Environmental Biology of Fishes 51: 429–444, 1998.

R. Cull
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Physiological tolerances of juvenile robust redhorse, *Moxostoma robustum*: conservation implications for an imperiled species

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Received 24.1.1997 Accepted 20.6.1997

Key words: fish, Catostomidae, temperature, salinity, pH, hypoxia, conservation biology

Synopsis

The robust redhorse, *Moxostoma robustum* (Teleostei: Catostomidae), is an imperiled sucker native to large rivers of the Atlantic slope of the southeastern United States. Juvenile *M. robustum* were tested for tolerances to temperature, salinity, pH, and hypoxia in order to evaluate basic early life-history requirements. Static (acute) tests resulted in estimates of mean lower temperature tolerances (5.3–19.4 °C) that varied with prior thermal acclimation and indicated no apparent difference in tolerance among fish 30, 60, and 90 days old. Fish acclimated to 20 °C and 30 °C had significantly different mean critical thermal maxima (34.9 °C and 37.2 °C, respectively) and exhibited pronounced increased opercular ventilation rates with elevated temperatures. Fish exposed to acute and chronic increases in salinity showed unusual patterns of mortality above the isosmotic point (9 ppt) that reflected possible differences in body mass and prior acclimation conditions (i.e., water ionic composition); small fish and those held in soft water were the least tolerant of increased salinity. Abrupt exposure to extreme pH values resulted in greater than 50% mortality at pH values below 4.3 and above 9.5 within a 96-hour period. Fish exposed to progressive hypoxia utilized aquatic surface respiration at a mean oxygen concentration of 0.72–0.80 mg O_2 l^{-1} (20 °C and 30 °C acclimated fish, respectively), and lost equilibrium at 0.54–0.57 mg O_2 l^{-1}. Juvenile *M. robustum* are moderately tolerant of a wide range of ambient physicochemical parameters, but further research is needed to determine how both abiotic and biotic factors have contributed to population decline and extirpation of this species.

Introduction

The robust redhorse, *Moxostoma robustum*, is the largest catostomid species occurring in Atlantic slope drainages of the southeastern United States, with adults reaching sizes of at least 7 kg and 72 cm. Recently this species was known from only two specimens collected in the early 1980's and was originally thought to be an undescribed and rare or possibly extinct species, probably representing an Atlantic slope sister taxon of the river redhorse, *M. carinatum*. In 1991 personnel of the Georgia Department of Natural Resources discovered an extant population of robust redhorse in the Oconee River, a tributary of the Altamaha River, Georgia. Availability of recently collected specimens and reevaluation of southeastern catostomid systematics led to the conclusion that *M. robustum* was rediscovered since its original taxonomic description by E.D. Cope in 1870 (Jenkins & Burkhead 1994).

The species formerly had a range spanning large southeastern Atlantic coastal drainages from the Pee Dee River, North and South Carolina, to the Altamaha River, Georgia. The Oconee River population is the only known extant population, although remnant populations may exist in some isolated portions of other coastal drainages.

Historically, *M. robustum* probably occurred primarily in riverine segments of the Piedmont physiographic province, but the existing Oconee River population is limited to an 85 km river stretch within the Coastal Plain physiographic province. The population exists only downstream of Lake Sinclair, a large (62 km^2) reservoir impounded by Sinclair Dam, a hydroelectric facility completed in 1952 and operated by the Georgia Power Company. The Oconee River population is senescent and there is no evidence of natural recruitment, based on annual sampling since 1992 by the Georgia Department of Natural Resources and despite recent observations of in-stream spawning and the deposition of viable eggs in spawning redds (B.J. Freeman & C.A. Jennings personal communication). Moreover, the stock of adult fish is relatively small and considered to be highly vulnerable to mortality associated with stream discharge and fluctuating hydrogeneration flow, the introduced predator *Pylodictis olivaris*, riparian habitat loss, land-use changes in the watershed, sedimentation, and declining native mussels on which *M. robustum* feeds. The current level of jeopardy of *M. robustum* in the Oconee River and the widespread decline of the species throughout its historical range has stimulated extensive research and recovery efforts to monitor the population, identify critical threats, evaluate habitat requirements and life history, and to propagate the species in captivity (Bryant et al. 1996).

Information about tolerances of fish species to stressful physicochemical conditions is essential to understanding historical distributions, microhabitat requirements, and possible factors that contribute to or help explain causes of population decline. Basic ecophysiological data are often lacking for endangered or threatened fishes at the time that their imperiled status is recognized. Among eastern North American catostomids there is one extinct species, the harelip sucker, *M. lacerum*, for which there is relatively scant detailed biological information (Ono et al. 1983, Miller et al. 1989, Jenkins & Burkhead 1994). Currently more catostomids are recognized as endangered, threatened, or of special concern in the western United States and Mexico than in the eastern United States (Williams et al. 1989). Consequently, a greater share of conservation-oriented research has been directed toward evaluating adaptations and environmental requirements of western suckers than some of the species-rich genera of eastern North America, such as *Moxostoma, Thoburnia, Scartomyzon, Hypentelium*, and *Ictiobus*. Important physiological studies have included imperiled species belonging to the genera *Catostomus, Chasmistes,* and *Xyrauchen* (Vondracek et al. 1982, Bozek et al. 1990, Falter & Cech 1991, Scoppetone et al. 1993), as well as a few common catostomids such as *Catostomus commersoni* (e.g., Beamish 1972, Wilkes & McMahon 1986a, b, Hobe et al. 1984, Hobe & McMahon 1988, Walker et al. 1989). Similar detailed studies do not exist for most eastern catostomids, especially imperiled species or large-river forms, including *M. robustum*. Comparative data are especially needed to better understand ecological requirements, since catostomids of eastern North America evolved under dramatically different hydrological conditions (e.g., flow regimes and sedimentation rates) than western species and might be expected to exhibit significant differences in physiological adaptation.

The purpose of this study was to determine minimum physicochemical conditions required for early life-history intervals and the implications of those requirements for the conservation of *M. robustum*. Availability of artificially spawned fish allowed us to examine tolerances of juveniles to experimental manipulation of temperature, pH, salinity, and dissolved oxygen. These parameters were chosen because of their potential relevance in explaining historical distribution patterns and in understanding possible factors involved in the demise of populations across the range of the species. Many of the large rivers historically inhabited by *M. robustum* have been significantly altered by the construction of reservoirs, sedimentation, and other forms of physical habitat modification. We sought to eval-

uate the tolerances of *M. robustum* to temperature and oxygen because these physical parameters are hypothesized to have contributed to reproductive failure of the Oconee River population or to declines elsewhere in the geographic range of the species. Habitat perturbations in the spawning redds or in nursery areas may have resulted in unfavorable thermal or dissolved oxygen conditions for early life-history intervals of *M. robustum*. Salinity and pH tolerances were examined primarily to assess osmotic and ionoregulatory adaptation in the context of understanding historical distribution patterns and potential dispersal capability within and among coastal drainages. Additionally, information on salinity and pH tolerance could be important in evaluating possible life-history impacts resulting from ecological conditions associated with land-use changes that negatively affect water quality.

Methods

Experimental fish were obtained from artificial crosses of adults collected from the Oconee River upstream of Dublin, Georgia (approximately between river km 121–147), during reproductive periods in May 1993 and 1995. Half-sibling fish from 1993 broodstock were obtained by fertilizing the ova of a single large female with milt of several males on 25 May 1993. The fertilized eggs were transported to Warm Springs Fish Technology Center (United States Fish and Wildlife Service) in Warm Springs, Georgia, and incubated at 20–23 °C until hatching on 28–29 May 1993. The offspring were either laboratory-reared and used as early post-metamorphosis juveniles (4–12 weeks), or both laboratory- and pond-reared juveniles (9–24 months) in experimental tests of temperature and salinity tolerance and response to hypoxia. Laboratory-reared fish were transported to our facility in Gainesville, Florida on 9 June 1993 and maintained and acclimated in fiberglass or glass aquaria supplied with flow-through well water, except as noted below for fish used in salinity and pH experiments. Early post-metamorphosis fish were initially fed twice daily using a commercial juvenile-salmonid [1] and live *Artemia*, and slowly converted to a mixed diet consisting of commercial pelleted feed, pre-frozen brine shrimp, and minced live California blackworms, *Lumbriculus variegatus*. Pond-acclimated fish were stocked as young-of-the-year into aquaculture ponds at Walton State Fish Hatchery (Georgia Department of Natural Resources, Social Circle, Georgia) on 15 June 1993. Fish were harvested from ponds on 8 March 1994, transported immediately to our laboratory, and maintained in the laboratory under the same conditions as above. Larger juvenile fish were fed a mixed diet of pelleted feed, live blackworms, and pre-frozen brine shrimp ad libitum every 1–2 days.

Lower temperature tolerance was determined using acute (static) methods in which prior-acclimated fish were exposed to sudden temperature extremes near their lethal or sublethal levels, following Fry (1947, 1971), Brett (1956), and subsequent investigators. In each exposure ten (rarely eight or four) early post-metamorphosis fish were placed in each test chamber maintained at constant temperature, with 14 test chambers spanning a 4°–18 °C range of temperatures. Test chambers consisted of 1 l glass jars each containing 750 ml of aerated well water. Jars were placed in an aluminum trough with a 4°–18 °C thermal gradient (Meldrim & Gift 1971, Stauffer 1986, Stauffer et al. 1989) at positions corresponding to approximately 1 °C differences between jars. Following initial exposure to test temperatures, fish were monitored at regular intervals over a 27 hour period and the number dead in each jar recorded. Mortality was defined as failure of an individual to respond to a gentle tactile stimulus, using a smooth glass rod, over a 15 min period following loss of equilibrium. This criterion was used because most fish had a heartbeat and reacted to stimuli for varying time periods following exposure to lethal or sublethal low temperatures.

Fish used in lower temperature tolerance tests were initially held at 22–23 °C. Short-term acclimation was achieved by raising or lowering water temperature 1 °C day^{-1} and holding fish at constant temperatures of 15°, 20°, 25°, and 30 °C one week prior to plunging into test chambers. Lower temperature tests were repeated on fish 30, 60, and 90 days old.

To estimate physiological tolerances of fish exposed to different levels of lower temperature ex-

tremes, we used statistical models commonly applied in bioassay or dose-response studies (Collett 1991). In our experiments mean tolerance is defined as the effective temperature at which 50% mortality was observed among the groups of fish exposed to acute changes in temperature. Tolerance was estimated by logistic regression using temperature as the independent variable and number of deaths as the binomially distributed response variable. An estimate of the mean tolerance, $\hat{\mu}$, was computed from estimates of the regression intercept and slope (denoted $\hat{\beta}_0$ and $\hat{\beta}_1$, respectively) as follows:

$$\hat{\mu} = \frac{-\hat{\beta}_0}{\hat{\beta}_1} \tag{1}$$

(Collett 1991). The standard error of $\hat{\mu}$ was estimated using

$$\mathrm{SE}(\hat{\mu}) = \sqrt{\frac{\hat{\gamma}_{00} + 2\hat{\mu}\hat{\gamma}_{01} + \hat{\mu}^2\hat{\gamma}_{11}}{\hat{\beta}_1^2}}, \tag{2}$$

in which, $\hat{\gamma}_{00}$, $\hat{\gamma}_{11}$, and $\hat{\gamma}_{01}$ are the regression estimates of $\mathrm{Var}(\hat{\beta}_0)$, $\mathrm{Var}(\hat{\beta}_1)$, and $\mathrm{Cov}(\hat{\beta}_0, \hat{\beta}_1)$, respectively.

The range of temperatures used in our experiments included extremes relative to acclimation conditions. At the extremes complete mortality or complete survival was observed. To estimate the temperature at which 50% mortality occurred (i.e., the mean tolerance), only temperatures that appeared to fit the linear-logistic model were included in the regressions. In these regressions a linear relationship is expected between temperature and the empirical logit of the proportion of animals that died during the experiment. The empirical logit is a reparameterization of this proportion and is appropriate for binomial responses (Agresti 1990). Given y deaths of n animals, the empirical logit of the proportion of deaths, y/n, is defined as $\log [(y + 0.5)/(n - y + 0.5)]$. Empirical logits were plotted against temperatures to determine the range of observations that appeared to fit the logistic regression model. Observations at extremes of temperature that did not fit the model were excluded from statistical analysis to ensure that the relationship between mortality and temperature, and therefore mean tolerance, was estimated accurately. Some precision was lost in the process, but this was unavoidable without prior knowledge of even approximate estimates of temperature tolerance.

Upper thermal tolerance was determined by measuring the critical thermal maximum (CTMax) of pond-reared juveniles (22 months old) that were acclimated in the laboratory for a minimum of two weeks to temperatures of 20 ° and 30 °C immediately prior to testing. Tests were conducted in individual 3.8 l glass vessels containing 1.9 l aerated well water and submerged in a glass aquarium that served as an external water bath. Water in the encircling bath was heated at a rate of 0.3 °C min^{-1} (Becker & Genoway 1979) with a submersible titanium heater connected to an Omega® Series 2010 programmable temperature controller. During trials fish were monitored every 5 min and the time (sec) required for each individual to complete 20 opercular ventilations was recorded. The CTMax was recorded as the temperature at which a fish lost equilibrium, using mercury thermometers calibrated to ± 0.1 °C with a National Bureau of Standards certified thermometer. Following loss of equilibrium fish were immediately returned to acclimation temperatures and allowed to recover before being blotted dry, weighed (g) and measured (mm, standard length).

Salinity tolerance was evaluated in both laboratory-reared and pond-reared fish using acute (static) and chronic (dynamic) exposures to elevated ionic concentrations. Commercial synthetic aquarium salts marketed with similar elemental constituents as natural sea water (Forty Fathoms®, Aquatic Eco-Systems, Inc.) were mixed with hard well water having the following approximate major ion content: total alkalinity 172–191 mg $CaCO_3$ l^{-1}; 11.8–19.2 mg Cl l^{-1}; 3.5–7.4 mg SO_4 l^{-1}; 54–58 mg Ca l^{-1}; 9.5–15.2 mg Mg l^{-1}; 5.0–9.3 mg Na l^{-1}. Salinity of water used in all experiments was adjusted precisely to within 1.0 part per thousand (ppt) using a Wescor® 5500 vapor pressure osmometer. Fish were offered food daily in all salinity experiments.

Acute salinity experiments were conducted similarly to lower temperature tolerance tests. A group of ten fresh-water acclimated fish was plunged into each test chamber maintained at constant salinity, using 13 test chambers ranging from salinities of 0 (control) to 18 ppt. Mortality in each test chamber

recorded at periodic intervals over a time span of 120 hours (laboratory-reared fish) or 484 hours (pond-reared fish). The physiological tolerances of fish exposed to acute changes in salinity were estimated somewhat differently than lower temperature tolerance due to differences in experimental design. The maximum exposure of pond-reared fishes to acute changes in salinity was 5 times longer (20 versus 4 days) than that of laboratory-reared fishes. Using 50% mortality to define the salinity tolerance of these two groups of fishes would have induced an unfair comparison, given the longer period of risk for the pond-reared fish. For example, if both groups of fish experienced approximately equal rates of mortality, a higher proportion of deaths is expected among the pond-reared fish simply because of their increased time at risk.

To compare the acute salinity tolerances of pond- and laboratory-reared fishes, the rate of mortality, rather than the proportion of deaths, was modeled as a function of salinity. In each experimental container of constant salinity, a Poisson distribution was assumed for the number of fish that died during their collective time at risk; therefore the probability that y fish died during t hours at risk was modeled in terms of the mean mortality rate λ (in deaths per hour) as follows:

$$f(y; \lambda, t) = \frac{e^{-\lambda t}(\lambda t)^y}{y!}. \quad (3)$$

Mean mortality rates of pond- and laboratory-reared fishes were formulated in terms of salinity using the following generalized linear regression model (McCullagh & Nelder 1989),

$$\log_e(\lambda) = \beta_0 + \beta_1 S + \beta_2 X, \quad (4)$$

in which S corresponds to salinity (parts per thousand) and X indicates whether fish were pond-reared ($X = 0$) or laboratory-reared ($X = 1$). In this model mortality rates of pond- and laboratory-reared fish have different intercepts and a single slope. The assumption of a common slope was tested by comparing model (4) with a second regression model that included an additional parameter for an interaction between S and X.

Using estimates of the parameters of regression model (4), we compared differences in mortality rates at constant salinity and differences in salinity tolerance at a constant rate of mortality. For example, the ratio of the mortality rate of laboratory-reared fish to the mortality rate of pond-reared fish was estimated by $\exp(\hat{\beta}_2)$. An estimate of its standard error is $\exp(\hat{\beta}_2)\sqrt{\widehat{\mathrm{Var}}(\hat{\beta}_2)}$. The salinity tolerances of laboratory- and pond-reared fishes were compared by estimating their difference at a fixed rate of mortality. This is similar to the way that temperature tolerances were defined using 50% mortality as a reference level of mortality. The difference in salinity tolerances, δ, was estimated by $\hat{\delta} = \hat{\beta}_2/\hat{\beta}_1$. An estimate of its standard error was computed using

$$\mathrm{SE}(\hat{\delta}) = \sqrt{\frac{\hat{v}_{00} - 2\hat{\delta}\hat{v}_{01} + \hat{\delta}^2\hat{v}_{11}}{\hat{\beta}_1^2}}, \quad (5)$$

in which $\hat{v}_{00}$, $\hat{v}_{11}$, and $\hat{v}_{01}$ are the regression estimates of $\mathrm{Var}(\hat{\beta}_2)$, $\mathrm{Var}(\hat{\beta}_1)$, and $\mathrm{Cov}(\hat{\beta}_1, \hat{\beta}_2)$, respectively.

Groups of both laboratory- and pond-reared fish were used in chronic salinity trials by exposing them to water of progressively increasing salt concentrations of 1 ppt every 2 days and recording mortality daily. Recirculated water in chronic trial aquaria was filtered using sponge filters and partially replaced every 3–5 days. Because of inconsistent results in initial salinity tolerance experiments, chronic tests were repeated on pond-reared fish exposed to different prior acclimation conditions. The first group (trial 1) was tested immediately following capture from ponds in which they were acclimated to soft water (conductivity = 132 μmho cm^{-1}; total Ca = 11.4 mg l^{-1}; total Mg = 2.11 mg l^{-1}; total Na = 3.44 mg l^{-1}) for approximately 9 months. The second group (trial 2) was maintained in hard well water (conductivity = 416 μmho cm^{-1}; total Ca = 50.6 mg l^{-1}; total Mg = 13.6 mg l^{-1}; total Na = 9.54 mg l^{-1}) within the laboratory for 5 weeks prior to testing.

Fish exposed to chronic increases in salinity were subsampled during the experiment to monitor changes in serum osmolality as ambient salinity was raised. Early post-mortem fish and subsamples of 5 live fish from the laboratory-reared group and the initial pond-reared group used in chronic salinity tolerance trials were removed at 3 ppt increments

and sacrificed for blood analysis. Blood was removed by cardiac puncture with a heparinized capillary tube drawn to a fine point. Formed elements and plasma were separated by centrifugation and the hematocrit and plasma osmolality (mOsm kg^{-1}) then determined. In a few cases plasma samples of individual fish were pooled to achieve a minimum required sample size of 5 μl for determining plasma osmolality; where possible, a mean of three separate readings per sample was used. Occasional hemolyzed samples from post-mortem fish were discarded from final analyses.

The pH tolerance experiments were conducted with early post-metamorphosis juveniles (3 months old) originating from random mixed crosses (17 females × 12 males) of 1995 broodstock. Parental fish were obtained from the same river reaches as the 1993 broodstock. Young hatched from artificial spawnings on 3–8 May 1995 were held indoors at the Warm Springs fish hatchery until being transported to the Gainesville laboratory on 13 July 1995, where fish were then maintained under identical conditions as the 1993 cohort. Young used in pH tolerance experiments were acclimated for two weeks at 25 °C in reconstituted soft water, made by adding appropriate salts to deionized water to achieve water with the following physical characteristics: pH 6.8–7.2; conductivity 170–200 μmho cm^{-1}; alkalinity 20–22 mg l^{-1} $CaCO_3$; hardness 25–27 mg l^{-1} $CaCO_3$. A series of aerated 3.8 l glass jars each containing 1.9 l of reconstituted water were maintained at constant values ranging from pH 4 to 12; pH of each jar was adjusted daily by titration of dilute H_2SO_4 or NaOH. Groups of 10 fish were placed in each jar and mortality was recorded at regular intervals over a 96 hour period, using the same methodology as lower temperature and acute salinity tolerance experiments.

Responses to reduced dissolved oxygen concentrations were examined by conducting progressive hypoxia trials on pond-reared fish selected randomly and acclimated in the laboratory to 20 °C and 30 °C. For each trial, a group of three fish was acclimated for 16 hours in a glass (61 × 41 × 32 cm) aquarium containing aerated well water. Dissolved O_2 concentration was progressively reduced at a rate of 20–25 mm Hg h^{-1} during each approximately 6-hour trial by slowly bubbling nitrogen into the bottom of two side compartments of the aquarium. The test tank was shielded except for a small observation port to minimize disturbance of fish from external visual stimuli. Partial pressure of O_2 (pO_2) was measured using a Strathkelvin® Instruments Model 781 oxygen meter. Fish were monitored once per hour at pO_2 above 25 mm Hg and every 15 min thereafter until reaching loss of equilibrium. The number of fish swimming or performing aquatic surface respiration (Kramer & Mehegan 1981) within five 20 sec intervals were recorded at each observation. In addition, at each observation time five random measurements were made of the time (sec) required for an individual to complete 20 opercular ventilations. Tests were terminated when fish lost equilibrium, at which point the elapsed time and pO_2 was recorded. Fish were then immediately removed and placed into a well-oxygenated tank, then measured (mm, SL) and weighed (g, wet mass) following recovery. Mean pO_2 values of responses at each acclimation temperature were converted to O_2 concentration (mg l^{-1}) using conversion factors in Colt (1984) to facilitate comparisons with other species.

Table I. Logistic regression estimates of mean lower temperature tolerance (°C) of juvenile half-sibling *M. robustum* at different ages and experimental acclimation temperatures. Estimates of SE are in parentheses.

Age (d)	Acclimation temperature (°C)			
	15°	20°	25°	30°
30	6.02 (0.14)	7.93 (0.27)	12.97 (0.30)	14.68 (0.31)
60	6.41 (0.29)	–	11.48 (21.0)	19.42 (2.57)
90	5.31 (22.43)	–	12.23 (0.31)	11.66 (0.44)

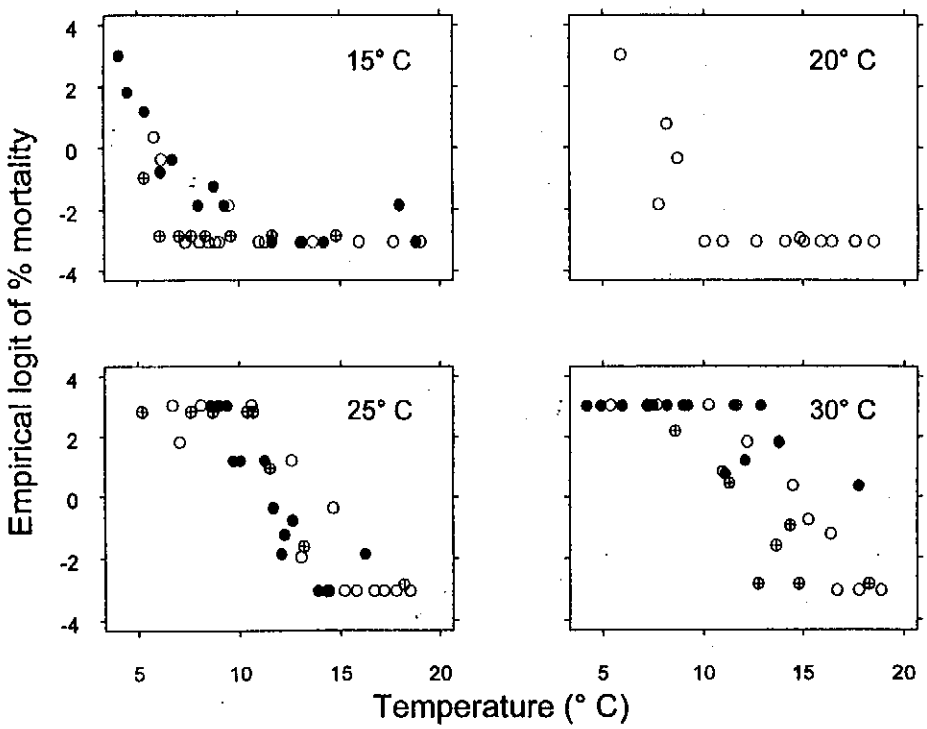

Figure 1. Empirical logit of percent mortality of half-sibling cohorts of juvenile *M. robustum* of different ages and temperature acclimations (mortality approaches 50% at empirical logit values approaching 0). Open circles = 30 days; closed circles = 60 days; cross hairs = 90 days.

Results

Temperature tolerance

Estimates of lower temperature tolerance ranged from a minimum of 5.3 °C in 3-month old 15 °C-acclimated fish to a maximum of 19.4 °C in 2-month old 30 °C-acclimated fish (Table 1). Tolerances were directly correlated with prior acclimation temperature as expected. Fish in abrupt lower-temperature tolerance tests ranged in body mass from 12–42 mg (1 month), 20–150 mg (2 month), and 32–222 mg (3 month). Across each acclimation temperature there appeared to be little difference in thermal tolerance of fish between one and three months of age (Figure 1).

Fish used in CTMax determinations ranged in SL from 8.7–11.2 cm ($\bar{X}$ = 9.8) and in mass from 6.9–17.2 g ($\bar{X}$ = 10.9). There was no difference in body size but there was a significant difference (p = 0.037) in upper temperature at loss of equilibrium between the two acclimation groups: mean CTMax for the 20 °C-acclimated fish was 34.93 °C (SE = 0.95; n = 12) and for 30 °C-acclimated fish was 37.16 °C (SE = 0.11; n = 11). Thermal stress was generally indicated by increased opercular ventilatory frequency during the course of CTMax trials, with fish in the 20 °C acclimation group exhibiting slightly higher ventilatory rates than 30 °C-acclimated fish (Figure 2).

Salinity tolerance

Laboratory-reared and pond-reared fish used in acute salinity experiments differed in body size as a result of prior exposure conditions, nutrition, and differential growth rates. Laboratory-reared fish (n = 110) ranged in SL from 20–79 mm ($\bar{X}$ = 33) and mass from 8–448 mg ($\bar{X}$ = 71); pond-reared fish (n = 130) ranged in SL from 80–128 mm ($\bar{X}$ = 98) and mass from 920–2660 mg ($\bar{X}$ = 1472). Fish exposed to salinities greater than 9 ppt exhibited 50% mortality or greater within 53 hours of exposure in both

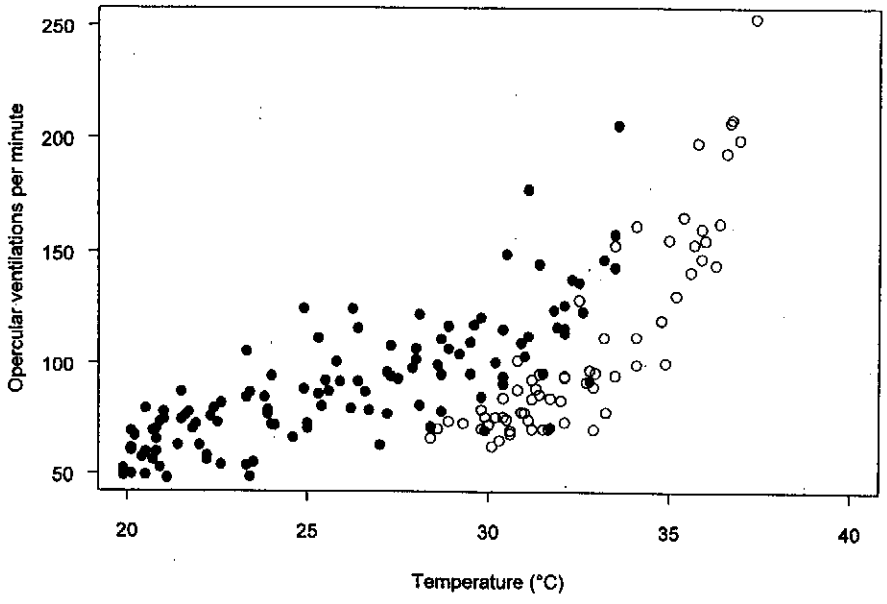

Figure 2. Opercular ventilation rate of *M. robustum* versus ambient temperature during critical thermal maxima trials. Closed circles = 20 °C acclimation; open circles = 30 °C acclimation.

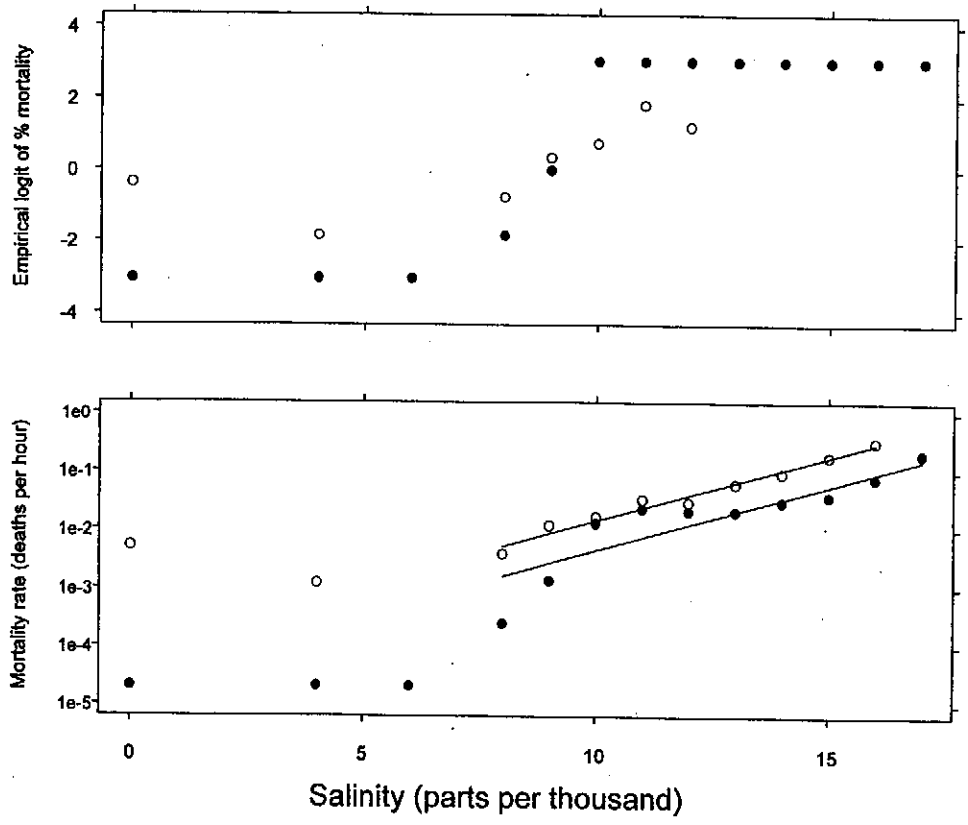

Figure 3. Empirical logit of percent mortality (upper panel) and mortality rate (lower panel) versus salinity for acute tolerance trials of juvenile *M. robustum* (mortality approaches 50% at empirical logit values approaching 0). Closed circles = pond-reared group; open circles = laboratory-reared group.

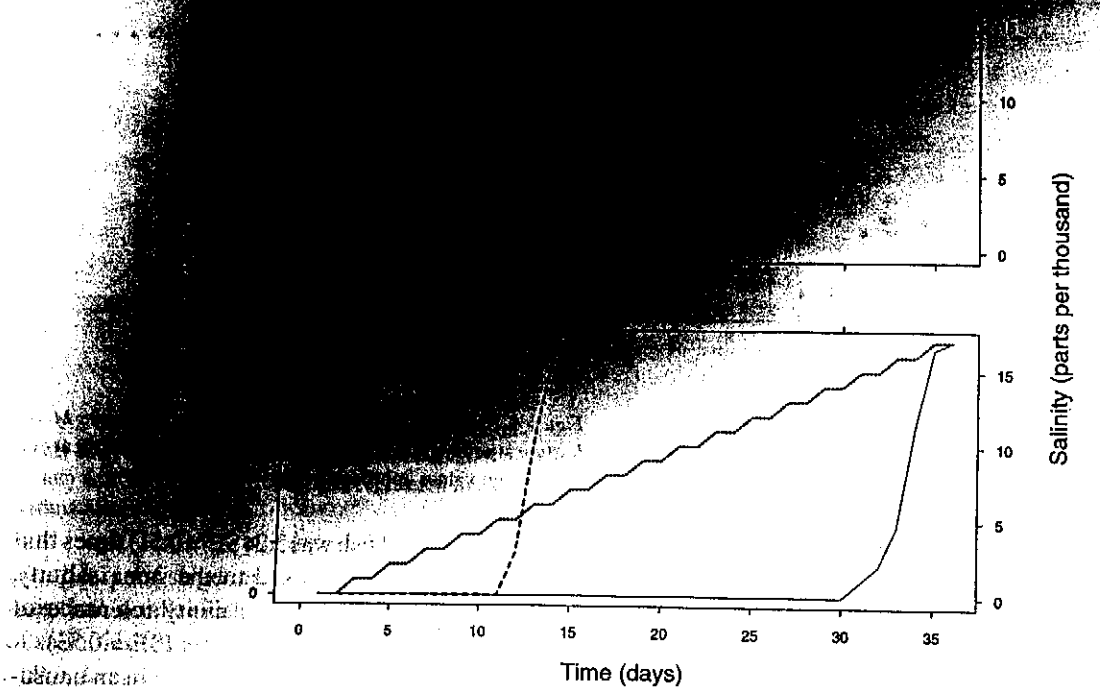

Figure 4. Cumulative mortality of laboratory and pond reared juvenile *M. robustum* exposed to chronic incremental increases in salinity. Dashed line = mortality of pond group acclimated to soft water (lower panel; trial 1; n = 165); solid lines = mortality of laboratory (upper panel; n = 348) and pond group (lower panel; trial 2; n = 33) acclimated to hard water; short dotted lines = ambient salinity.

Table 2. Mean plasma osmolality and mean hematocrit of *M. robustum* at chronically elevated salinities. Values within each treatment group differed significantly ($p < 0.05$, Sheffe's S post hoc ANOVA) among all salinities except for those designated with the same superscript letter. Estimates of SE are in parentheses following mean values.

Salinity (ppt)	Laboratory-reared		Pond-reared (trial 1)	
	Plasma osmolality (mOsm kg^{-1})	Hematocrit (% erythrocyte)	Plasma osmolality (mOsm kg^{-1})	Hematocrit (% erythrocyte)
0	272.1^{a} (6.1) (n = 14)	29.4^{a} (1.2) (n = 14)	296.4 (3.8) (n = 15)	42.7^{a} (1.0) (n = 15)
3	286.0^{a} (8.0) (n = 2)	29.7^{a} (2.3) (n = 4)	326.7 (3.1) (n = 5)	35.2^{a,b} (0.7) (n = 4)
5	–	–	422.7^{a} (3.4) (n = 5)	41.6^{a,b} (3.6) (n = 5)
6	360.9^{b} (4.0) (n = 5)	27.2^{a} (3.5) (n = 5)	424.0^{b} (4.1) (n = 20)	35.8^{b} (1.1) (n = 19)
7	–	–	544 (n = 1)	37^{a,b} (n = 1)
9	342.3^{b} (3.3) (n = 3)	34.6^{a} (1.3) (n = 5)	–	–
12	399.4 (5.1) (n = 5)	25.8^{a} (1.7) (n = 5)	–	–

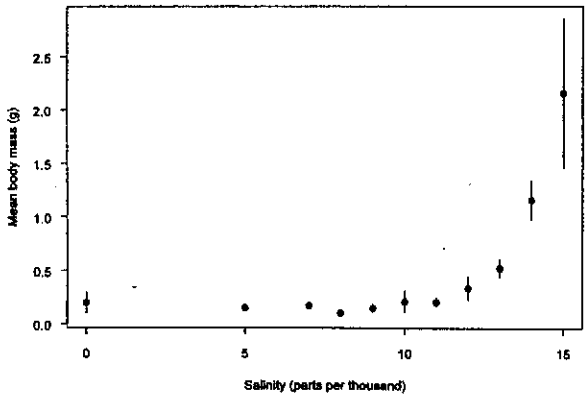

Figure 5. Mean post-mortem body mass of laboratory-reared juvenile *M. robustum* at chronic lethal salinities, ± 2 SE (vertical lines).

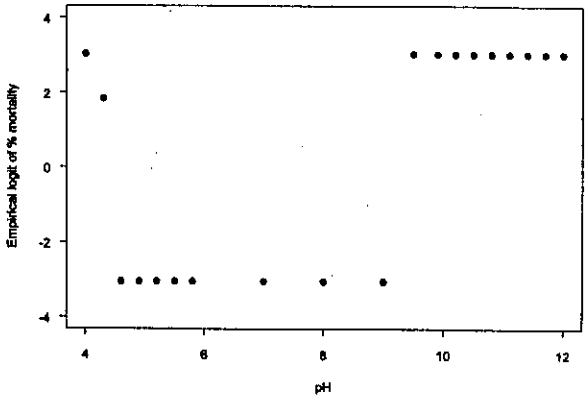

Figure 6. Empirical logit of percent mortality of juvenile *M. robustum* at acute pH exposures (mortality approaches 50% at empirical logit values approaching 0).

experimental groups. Most laboratory-reared fish succumbed within 16 hours of exposure to salinities in the range of 12–16 ppt; by the end of the experiment, there was 100% mortality in all laboratory-reared fish exposed to salinities of 13 ppt and above (Figure 3). In contrast, pond-reared fish acclimated to hard well water for at least two weeks prior to initiation of the experiment exhibited a much lower mortality rate across all salinities but had a lower overall tolerance than laboratory-reared fish; all pond-reared fish placed in salinities above 9 ppt died before the end of the 484 hour exposure period. Regression slopes of mortality rate versus salinity were not significantly different ($p = 0.75$) between laboratory- and pond-reared fish across the ranges where death was assumed to be caused by salinity stress. However, the mean mortality rate of laboratory-reared fish was 3.2 (SE = 0.51) times that of pond-reared fish exposed to the same salinity. The estimated difference in salinity tolerance of these two groups of fish was 2.3 ppt (SE = 0.33).

Chronic increase in salinity resulted in an unusual pattern of mortality among experimental groups (Figure 4). Large juveniles (9–26 g; $\bar{X} = 14.7$ g; n = 175) that were exposed to salinity increases immediately following capture from ponds exhibited 100% mortality between 4-6 ppt (trial 1). In contrast, all pond-reared fish (11–19 g; $\bar{X} = 14.2$ g; n = 345) acclimated for 5 weeks in hard well water (trial 2), and 80% of small (5–455 mg; $\bar{X} = 61.1$ mg; n = 345) laboratory-reared juveniles survived salinity increases until concentrations exceeded 12 ppt. No fish in either of the latter groups survived at concentrations above 16 ppt. In the laboratory-reared

Table 3. Mean body mass and mean pO_2 (mm Hg) at time of stress responses exhibited by juvenile *M. robustum* exposed to hypoxic water under different temperature acclimations. Scheffe's S post hoc ANOVA comparisons between acclimation groups denoted with superscript letters.

	Acclimation temperature	
	20 °C	30 °C
N	24	15
Mean mass (g ± 1 SE)[a]	18.2 ± 1.2	10.8 ± 0.5
Aquatic surface respiration[b]	13.7 mm Hg (0.80 mg O_2 l^{-1})	14.7 mm Hg (0.72 mg O_2 l^{-1})
Loss of equilibrium[b]	9.2 mm Hg (0.54 mg O_2 l^{-1})	11.5 mm Hg (0.57 mg O_2 l^{-1})

[a] $F = 21.09$ ($p < 0.001$).
[b] nonsignificant at $p < 0.05$.

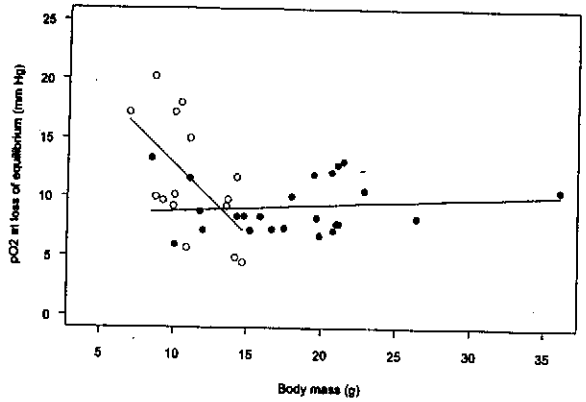

Figure 7. Oxygen partial pressure (mm Hg) at loss of equilibrium versus body mass of *M. robustum* acclimated to 20 °C (closed circles) and 30 °C (open circles), and regression lines for each acclimation group.

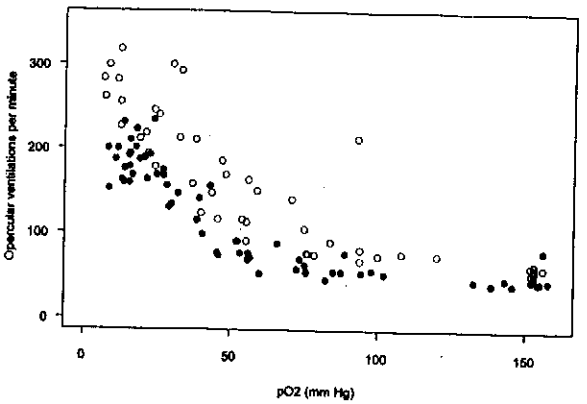

Figure 8. Opercular ventilation rate of *M. robustum* versus oxygen partial pressure during progressive hypoxia trials. Closed circles = 20 °C acclimation; open circles = 30 °C acclimation.

group larger fish generally survived to higher salinities than smaller individuals in the 12–16 ppt range of salinities (Figure 5).

Pond-reared (trial 1) fish exposed to chronic increase in salinity exhibited markedly higher plasma osmolality values than laboratory-reared fish (Table 2). In both groups there was a general trend of increased plasma osmolality with elevated salinity, but the pond-reared group showed a more acute increase at lower salinities. For each experimental group, mean plasma osmolality values differed significantly across most salinities. In contrast to the trend noted in plasma osmolality, both experimental groups exhibited no significant differences in mean hematocrit across salinities ($0.12 \leq p \geq 0.99$), although a slight difference was observed between pond-reared fish at 0 and 6 ppt ($p = 0.005$).

pH tolerance

Juvenile fish used in acute pH tolerance tests ranged in mass from 10.5–98.3 mg ($\bar{X} = 43.4 \pm 1.0$ SE, n = 200) and SL from 17–37 mm ($\bar{X} = 29.3 \pm 0.2$ SE). Fish acutely exposed to extreme pH values exhibited extreme distress and generally died within the first few hours of exposure, especially at high pH values (Figure 6). At pH 10.5–12.0 there was 100% mortality in the first 3 hours; at pH 9.9, mortality was 50% at 48 hours and 100% at 55 hours. All fish at pH 9.5 died between 55 and 70 hours. There was no mortality over the trial period in each of the test chambers with pH between 4.6–9.0. In contrast to rapid death at high pH, fish exposed to pH values of 4.0 and 4.3 survived longer: at pH 4.0, mortality totalled 30% at 6 hours and 100% at 11 hours; at pH 4.3 mortality totalled 50% at 22.5 hours, 90% at 28 hours, but never reached 100%. The abrupt gradient in mortality rates with differences in pH prevented computation of mean pH tolerances in the manner that was used for temperature. However, based on Figure 6 it appears that 50% mortality occurred between pH 4.0–4.3 and pH 9.0–9.5.

Hypoxia tolerance

Despite random selection of pond-reared fish for evaluating behavioral responses to hypoxia, mean body masses of the two temperature acclimation groups (20 °C and 30 °C) were significantly different (Table 3). Tolerances to low dissolved oxygen, measured as the mean pO_2 at which fish either initiated aquatic surface respiration or lost equilibrium, were not significantly different; however, the pO_2 at loss of equilibrium appeared to decrease with greater body mass among smaller fish (Figure 7). The limited number of small fish in the 20 °C acclimation group precluded our ability to examine this relationship in both groups. Fish ventilation

creased their ventilation rates as pO_2 decreased, with individuals acclimated to 30 °C showing a slightly higher rate than those acclimated to 20 °C (Figure 8).

Discussion

There is very limited comparative information on physiological tolerances of catostomids from large rivers of the eastern United States. Our results suggest that juveniles of *M. robustum* are moderately tolerant of a broad range of environmental physicochemical conditions. Based on experimental results, none of these factors alone appears to be likely in limiting natural recruitment of robust redhorse in the Oconee River or to be solely involved in the widespread decline of the species over its historical range. A combination of factors affecting physiological and ecological requirements of *M. robustum* have probably acted synergistically in contributing to declining populations of this species.

Juvenile *M. robustum* appear to tolerate a broad range of temperatures, depending on prior acclimation conditions and possible other factors such as age or nutritional state. Adults spawn in the Oconee River when rising water temperatures reach about 20 °C, typically in mid- to late-May. Hatching success of artificially-cultured eggs decreases with increasing acclimation temperatures, especially above 23 °C (J.L. Shelton unpublished data). Moreover, there is an increased incidence of spinal deformities in larvae and juveniles reared at temperatures above 25 °C, similarly known to occur in the white sucker, *Catostomus commersoni* (McCormick et al. 1977). Although large juveniles of *M. robustum* tolerated relatively high temperatures in our experiments, it is possible that individuals during very early life-history stages would be more sensitive to elevated temperatures. Other catostomids may experience developmental anomalies or are intolerant of extreme or fluctuating water temperatures outside of species-specific optima during prehatching and swim-up stages (McCormick et al. 1977, Bozek et al. 1990, Scoppettone et al. 1993). Low temperatures tolerated by juvenile *M. robustum* in our experiments were far below normal ambient stream temperatures encountered by wild fish within approximately the first six months following spawning.

Stream temperatures downstream from Sinclair Lake may exceed historical pre-impoundment levels by as much as 2–5 °C during the normal spawning and swim-up periods (J.W. Evans personal communication). Thus, elevated or fluctuating water temperatures produced by hydropeaking discharge from Sinclair Dam could conceivably have contributed to diminished reproductive success by reducing hatching success or by increasing mortality of larvae during critical early life-history. Many of the rivers that *M. robustum* historically occupied have large reservoirs, and it is possible that a combination of altered stream temperatures and other factors associated with regulation of these rivers, including altered flow regimes and blockage of spawning migrations may have been partly responsible for the decline or extirpation of populations of this species throughout its former range. Currently there are plans to mitigate in-stream flow fluctuations in the Oconee River below Sinclair Dam during the spring spawning period. Further data are needed to fully assess possible thermal effects on hatching success and survival. Such efforts are underway by researchers of the University of Georgia to study combinations of the effects of temperature, flow, and other physical factors on reproductive success.

Relatively few studies have examined the salinity tolerance of North American freshwater fishes, partly because of a common misconception that many freshwater species are largely intolerant of salt levels much greater than 0 ppt. However, there is strong evidence that freshwater species exhibit a continuum of tolerances to salinity that reflects physiological adaptation and evolutionary history (Maceina et al. 1980, Peterson 1988, Dunson & Travis 1991, Hart et al. 1991, Peterson & Meador 1994). Many freshwater species can withstand extended exposure to salinities at or below the approximate isosmotic point (9 ppt) and brief exposure to about 15 ppt, but most cannot survive chronic exposure to salinities above 9 ppt (Peterson & Meador 1994). The results of this study indicate that *M. robustum* is relatively stenohaline but capable of tolerating sa-

ities well within the range reported for many freshwater species. There was a significant size effect among laboratory-reared juveniles chronically exposed to increasing salinity, with larger individuals capable of tolerating higher salinities (Figure 5). A similar trend was apparent when comparing pond-reared fish (11–19 g) acclimated to soft water and laboratory-reared fish (5–455 mg) acclimated to hard water. However, no size effect in survivorship was observed among pond-reared (hard-water acclimated) fish exposed to either acute or chronic increases in salinity. We conclude that prior acclimation to different water conditions may account for the significantly lower salinity tolerance observed in juvenile fish tested immediately following harvest from ponds (trial 1). These fish were held for over 9 months in relatively soft water (see Methods). Following acclimation for over 1 month in hard well water, a group of the same fish (trial 2) exhibited similar tolerance to chronic increase in salinity as juveniles that had been reared entirely in well water. In the acute salinity experiments, the observed differences in survivorship and mortality rate (Figure 3) between experimental groups may be attributable to both size and acclimation effects. The pond-reared fish were larger and not fully acclimated to water of the same ionic composition as the laboratory-reared group. Although data were limited, fish acclimated to hard-water conditions appeared to regulate plasma osmolality at low salinities more efficiently than soft-water acclimated fish during chronic exposure to increasing salinity (Table 2).

Results of salinity experiments indicate that *M. robustum* is probably capable of tolerating any potential short-term increases in salinity that might be expected to occur in the Oconee River or in other Coastal Plain rivers as a result of foreseeable hydrological or land-use changes. Our results further suggest that, excluding consideration of other factors, possible barriers imposed by saline coastal waters should not have precluded the occurrence or dispersal of the species in the downstream portions of the major rivers historically occupied.

The former range of *M. robustum* includes rivers that traverse alkaline to neutral waters of the Piedmont physiographic province, to slightly acidic waters of the Coastal Plain. Juvenile robust redhorse appear to be moderately tolerant of acute exposure to pH values that extend beyond the range expected to occur in natural habitats. In other studies juveniles or adults of some catostomid species have been found to tolerate pH values ranging from 4.2 to 10.7 (Beamish 1972, Trojnar 1977, Falter & Cech 1991); however, because of different methodologies, results of our work may not be directly comparable with these studies. Moreover, results of acute exposures in laboratory studies are difficult to relate to possible effects of chronic exposure under natural conditions. Falter & Cech (1991) found significant differences in pH tolerance between two sucker species and suggested that declines of one species, *Chasmistes brevirostris*, might be linked in part to high pH environments resulting from eutrophication and massive algal blooms caused by anthropogenic changes in water quality. Conversely, acidification of natural waters has been implicated in the decline or extirpation of *Catostomus commersoni* and other species in lakes and rivers of eastern North America (Beamish 1972, 1974, Beamish & Harvey 1972, Trojnar 1977). Both low and high pH values, especially when accompanied by low water hardness or conductivity, cause severe ionoregulatory disruption and may significantly affect growth, reproduction, yolksac absorption, and survival of early life-history stages (Beamish 1974, Trojnar 1977, Fromm 1980, Fraser & Harvey 1984, Hobe et al. 1984, Trippel & Harvey 1987, Hobe & McMahon 1988, Falter & Cech 1991). Because of existing and potential changes in water quality resulting from impoundments, sedimentation, kaolin mining, and other forms of habitat modification in the Altamaha River watershed and throughout southern Atlantic slope rivers, further research is needed to address possible impacts of altered pH environments on *M. robustum*.

Moxostoma robustum spawns in well-aerated river shoals. We examined behavioral response to low oxygen levels in order to address the hypothesis that lack of recruitment might be due in part to low dissolved oxygen experienced by juvenile fish. Such hypoxia can occur in association with fluctuations in stream flow, water temperature, or sedimentation. Based on our results, *M. robustum* tolerates

low dissolved oxygen in a range similar to that known for other catostomids of the genera *Chasmistes* and *Catostomus*, which have critical oxygen minima (evaluated as loss of equilibrium or death) in an approximate range of 0.56–1.16 mg O_2 l^{-1} (Black et al. 1954, Castleberry & Cech 1992, Smale & Rabeni 1995a, b). Juvenile *M. robustum* acclimated to 30 °C were slightly less tolerant of hypoxic conditions than fish acclimated to 20 °C as predicted. Unexpectedly, juvenile *M. robustum* initiated aquatic surface respiration under low oxygen conditions, despite being morphologically unsuited for such behavior. While aquatic surface respiration may increase survival time in hypoxic water, it may also result in increased susceptibility to predation. The critical dissolved oxygen concentration represented by loss of equilibrium in *M. robustum* is considerably below the minimum lethal concentration (0.79 mg O_2 l^{-1}) within a 95% confidence interval reported for *Catostomus commersoni* by Smale & Rabeni (1995a). Compared to the values reported for 35 species by Smale & Rabeni (1995a), *M. robustum* appears to fall within a range of species considered by these authors to be intermediate to tolerant of hypoxia. However, laboratory survival tests typically underestimate minimal dissolved oxygen levels required by warmwater stream fishes for long-term persistence (Davis 1975, Smale & Rabeni 1995b). Thus, while juvenile *M. robustum* did not exhibit greatly reduced tolerance to hypoxia in the laboratory, oxygen concentration may still play an important role in survival of early life-history stages within natural microhabitats. For example, when exposed to low oxygen levels, developing fish eggs and embryos may exhibit retarded growth, reduced yolksac absorption and developmental deformities (Davis 1975). Additional research is critically needed to address minimum oxygen requirements of *M. robustum* embryos and larvae, to determine dissolved oxygen levels within spawning redds and juvenile drift areas, and to evaluate possible temporal changes in physiological requirements and ambient stream oxygen levels.

Results of this study provide general, preliminary estimates of physiological tolerances and important information to be considered in identifying suitable segments of river systems targeted for reintroductions of *M. robustum*. Further research is required to fully address the interactions of abiotic and biotic factors that have contributed to the large-scale demise of *M. robustum* from southeastern Atlantic slope rivers. Future studies should incorporate experimental designs to evaluate the combined effects of temperature, low dissolved oxygen, flow, sedimentation, and other water-quality parameters on the hatching success and survival of early life-history stages of this species. A thorough understanding of factors that have contributed to the decline of *M. robustum*, and a successful recovery and conservation program, are contingent on an integrative approach to carefully identify the critical ecological and physiological requirements of the species and identification of optimal microhabitat refugia for juvenile fish.

Acknowledgements

We are grateful to James W. Evans, Les Ager and the Georgia Department of Natural Resources for facilitating our research involvement in recovery efforts for the robust redhorse. Gregory L. Looney coordinated artificial spawning activities and, together with Dennis Young, provided and transported fish embryos, larvae and juveniles for experimental purposes. We are indebted to Tina L. Yanchis for laboratory assistance. Lauren J. Chapman offered helpful comments on an earlier version of the manuscript. Additional advice was provided by members of the Robust Redhorse Conservation Committee, representing a coalition of scientists, government agencies, power companies, and private interests devoted to conservation of this unique species. In particular, we thank Byron J. Freeman, Cecil A. Jennings, and James L. Shelton for constructive comments and engaging dialogue.

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