial test were among the largest fish in our holding aquaria in both 1999 and 2000. The highest DO concentration in each test was near the saturation concentration and served as the control. The temperature, pH, salinity, and DO content of the water and the mortality of the test animals were usually recorded at 0, 2, 4, and 24 h. Four acute tests were performed; the test conditions are presented in Table 1. Fish were not fed during testing except in the 72-h test, in which they were provided bloodworms at least once daily. The mean wet weights of a sample of fish from each test are provided in Table 1; the sample was usually the fish that succumbed during the test. As a quality assurance measure, two control fish from the June 28, 2000, test were preserved in Lugol's solution for subsequent histological examination of gill tissues.

TABLE 2.—Estimated median lethal concentrations (LC50s) and 95% confidence intervals (CIs) for young-of-year shortnose sturgeon of different ages exposed to low dissolved oxygen at selected temperatures.

| Fish age (d) | Temperature (°C) | Duration (h) | LC50 (mg/L) | 95% CI (mg/L) |
|---|---|---|---|---|
| ~77 | 24.6–25.0 | 24 | 2.7 | 2.3–3.1 |
| ~100 | ~28.4–29.2 | 24 | 3.1 | [a] |
| ~104 | 21.8–22.4 | 24 | 2.2 | 2.2–2.3 |
| ~134 | 26.0–26.4 | 24 | 2.2 | 2.0–2.4 |
| | | 48 | 2.2 | 1.9–2.4 |
| | | 72 | 2.2 | 1.9–2.4 |

[a] Not determined.

Dissolved oxygen concentrations were determined by titration (APHA et al. 1995) and by use of Nester Model 8500 (Nester Instruments, North Wales, Pennsylvania) and Orion Model 862A (Orion Research, Inc., Boston, Massachusetts) DO meters. Estimated LC50 values and 95% confidence intervals (CIs) were derived by linear interpolation combined with bootstrapping using ToxCalc (TidePool Scientific Software 1994).

## Results

Most of the mortality in our experiments occurred fairly rapidly. For the four tests, 53–90% of the mortality occurred within the first 2 h and 79–96% within the first 4 h. The signs of stress observed in fish exposed to low DO were reduced swimming and feeding activity and increased ventilation frequency.

The median LC50 values obtained in the four tests ranged from 2.2 to 3.1 mg/L (Table 2). The youngest age-group tested, which was approximately 77 d old, yielded a 24-h LC50 of 2.7 mg/L (95% CI = 2.5–2.8 mg/L) when exposed to low DO at 25°C. Fish that were approximately 104 d old and that were tested at 22°C and fish that were approximately134 d old and tested at 26°C had identical 24-h LC50 values of 2.2 mg/L with 95% confidence intervals that differed only slightly (2.2–2.3 mg/L and 2.0–2.4 mg/L). In the 72-h test conducted with approximately 134-d-old fish, the 24-h, 48-h, and 72-h LC50 values were also 2.2 mg/L, with only slight differences in the 95% confidence intervals (Table 2). Fish that were approximately100 d old and that were tested at 29°C were the most sensitive to low DO, yielding a 24-h LC50 of 3.1 mg/L. However, during their acclimation to

a nominal 30°C, the fish were exposed to temperatures as high as 31°C, which may be near their upper temperature tolerance limit. The survival of fish in control treatments, which contained near-saturation concentrations of DO, was 100% during each test.

The concentrations of chlorine-produced oxidants in our low-salinity dilution water were less than the U.S. Environmental Protection Agency's water quality criteria final chronic value of 7.5 μg/L (EPA 1985). Histological examination of gill arches from two control fish from the June 28, 2000, test revealed no evidence of any pathological abnormalities.

## Discussion

Our data and those of Jenkins et al. (1995) demonstrate that juvenile shortnose sturgeon up to 134 d old are quite sensitive to low DO in acute tests at low salinities. Adult and older juvenile shortnose sturgeon are known to inhabit low-salinity areas (Gilbert 1989; Flournoy et al. 1992; Collins and Smith 1997), but there are uncertainties regarding the habitats used by fish of the ages tested in our experiments and in those of Jenkins et al. (1995). The LC50 values derived in our studies and those of Jenkins et al. (1995) are greater than those for 10 species of juvenile estuarine fish tested by Miller et al. (2002). However, the mean LC50 value of 2.4 mg/L reported for postlarval striped bass *Morone saxatilis* (Miller et al. 2002) is similar to the LC50 values that we derived for shortnose sturgeon at 22–26°C.

The LC50 values derived in our studies tend to support the findings by Jenkins et al. (1995) that younger sturgeon are more sensitive to low dissolved oxygen than are older fish. However, factors such as different test conditions, genetic variability, and test variability may have contributed to the apparent difference in sensitivity between the fish that were approximately 77 d old and those that were approximately 104 and 134 d old.

Shortnose sturgeon appear to be particularly vulnerable at high water temperatures. Flournoy et al. (1992) reported that shortnose sturgeon in the rivers of the Deep South seek out certain deep holes when water temperatures approach lethal levels. They also reported that at water temperatures exceeding 28°C many recaptured sturgeon from such holes in the Altamaha River, Georgia, exhibited weight loss. Secor and Gunderson (1998) reported that juvenile Atlantic sturgeon are vulnerable to conditions of hypoxia and high temperature; all fish exposed to a temperature of 26°C and a DO concentration of approximately 3 mg/L without access to the air died within the first 30 h of their experiment.

Although the information obtained in our studies will be useful to environmental managers involved in protecting the endangered shortnose sturgeon, there remain a number of questions about the effects of DO. These include (1) the acute sensitivity of older juveniles and adult fish to low DO and the influences of salinity and temperature on their sensitivity; (2) possible chronic effects of low DO; (3) the age-specific ability of shortnose sturgeon to avoid low DO concentrations and the possible effect of temperature on such responses; and (4) the habitat of young-of-year fish with respect to salinity during approximately their first 200 d after hatching.

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