

Winterkill Simulation on Three Size Classes of
Spotted Seatrout

by

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Abstract.—Three size classes of spotted seatrout, *Cynoscion nebulosus*, were exposed to a winterkill simulation (water temperature Δ $-0.33^{\circ}\text{C}/\text{hour}$) in order to determine the dynamic critical thermal minimum. In addition, size dependence to cold tolerance was investigated for this species. The adult fish ranged from 276-333 mm in total length (TL) and had a median lethal water temperature (LT50) of 1.8°C . Young adult fish ranged from 117-149 mm TL and had a LT50 of 1.85°C ; juvenile fish ranged from 33-79 mm TL and had a LT50 of 2.95°C . Significant differences existed between size classes for death ($P < 0.001$), but not for stress ($P = 0.45$) or loss of equilibrium ($P = 0.49$). Signs of stress were first displayed at 6.3°C for juvenile and young-adult sized fish; adults did not display clear signs of stress in this study. Signs of losing equilibrium were first displayed at 5.5°C for all size classes. Positive size dependence for low temperature tolerance may be a selective disadvantage for these spring and summer spawners as young-of-the-year individuals could be as small as 33 mm TL before being exposed to winter temperatures.

Introduction

The spotted seatrout *Cynoscion nebulosus* is one of most recreationally sought after gamefish within the inshore Gulf of Mexico (NMFS 2008). The abundance of spotted seatrout has been impacted due in part to periodic low temperature kills (Storey and Gudger 1936; Gunter and Hildebrand 1951; Holt and Holt 1983). These winter die-offs negatively impact future catch rates as evidenced by fishery dependent (Green and Campbell 2005) and fishery independent data collected after major cold fronts in Texas (Martinez-Andrade et al. 2005). Water temperatures resulting in spotted seatrout mortality have been recorded during these freeze events in Texas by numerous authors (Simmons 1957; Holt and Holt 1983; McEachron et al. 1994), however the specific critical thermal minimum for spotted seatrout remains unknown.

Observations specific to winter freezes have resulted in a rudimentary threshold for spotted seatrout cold tolerance; thermal stress mortality occurs at water temperatures $\leq 7.2^{\circ}\text{C}$ for long periods (> 24 h), whereas fish exposed to water temperatures equaling 8.8°C for short periods (12 h) have recovered (Gunter and Hildebrand 1951; Tabb 1958; Moore 1976). However, spotted seatrout have been reported in the literature to be taken alive from waters as cool as 3°C in Texas (Simmons 1957; Bumguardner et al. 1992) and 5°C in Mississippi (Etzold and Christmas 1979), unfortunately these reports do not give any information of fish condition, size or prior acclimation. It has been noted that mortality is most severe earlier in the winter as compared to later in the season, suggesting a combination of factors, including smaller size (Texas Parks and Wildlife unpublished data) and short or no acclimation period, may result in differential juvenile spotted seatrout cold mortality in early and late winter (Gunter 1941; McEachran et al. 1994). Further studies suggest that a size-dependence may occur within a species; allowing different life stages to withstand thermal shock better than others. Various authors have suggested that based on allometric relationships between gill surface and body mass, that smaller fish would be more vulnerable to low temperature-induced osmotic stress than larger fish (Shekk et al., 1990; Johnson and Evans 1996; Hurst 2007).

Bennett and Judd (1992) determined that a dynamic critical thermal minimum (CTMin) test (steady decreasing rate) more accurately predicted responses *in situ* rather than static cold tolerance tests. Thus, the objective of this research was to determine the CTMin of three size classes of spotted seatrout based on a rapid water temperature decrease. Although previous observational studies offer crude estimates of the cold tolerance of spotted seatrout, they fail to identify the CTMin or account for size effects. Winterkill simulations were conducted on three size classes of spotted seatrout to determine if any size dependence occurs in relation to winter mortality. This baseline cold tolerance data is essential to assist regulatory agencies in assessing winterkill severity and for developing a mitigation response.

Methods

Young-of-the-year (YOY) spotted seatrout (< 150 mm, total length [TL]) were collected by bag seine along the south shoreline of Keller Bay (a secondary bay of Matagorda Bay), during October of 2007. Fish were then transported, separated into two size classes (< 100 mm TL, and > 100 mm TL) and held in separate aerated 3,500 L recirculation tanks maintained at 18° C at the Perry R. Bass Marine Fisheries Research Station, Palacios, Texas. Fish were fed to satiation using both live grass shrimp (*Palaemonetes sp.*) and a dry floating fish feed (Kaytee, Inc., Chilton, Wisconsin; protein \geq 35%, fat \geq 5%, fiber \geq 4%), 1 – 2 times per day, until start of each experiment. Adult fish were captured in Mesquite Bay (a secondary bay of Aransas Bay), by rod and reel in November 2008 and transported and maintained in a similar fashion as the previously captured fish.

The first experiment was directed at the juvenile size class using 36 individuals (between 33 – 79 mm TL). This minimum size range for juvenile fish were representative of spotted seatrout collected within Texas bays during winter months (Table 1). These fish were maintained in recirculating tanks for approximately 30 days. Fish were randomly placed in twelve 5.5 L aerated glass aquaria (three per aquarium) with 5 L of water from the initial holding tank. Feeding was discontinued and fish were maintained in these aquaria for ~12 h at 18° C prior to initiation of the experiment. Ammonia concentrations were monitored during all experiments and water exchanges with pre-tempered water were conducted if ammonia levels considered unsuitable (\geq 0.5 mg/L) (Daniels and Boyd 1987).

Aquaria were placed in a temperature programmable environmental chamber (Luwa Environmental Specialties, model ES2000 C-LT-R, Raleigh, NC). The interior dimensions of the environmental chamber used in this study were (Width-Depth-Height; 86.5 x 71 x 151.5 cm) with a viewing window to monitor the biotic responses of the fish. The air temperature within the environmental chamber was lowered at a rate of 0.33°C/hour for all experimental studies (McDonald and Bumguardner 2010). This temperature adjustment rate was chosen because it is within the range of water temperature decreases observed for shallow Texas bays (Aransas Bay - 0.29°C/h and Upper Laguna Madre - 0.45°C/h), which had high cold induced mortality of spotted seatrout during the December 1989 freeze (McEachron et al. 1994). The water

temperature of each individual aquarium was recorded every four min using a 12-channel scanning digital thermometer (Model 92 0000-00 Digi-Sense, Barnant Co. Barrington IL). Once the experiment began an observer monitored the condition of each fish every 15 min for three biotic responses; signs of stress (erratic swimming), loss of equilibrium and death (cessation of respiratory movement) (Close et al. 1997). The second experiment utilized a larger size class (classified as young-adults) and was conducted in the same manner as the first experiment, using 12 individuals (117 – 149 mm TL). These fish were maintained in our recirculation tanks for approximately 60 days. The extended holding time, as compared to the previous study, was necessary for fish to grow to the desired larger size. The third experiment targeted adult size class and was conducted in the same manner, using 12 individuals (276 – 333 mm TL) in larger aquaria (18.9 L), which held 18 L of water from the initial housing tank. These fish were maintained in our recirculation tanks for approximately 30 d. Fewer fish were used in the second and third experiments than in the first experiment in order to reduce any overcrowding effects in aquaria due to the larger size of individual fish. In our first experiment, individual lengths and water temperatures resulting in stress, equilibrium loss and death were averaged by aquaria. In order to make data comparable, individual aquaria in each experiment were treated as replicates. Prior to data analysis, hourly rates of temperature change between aquaria of each experiment and between experiments were compared and showed no statistical difference with one factor ANOVAs ($P < 0.05$). A series of one-factor ANOVAs and post-hoc tests (Tukey) ($P < 0.05$) were used to investigate significant differences for each pre-death biotic response and death between the three different size classes. Median lethal water temperature (LT_{50}) was used as a proxy for CTMin, and was determined for all fish in each size class. Statistics were calculated using SAS (e.g., SAS vers. 8.02, SAS Inst., Inc., Cary, NC).

Results

All water temperature change rates were not statistically different among aquaria for each experiment and between experiments. Water temperature change rates per hour were not different between all aquaria for each experiment; juvenile size experiment ($F_{11,540} = 0.04$, $P = 1.00$), young-adult size experiment ($F_{11,624} = 0.02$, $P = 1.00$) and adult size experiment ($F_{11,612} = 0.10$, $P = 0.99$). Water temperature change rates for juvenile size experiment (mean \pm standard error) (-0.31 ± 0.01), young-adult size experiment (-0.32 ± 0.01) and adult size experiment (-0.30 ± 0.01) were not different between experiments ($F_{2,1809} = 1.20$, $P = 0.30$).

Stress induced water temperatures were not statistically different between juvenile-sized fish ($5.5^{\circ}\text{C} \pm 0.39$) and young adult-sized fish ($5.6^{\circ}\text{C} \pm 0.38$) ($F_{1,22} = 0.60$, $P = 0.45$). Adult-sized fish were not included in this analysis, since they did not display any clear signs of stress. The water temperatures resulting in signs of stress were first observed at 6.1°C for the juvenile fish and 6.3°C for the young adult-sized fish.

Loss of equilibrium related water temperatures were not statistically different between juvenile-sized fish ($4.7^{\circ}\text{C} \pm 0.28$), young adult-sized fish ($4.6^{\circ}\text{C} \pm 0.52$) and adult-sized fish ($4.4^{\circ}\text{C} \pm 1.10$) ($F_{2,33} = 0.71$, $P = 0.49$). The water temperatures resulting

in loss of equilibrium were first observed at 5.5°C for both juvenile fish and adult-sized fish and 5.3°C for the young adult-sized fish.

Water temperatures at death were lower for adult-sized fish ($1.72^{\circ}\text{C} \pm 0.36$) and young adult-sized fish ($1.94^{\circ}\text{C} \pm 0.39$) as compared to the juvenile-sized fish ($2.96^{\circ}\text{C} \pm 0.36$) ($F_{2,33} = 38.38$, $P < 0.001$). The median temperatures representing the LT50s of each of the size classes are illustrated (Figure 1). No differences occurred between adult-sized fish and young adult-sized fish. The water temperatures resulting in death were first observed at 3.5°C for juvenile-sized fish, 2.6°C for young adult-sized fish and 2.2°C for adult-sized fish.

Discussion

Evidence from this study suggests that no size dependent differences exist for stress and loss of equilibrium in spotted seatrout using lower water temperatures as criteria. However, these results have two major caveats. First, observational monitoring may be subjective and are dependent upon the criteria designated by the observer. Second, the lack of observational differences between size classes with these criteria does not necessarily indicate that differences were not occurring. Fish withstanding rapid cooling may look and act normal even while their energy reserves are being depleted (Oliver et al. 1979; Thompson et al. 1991; Schultz et al. 1998) and may explain why adult-sized fish did not display any signs of stress in our experiment. Because these criteria are subjective and imprecise, many lethal water temperature studies focus on death (cessation of gill movement) rather than stress (Close et al. 1997).

Larger-sized spotted seatrout displayed a higher tolerance of lower water temperatures, indicative of positive size-dependent mortality. This type of size-dependence caused by low temperature mortality was almost universally accepted due to the numerous accounts in laboratory and field surveys suggesting that metabolic differences or osmotic stress as the cause (Oliver et al. 1979; Post and Evans 1989; Malloy and Targett 1991). However, this is contradictory to the size dependent thermal tolerance of a similar species of the same family *Sciaenidae*. Lankford and Targett (2001) demonstrated that Atlantic croaker *Micropogonias undulatus* juveniles exhibit a negative size-dependant response to cold, suggesting this as a response to life history differences between these species. Although, Atlantic croaker do use estuaries as a nursery during winter and spring seasons (TPWD-CF data), as they mature they migrate to the more thermally stable offshore waters to spawn (Gutherz 1977; Norcross 1991). Vetter (1982) found that adult sand seatrout, a species that shares a similar life history to Atlantic croaker, are more susceptible to the temperature extremes of the inshore bays than adult spotted seatrout. Other accounts suggest that species that use the offshore for part or all of their life history tend to be less tolerant of the temperature extremes of the inshore bays (Vetter 1982; McDonald et al. 2009; McDonald and Bumguardner 2010).

These results establish a baseline for determination of the critical thermal minimum of spotted seatrout, and suggest that size-dependent mortality occurs in YOY individuals. It is recommended that regulatory agencies and aquaculture parties

overwintering spotted seatrout in outdoor ponds be aware of ambient water temperatures during cold weather events. Bumgardner and Maciorowski (1989) document a considerable decrease in the growth rate of spotted seatrout at 11.5°C as compared to water temperatures $\geq 15.5^{\circ}\text{C}$ signifying less favorable conditions. Recommendations for preparation of fish kill due to freezes are 1) exercise caution when restocking at water temperatures below 11.5°C due to the risk of metabolism overexertion and/or cessation of feeding and 2) prepare for fish kill response once water temperatures drop below 6.3°C. Further work is needed to determine if positive size dependence continues as fish size increases, in order to determine the overall critical thermal minimum for all size classes.

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TABLE 1.—Minimum (min), maximum (max), mean \pm standard error of lengths (ln) specific to spotted seatrout collected within Texas bays during the winter months for over 30 years (1977-2008). Data represented here comes from bag seine data conducted by the Texas Parks and Wildlife-Coastal Fisheries Division resource monitoring program.

Months	Total length range and average		
	Min. Ln. (mm)	Max. Ln (mm)	Mean \pm S.E.
December	33	170	87.0 \pm 2.6
January	37	187	94.4 \pm 6.4
February	42	185	102.8 \pm 8.1

FIGURE 1.—Critical temperature minimums of three size-classes of spotted seatrout. Solid lines represent non-linear regressions of lethal temperatures per aquarium. Dashed lines represent median lower lethal temperatures.

