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Acute Responses to Salinity for Sunshine Bass and Palmetto Bass

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Abstract.—Differences in salinity tolerance and osmoregulatory performance between sunshine bass (white bass *Morone chrysops* ♀ × striped bass *M. saxatilis* ♂) and palmetto bass (striped bass ♀ × white bass ♂) were evaluated by using direct-transfer toxicity testing and monitoring of plasma osmolality during salinity elevation. The 24-h LC50 (concentration lethal for 50% of test animals) for sunshine bass was a salinity of 27.9‰ with a 95% lower and upper confidence interval (CI) of 24.5‰ to 36.2‰. The 24-h LC50 for palmetto bass was 28.0‰ with a 95% CI of 26.2‰ to 30.8‰. Test temperatures during this interval ranged from 19.6°C to 21.7°C, and the LC50 values for both crosses did not differ significantly. Differences in osmoregulatory performance were determined by sampling blood plasma osmolality (mmol/kg) during a stepwise elevation in salinity from freshwater to 52‰. Plasma osmolality of sunshine bass was significantly higher at 11‰, but osmolality of palmetto bass was higher at 41‰ and 52‰. Sunshine bass maintained baseline plasma osmolality through 41‰, whereas palmetto bass could only maintain baseline osmolality through 31‰. Although both crosses have similar tolerance to increasing salinity in terms of survival, sunshine bass appear to tolerate osmoregulatory stress better than palmetto bass, based on blood plasma osmoregulation.

Intensive research efforts in aquaculture have been devoted toward the family Moronidae (formerly included within Percichthyidae [Johnson 1984]) since the 1960s. Although most of the early attention was focused on restoring populations of striped bass *Morone saxatilis* (Stevens 1989), more than 19 hybrid crosses, backcrosses, and trihybrid crosses have been artificially produced among members of the genus *Morone* (Kerby and Harrell 1990). The most popular crosses for food fish production have been hybrids between the striped bass and the white bass *M. chrysops*. Crossing a female striped bass with a male white bass yields the original cross hybrid striped bass, or palmetto bass. Crossing a female white bass with a male striped bass produces the reciprocal cross, or sunshine bass.

Pond culture of hybrid striped bass is typically divided into three phases. Phase I involves stocking fry into fertilized ponds and harvesting fingerlings of about 1 g in size 45–60 d after stocking. These fish are graded and redistributed into ponds for phase II, which is complete when the fish are harvested at the end of the first growing season at a size of 0.1 kg (5–6 months poststocking). After a second grading, fish are stocked and harvested at market size of about 0.75 kg at the end of the second growing season (12 months poststocking; phase III).

Popularity of hybrid striped bass in domestic and international food fish markets has expanded aquaculture efforts of these fish in a wide range of environmental salinities. Hybrid striped bass have been successfully grown to a market size in freshwater, brackish water, and seawater, but fish at different production phases have different salinity requirements (Tomasso 1997). An ontogenic shift in salinity tolerance has also been documented among various tilapia species and hybrids in the genus *Oreochromis* (Watanabe et al. 1985). Salinity tolerance of the developmental stage in question should be considered because salinities of production facilities may be much different than salinities of nursery waters.

Saline water may be beneficial in *Morone* culture. In two contrasting studies assessing estuarine cage culture of hybrids of striped bass (cross not specified), Williams et al. (1981) demonstrated growth to be positively correlated with salinity, whereas Woods et al. (1983) reported that salinity negatively affected growth of palmetto bass. Growth of white bass at salinities of 0–12‰ was significantly higher than at 16–20‰ (Heyward et al. 1995).

Although little literature exists on the salinity requirements of hybrid striped bass embryos and fry, survival of striped bass in these early stages

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at different salinities has been documented. Low to moderate salinities (1–10‰) increase striped bass embryo and fry survival (Albrecht 1964; Lal et al. 1977; Morgan et al. 1981). Salinities greater than 9‰ were shown to compromise the development of striped bass embryos and fry in the Savannah River. Salinities greater than 24‰ resulted in 100% mortality (Winger and Lasier 1994).

Tolerance to salinity increases as moronids grow into the fingerling stages (Kerby 1986). Fish are frequently handled and transported at these phases of production. Increasing the salinity of the transport water is one technique used to ease osmoregulatory stress associated with handling (Wedemeyer et al. 1990). Wattendorf and Shafland (1982) reported that phase II original and reciprocal-cross hybrid striped bass can survive direct transfers between freshwater and seawater. This extreme change in salinity exceeds the recommended salinities used to ease osmoregulatory stress, but demonstrates that hybrid striped bass are considerably euryhaline. Rebush and Heath (1997) discovered that a salinity of 5‰ is sufficient to ameliorate stress responses in hybrid striped bass fingerlings. White bass are traditionally found in freshwater, but also exhibit some degree of euryhalinity. Heyward et al. (1995) found that white bass survival was greater in low to moderate salinities (0–12‰) than in higher salinities (16–20‰).

Increasing the hardness of transport water is another technique used to ease osmoregulatory stress during handling. Grizzle and Mauldin (1995) demonstrated that phase I striped bass succumb in 5‰ sodium chloride if the calcium concentration is below 15 mg/L. Calcium did not affect survival of phase II and larger striped bass (Grizzle et al. 1990; Mazik et al. 1991). Calcium is also important to the survival of striped bass fry (Kane et al. 1990; Grizzle et al. 1992). In addition to hardness, various trace elements affect the salinity tolerance of striped bass (Dwyer et al. 1992). No literature could be located on the effect of hardness or trace elements on salinity tolerance of hybrid striped bass.

Rebush and Heath (1997) used plasma osmolality to monitor the recovery of fed and fasted sunshine bass in recovery water of different salinities. Plasma osmolality did not differ in sunshine bass exposed to different concentrations of environmental calcium (Seals et al. 1994). Plasma osmolality of sunshine bass decreased at low salinities (1‰), remained near levels at 8‰, and increased at higher salinities (16‰ and 24‰; Weirich et al. 1992). In the same study, plasma osmolality decreased from baseline levels with increasing calcium in freshwater. Osmolality has also been used as an indicator of stress in striped bass (Davis and Parker 1990; Hunn et al. 1992). Salinity tolerance has been monitored by measuring plasma osmolality in other teleostean species such as largemouth bass *Micropterus salmoides* (Meador and Kelso 1990), red drum *Sciaenops ocellatus* (Crocker et al. 1983), and among five species of sciaenids (Mavares and Pérez 1984).

Although economically feasible production has been achieved with both the sunshine and palmetto bass, most production in recent years has shifted toward the sunshine bass. In 1996, 19 of 23 (82.6%) commercial producers surveyed produced sunshine bass (Kahl 1997). The main reasons for this trend result from efforts to increase the efficiency of commercial hybrid production. Female white bass are easier to obtain and handle than female striped bass, and striped bass semen can be extended or cryopreserved and easily shipped to inland production facilities (M. Freeze, Keo Fish Farms, personal communication). Rudacille and Kohler (2000) provide evidence based on performance for this production bias. Compared with palmetto bass, phase II and phase III sunshine bass in water reuse systems display significantly higher relative growth, average daily growth, relative weight, and feed conversion ratio to a market size. Distinctions between sunshine bass and palmetto bass have also been made using meristics and morphometrics (Harrell and Dean 1988). Differences in leukocyte counts, hematocrit, and hemoglobin have been identified between sunshine and palmetto bass (Hrubec et al. 1996). Because striped bass are anadromous and white bass are freshwater inhabitants, differences in acute salinity tolerance may also exist between sunshine and palmetto bass.

Commercial producers of the hybrids prefer to produce sunshine bass to palmetto bass (Kahl 1997). Some distinctions have been made in the past between the two crosses (Harrell and Dean 1988; Hrubec et al. 1996), but only recent research efforts have identified differences in performance (Rudacille and Kohler 2000). The objective of this study was to determine if sunshine and palmetto bass differ in their responses to salinity.

Methods

Both sunshine and palmetto bass were received from Blackwater Fish Research and Development

Center in Holt, Florida, after the first phase of production. The fish originated from mixed spawns, and ranged from 45 to 60 d in age. Both crosses were shipped by next-day air in separate double-bagged and chilled Styrofoam boxes. Upon arrival, fish were acclimated to a water reuse system consisting of 2,000-L circular tanks with a pressurized sand filter and crushed limestone biofiltration. Fish were held for 2 weeks before testing and were fed a daily maintenance diet of #2 crumble trout diet (40% crude protein) until 24 h before experimentation. Cannibals were periodically removed from the tanks.

Dechlorinated municipal water was cycled for 2 weeks through a crushed limestone biofilter. This system contained no fish and served as the source of freshwater throughout the study. All total ammonia nitrogen and nitrite measurements were made with a DREL/1C portable colorimeter (Hach Company, Loveland, Colorado). Total ammonia-N was less than 0.1 mg/L, nitrite was measured at 0.007 mg/L, pH was 8.3, and free chlorine was undetectable in the source water. The salinity of the source water was measured at 1‰. Experimental salinities were established using a saturated synthetic seawater solution with a concentration of 33.5% (335‰). Synthetic sea salt was chosen over rock salt to eliminate any effect of divalent ion or trace element deficiencies on salinity tolerance. This solution was prepared by mixing Instant Ocean (Aquarium Systems, Mentor, Ohio) to saturation with the source water, and 24 h after mixing, no free chlorine was detected in this solution upon addition of *o*-toluidine to a sample. This solution was mixed thoroughly and transferred to a head tank where the solution could be delivered by gravity to the testing area and diluted to the desired test salinity. The proper concentration in each test aquarium was achieved by diluting the saturated sea salt solution to volume with the conditioned source water.

Range-finding salinity tolerance test.—A nonrenewal static toxicity test was designed following the procedures described by Peltier and Weber (1985). The testing area consisted of a series of 38-L aquaria. A range of salinities was established for each cross (5, 10, 15, 20, 25, 30, 35, 40, and 45‰) plus a control. The control tank contained only source water with no added sea salt. This dechlorinated, conditioned source water contained a 1‰ equivalent salinity. Salinities were measured with a model SR1 temperature-compensated salinity refractometer (Aquatic Eco-Systems, Apopka, Florida) to an accuracy of 1‰. Dissolved oxygen and temperature were measured in all test aquaria with a model 54A oxygen meter equipped with a thermistor (Yellow Springs Instruments, Yellow Springs, Ohio) and pH was recorded with a pH 57 ESD (Aquatic Eco-Systems, Apopka, Florida). All tanks received gentle aeration of compressed air through an air stone.

Ten plastic bags for each cross were assigned to each of 10 salinity treatments. Ten fish of each cross were randomly netted from the acclimation system and placed into individual plastic bags. Excess water was drained through a small net and all 10 fish from each bag were directly transferred to their assigned treatment within 5 min. Behavior and condition of fish were noted upon transfer. Mortalities were recorded and dead fish were removed at 6, 24, 48, 72, and 96 h postexposure. Salinities were measured and adjusted as necessary at these intervals. These measurements did not vary by more than 1‰ from the target salinity. Excluding sampling periods, all aquaria were covered with black plastic.

Definitive salinity tolerance tests.—Fish were graded every other day in the acclimation system, but significant mortality resulted from cannibalism, especially among the sunshine bass. Additional sunshine bass were obtained from Keo Fish Farms, Inc., Keo, Arkansas and combined with remaining Florida sunshine bass to perform the definitive tests. Fish were acclimated for 1 week before testing and fed daily until 24 h before experimentation. No additional palmetto bass could be obtained, so the remaining Florida fish were used in the definitive tests. Palmetto bass averaged 8.7 ± 1.3 g and sunshine bass averaged 6.2 ± 1.8 g. These sizes were significantly different ($P = 0.0006$). Fish used in the definitive tests were approximately 80 d of age.

A mean LC50 value of approximately 26‰ for both crosses was obtained from the range-finding test. There were no significant differences in mortality between the 24-h and 48-h intervals. The central value for the range of salinities in the definitive tests was approximately 26‰. A multiplier of 0.91 was used in constructing a geometric series of test concentrations. The salinities for the definitive test were 16, 18, 20, 22, 24, 26, 29, and 33‰ plus a control with only source water (1‰). These salinities were randomly assigned to test aquaria in triplicate for each cross. Salinities were established and 10 fish were transferred to each test aquarium in the same manner as in the range-finding test.

Salinities 1‰ (control) through 26‰ were run first, followed a week later by salinities 29–33‰

and an additional control of source water (1‰). For analytical purposes, the results from these two trials for each replicate were combined into one medial lethal test representing all salinities. Dead fish were removed and their numbers were recorded. Salinities were measured and adjusted as necessary, and ammonia was measured in the controls at 6, 24, 48, 72, and 96 h. Temperature was measured initially and at 48 and 96 h postexposure. Dissolved oxygen was measured initially and at 48 h postexposure.

Osmoregulatory performance.—A saturated synthetic sea salt solution was made by mixing Instant Ocean to excess with source water, and 24 h postmixing, no free chlorine was detected in the solution upon addition of *o*-toluidine to a sample.

A water reuse system consisting of a battery of 38-L aquaria was filled with dechlorinated municipal water and allowed to circulate with no fish for 3 weeks. The system was equipped with a submerged biofilter with crushed limestone and commercial plastic balls. Sunshine bass (9.5 ± 2.3 g) and palmetto bass (11.8 ± 4.0 g) differed significantly in size ($P = 0.018$). These fish were approximately 120 d of age and were transferred from a holding system to separate, randomly assigned aquaria. Six fish were stocked into each tank, and each cross was replicated four times. Each aquarium received compressed air through an air stone. Water flow into each aquarium was adjusted to 6.4 L/min. Fish were acclimated to the system for 2 weeks. All fish were fed to satiation two times per day during this period. Feed was withheld for the 24 h before testing began, and the biofilter was bypassed. This reduced the volume of the system and therefore decreased the amount of saturated seawater needed to achieve the desired salinity. Before the addition of any salt, one fish from each aquarium was removed and anesthetized in tricaine methanesulfonate (MS-222) buffered with sodium bicarbonate. Fish were fully sedated within 20 s and weights and lengths were measured and recorded for each fish. The tail was severed and up to three heparinized hematocrit tubes of blood were taken from the caudal artery and vein. Tubes were capped and centrifuged at 8,000 revolutions/min for 5 min. The plasma portion of the blood was collected in sample vials and stored on ice. Blood samples were collected and analyzed by methods described by Wedemeyer and Yasutake (1976).

Based on the calculated volume of the system, an appropriate volume of the saturated salt water was added to the system to elevate the salinity by 10‰ over 2 h. This was achieved by adding the saturated seawater to an extra aquarium within the system and initiating flow. Salinities were measured and adjusted as necessary 30 min before sampling. One fish from each aquarium was again removed and analyzed at the newly established salinity; a process that took about 1 h. The process of raising salinity and sampling fish was repeated until values from each aquarium were obtained at salinities of 1, 11, 21, 31, 41, and 52‰. The order in which the aquaria were sampled within the hour of sampling was randomly changed so that both crosses received, on the average, equal duration following a salinity elevation. Plasma osmolality was determined 24 h after collection with a 5100C vapor pressure osmometer (Wescor, Inc., Logan, Utah).

Data analysis.—Although tests were carried out to 96 h, only 24-h LC50 values for salinity are reported. For palmetto bass and sunshine bass, respectively, 96.6% and 93.3% of 96-h mortality occurred within 24 h. Cumulative mortality data for each cross at the 24-h interval were analyzed using Probit analysis (Finney 1971). Data from each cross were pooled and tested for normality using Pearson chi-square goodness-of-fit tests at $\alpha = 0.10$. Distributions for sunshine bass and palmetto bass were not normal ($P = 0$ and 0.0266, respectively), so 95% confidence intervals, CIs) for each 24-h LC50 were calculated using a *t*-value of 2.36. Size of fish and un-ionized ammonia concentrations in controls were also analyzed by cross using a one-way analysis of variance (ANOVA). Differences among means were detected with Tukey's Studentized range test at $\alpha = 0.05$.

Total osmolality was analyzed between crosses at each salinity and for each cross by salinity using a one-way ANOVA for each comparison. Percentage data were arcsine transformed before analysis. All statistical analyses were performed using SAS (SAS Institute, Cary, North Carolina).

Results

No differences in salinity tolerance could be detected between crosses. The 24-h LC50 of salinity for sunshine bass was determined to be 28.0‰ with a 95% CI of 26.3–30.8 (Figure 1). The 24-h LC50 of salinity for palmetto bass was determined to be 27.9‰ with a 95% CI of 24.5–36.2. Temperatures during this interval ranged from 19.6°C to 21.7°C.

Un-ionized ammonia (UIA-N) concentrations in controls were significantly different between sunshine and palmetto at the 24-h interval, but the

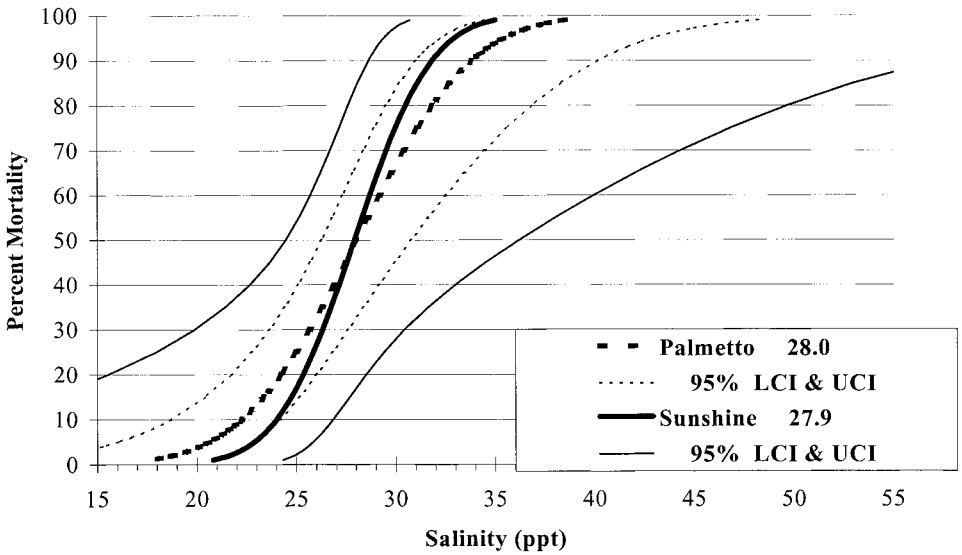

FIGURE 1.—Mean percent mortality versus salinity (ppt) at 24 h posttreatment for sunshine bass (solid line) and palmetto bass (broken line). Lines with lesser weight represent the lower (LCI) and upper (UCI) 95% confidence intervals. Mean LC50 values (ppt) for each cross are included in the legend.

effect of these differences is deemed negligible. The highest observed measurement was 0.037 mg/L UIA-N, which is less than one-tenth of the reported LC50 for sunshine bass (Oppenborn and Goudie 1993). Dissolved oxygen (DO) was adequate (>6.4 mg/L) in all aquaria, and pH ranged from 8.0 to 8.2 in test salinities and was 8.3 in controls.

Fish exhibited increased gill ventilation rates and swam erratically for several minutes upon transfer into the test salinities. The fish were neutrally buoyant in the freshwater holding system, but were initially positively buoyant upon transfer into the higher density salt water. The fish swam in a downward position until neutral buoyancy was achieved, usually within a few hours.

Sunshine bass displayed a significantly higher total osmolality than palmetto bass (Table 1) at 11‰. Palmetto bass had a significantly higher total osmolality than sunshine bass at the two highest salinities (41‰ and 52‰). The highest osmolality for sunshine bass was observed at 52‰, which was significantly higher than at all other salinities. The osmolality of palmetto bass, at 52‰, was higher than at all other salinities, and the observation at 41‰ was significantly higher than osmolality at 1‰ and 11‰.

TABLE 1.—Total osmolality (SD) at selected sampling intervals for sunshine and palmetto bass exposed to increasing salinity. Significance was determined at $\alpha = 0.05$. Within columns, values without a letter in common are significantly different; letters indicate significant differences between salinity transition within a cross (column), asterisks indicate a significant difference between hybrid types (row) at the salinity.

Salinity (‰)	Total plasma osmolality (mmol/kg)	
	Sunshine bass	Palmetto bass
1	360 (33) z	351 (20) z
11*	384 (26) z	348 (7) z
21	366 (14) z	361 (3) yz
31	343 (20) z	357 (14) yz
41*	366 (9) z	400 (9) y
52*	451 (20) y	530 (39) x

Discussion

No differences in salinity tolerance between sunshine and palmetto bass was detected using direct-transfer toxicity tests. Direct transfer of hybrid striped bass fingerlings into salinities of about 28‰ will cause mortality to half of the fish within 24 h at temperatures of about 21°C. Responses may differ at other temperatures. The lethal effects of salinity display a threshold effect. When fish are exposed to salinities approaching the LC50, small increases by a few parts per thousand could potentially cause significantly higher mortality. This threshold effect could also explain the nonnormal distribution of data for both crosses.

Monitoring plasma osmolality during salinity

acclimation was more revealing in identifying differences between sunshine and palmetto bass than direct transfer tests (Table 1). Baseline osmolality of both crosses before the addition of salt did not differ significantly, making comparisons during the elevation in salinity possible. Osmolality of sunshine bass was significantly higher after the initial salinity elevation (11‰). These observations indicate that both sunshine and palmetto bass differ in their responses to initial salinity exposure. This relationship changed at both 41‰ and 52‰, concentrations at which mean osmolality of palmetto bass was higher than osmolality of sunshine bass. The inability to regulate osmolality was reached at a lower experimental salinity by palmetto bass.

The highest salinities at which the fish were sampled exceeded levels that would typically be experienced in routine aquaculture operations. However, the differences in blood osmolaties observed at 41‰ would probably have manifested at a lower salinity during the transition from 31‰ to 41‰, possibly near to 35‰, the typical salinity for surface seawater (Spotte 1992). Additional studies are needed to determine the precise salinity osmoregulatory differences that occur between sunshine bass and palmetto bass.

Seals et al. (1994) reported an osmolality of 327–350 mmol/kg for juvenile sunshine bass held in freshwater with varying calcium concentrations. Baseline plasma osmolality for hybrid striped bass (cross not specified) phase I fingerlings of 18.5 g and 19.4 g held at 1‰ is reported to be 310 ± 4 and 344 ± 7 mmol/kg, respectively (Weirich et al. 1992). Baseline osmolality for sunshine bass (360 ± 33) in this study slightly exceeded those values.

Crocker et al. (1983) transferred juvenile red drum from seawater to freshwater (decreased salinity) and observed a drop in plasma osmolality. Both the sunshine and palmetto bass in this study demonstrated an increase plasma at the highest salinities. Some changes were observed at intermediate salinities with both crosses, but none of these were significant. Both crosses were moribund at these highest salinities, and osmolality greatly exceeded the previously reported value; therefore, abnormally high osmolality appears to be a cause of death from lethally high salinity.

With respect to osmolality, two observations from this study indicate that sunshine bass are more capable of handling osmoregulatory stress than palmetto bass. When exposed to increasing salinities, sunshine bass maintained a significantly lower plasma osmolality than palmetto bass, most notably during hypermarine conditions when high salinity becomes lethal. Sunshine bass also maintained plasma osmolality not different from controls, increasing in salinity through 41‰. Palmetto bass could only maintain plasma osmolality not different from controls through 31‰. These results, coupled with superior growth and performance as reported by Rudacille and Kohler (2000), provide evidence that sunshine bass appear to be a more vigorous hybrid than the palmetto bass, and consequently better suited for aquaculture.

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