Low temperature tolerance in white shrimp, *Litopenaeus setiferus,* in South Carolina

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Introduction

The penaeid shrimp fishery is an important commercial and recreational fishery along the Atlantic coast of the southeastern United States (National Marine Fisheries Service). In South Carolina, the fishery consists predominantly of the white shrimp, *Litopenaeus setiferus* (Linneaus, 1797), with commercial landings exceeding 1.9 million pounds per year in recent years (D. Whitaker, SCDNR, pers. comm.). The white shrimp fishery consists of mainly a fall fishery as well as spring "roe" fishery. White shrimp are abundant in shallow water estuarine habitats including marshes, preferring areas with *Spartina* vegetation, such as those along South Carolina (Giles and Zamora, 1973). Adults spawn offshore in the spring, and subsequent postlarvae recruit to estuaries and sounds where they grow in shallow marsh nursery areas (South Atlantic Fishery Management Council, 1993). Once shrimp reach a minimum size of 100mm, they migrate offshore beginning in August and continuing through the fall (Lindner and Anderson, 1956; McKenzie, 1981). Subadult white shrimp overwinter in estuarine and nearshore waters. These overwintering white shrimp are the majority of the spring spawning population (Whitaker, 1984).

Overwintering white shrimp are known to be vulnerable to cold kills in severe winters with cold water temperatures (Farmer et al, 1978; Gaidry, 1974; Whitaker, 1984). Previous studies have demonstrated a positive correlation between overwintering white shrimp population in South Carolina coastal estuaries and the following spring commercial catch, in which low yields of overwintering white shrimp in trawl surveys conducted in winter months corresponds with low landings in the spring commercial shrimp fishery (Farmer et al, 1978). The progeny of the spring spawn grow to adult size in the fall and comprise the fall fishery, thus a poor spring spawn can result in low fall landings (Lam et al, 1989). In years with severe winters, the South Carolina Department of Natural Resources may delay opening the spring fishery in an effort to protect remaining spring spawning stock (South Atlantic Fishery Management Council, 1993).

It has been reported in the literature that low water temperature is positively correlated with mortality in overwintering white shrimp populations in South Carolina (DeLancey et al, 2005; Farmer et al, 1978). In a study by Farmer et al (1978), weekly trawl surveys were conducted during winter months in Charleston Harbor for a period of two years, which included one of the most severe winters on record, 1977 (Whitaker, 1984). Catch data from that year suggested 8.5^{o} C as the critical temperature that begins to cause declines in white shrimp numbers, with relative percentages of catches declining sharply (Farmer et al, 1978). The study, which also examined historical catch and weather data, revealed that white shrimp mortality was higher in years with a minimum of ten consecutive days of low water temperature (below 8.5^{o} C). Additionally, the study suggested direct mortality of white shrimp as the temperature approaches 6^{o} C (Farmer et al, 1978; Whitaker, 1984). This work has contributed to a better understanding of how severe winters and low water temperatures impact white shrimp populations; however, there have been no studies that have empirically determined the low temperature limits of white shrimp.

Thermal tolerance studies empirically quantifying low temperature limits of marine organisms are critical for determining indices of environmental stress and causes for population declines, as temperature is one of the most important abiotic factors affecting growth and survival (Carveth et al, 2007). These studies generally employ one of four experimental approaches: the incipient lethal temperature (ILT) method, the critical thermal method (CTM), the chronic lethal method (CLM), and the acclimated chronic exposure (ACE) method. In the ILT method, a lethal temperature is determined by rapidly transferring organisms from several acclimation temperatures into different test temperatures until mortality occurs (Selong et al, 2001). In the CTM, organisms are subjected to a constant linear increase or decrease (rates varying from 1^{o} C/hour to 1^{o} C/min) in temperature until a predetermined sublethal endpoint (loss of equilibrium), as well as a lethal endpoint (Beitinger et al, 2000). The CLM uses slow, linear changes (1^{o} C/day) in water temperature from an acclimation temperature until mortality occurs (Carveth et al, 2007). The ACE method is a hybrid of the ILT method and CTM, and involves gradually adjusting water temperature (typically 1^{o} C/day) to allow organisms to acclimate to temperature changes, which according to Selong et al (2001) better simulates the actual thermal response of aquatic organisms in natural environmental conditions. Once a predetermined test temperature is reached, organisms are maintained at that constant temperature for an exposure time reflective of natural environmental conditions, or until mortality (Selong et al, 2001). The CTM has been used to determine critical thermal minima in Penaeid species including juvenile Pacific white shrimp, *Litopenaeus vannaemi* (Boone, 1931) and the green tiger prawn *Penaeus semisulcatus* (DeHaan,1844) (Kir and Kumlu, 2008; Kumlu et al, 2010). The CLM and ACE method have been used in several studies determining thermal limits in fish (Carveth et al, 2007; Selong et al, 2001). These methods are considered to be applicable for determining thermal tolerance in aquatic organisms in general (Selong et al, 2001).

An empirical study of low temperature tolerance of overwintering white shrimp would be useful for quantifying the effects of cold water temperatures during severe winters on South Carolina shrimp populations. Results of environmentally realistic test temperatures and mortalities would provide managers with an early detection tool and evaluate the success of current management practices for white shrimp. This research will be conducted using experimental procedures previously employed in thermal tolerance studies of marine organisms chosen because they most closely reflect the natural environmental conditions experienced by overwintering white shrimp. Using the four methods will provide a complete profile of white shrimp low temperature tolerance, as the CTM determines acute temperature tolerance, the CLM measures temperature tolerance with gradual changes more realistic with the environment, and the ACE method determines the effect of prolonged exposure to low temperatures. The goal of the study is to determine empirically the temperatures at which overwintering white shrimp begin to experience loss of equilibrium and mortality.

Hypothesis and Objectives

The objective of the study is to determine the low temperature tolerance of overwintering white shrimp using four experimental methods: the critical thermal method (CTM), the chronic lethal method (CLM), the acclimated chronic exposure (ACE) method with static temperatures, and the ACE method with fluctuating temperatures. These methods were chosen because they will most closely represent the natural environmental conditions experienced by overwintering white shrimp in Charleston Harbor. The H_0 is that water temperatures below 8.5^{o} C will have no effect on loss of equilibrium in white shrimp, and temperatures below 6^{o} C will have no effect on mortality. The H_1 in this study is that water temperatures below 8.5^{o} C will lead to a loss of equilibrium in white shrimp, and temperatures below 6^{o} C will lead to mortality.

Methods

Experimental facilities

Experiments will be conducted at the Marine Resources Research Institute (MRRI) in Charleston, South Carolina. Adult white shrimp ranging from 80-110mm TL will be obtained from trawl sampling in the central deep water basin of Charleston Harbor. This size range reflects the modal distribution of subadult white shrimp found overwintering in nearshore waters including Charleston Harbor (Farmer et al, 1978). Experiments will be performed using two Bahnson CCS-3000 environmental chambers. Lighting will be provided by overhead fluorescent fixtures set to a 10.5 h light: 13.5 h dark cycle which simulates a South Carolina January photoperiod. Each chamber will contain twenty-four acrylic, 75-liter tanks measuring 75cm x 30cm x 30cm. Tanks will be filled with 22 ppt artificial seawater made from Instant Ocean® sea salt (Instant Ocean, Cincinnati, OH, USA) and dechlorinated tap water. This salinity was chosen because it reflects the average salinity experienced by white shrimp in January in the central basin of Charleston Harbor.

All experimental shrimp will be transferred to tanks with a salinity of 22 ppt and temperatures ranging from 15-18^{o} C. Shrimp will be held for a 14 day acclimation period prior to low temperature trials. All shrimp will be measured and weighed as they are transferred to tanks. Water temperatures in tanks will be gradually lowered to 13^{o} C by lowering the air temperature in the chambers by 1^{o} C/day, and will be kept at that temperature for a minimum of 10 days to allow for acclimation. This temperature was chosen because it reflects the average Charleston Harbor water temperature at the beginning of January, as measured at the Customs House in the main channel of the Cooper River in Charleston Harbor, and on long-term trawl surveys conducted in Charleston Harbor. Shrimp will be offered a commercial pelleted shrimp diet, Zeigler™ Zero Exchange Super-Intensive shrimp feed (30% Protein, 9% Fat) (Zeigler Bros. Inc., Gardners, PA, USA) daily.

Water quality analysis will be performed weekly using a Hach® Laboratory Spectrophotometer DR/2500 (Loveland, CO, USA) and Hach Permachem® Reagent powder pillows to measure ammonia (Ammonia Cyanurate Reagent and Ammonia Salicylate Reagent) and nitrite (NitriVer® 3 Nitrite Reagent) levels. Temperature, salinity, pH and DO will be recorded weekly using handheld YSI model 556 MPS meter (Yellow Springs, OH, USA). Water temperature will be recorded every 30 minutes with HOBO Pendant® temperature loggers (Onset Computer Corp., Pocasset, MA, USA). Water pH will be maintained at 7.5-8.5, and DO will be maintained at a minimum concentration of 7.0 mg/L.

Throughout the experiments, loss of equilibrium and mortality events will be recorded at various intervals for each experimental method. After each mortality event, dead shrimp will be removed and weighed, measured, and examined to determine sex. Loss of equilibrium will be defined as the point at which shrimp become lethargic, fall to the bottom of the tanks on their backs and are unable to recover upright positions (Nelson and Hooper, 1982). Death will be defined as the lack of any body and gill movements and a failure to respond to tactile stimuli (D. Whitaker, SCDNR, pers. comm.).

Experimental Methods

The CTM involves a rapid linear change in water temperature from an acclimation temperature until a predetermined sublethal temperature (loss of equilibrium), and a lethal endpoint are reached (Beitinger et al, 2000). CTM is useful for determining acute temperature tolerance. A rate of 1^{o} C/hour will be used for this study. The CTM will be conducted using one environmental chamber with 24 tanks, with shrimp stocked as one individual per tank. After the acclimation period of 10 days, the water temperature will be decreased at a rate of 1^{o} C/hour until all shrimp reach the defined endpoint (loss of equilibrium and mortality). Water temperature will be decreased by reducing air temperature in the chambers and using bags of ice placed into the tanks. Shrimp will be checked for loss of equilibrium or mortality every 30 minutes. The mean temperature at which shrimp experience loss of equilibrium and mortality will be calculated.

The CLM involves a slow, linear change (1^{o} C/day) in water temperature from an acclimation temperature. The CLM uses temperature changes that allow acclimation to gradually changing temperatures, and more closely reflect natural environmental conditions (Selong et al, 2001). The CLM will be conducted in one environmental chamber with 24 tanks, with shrimp stocked as one individual per tank. After the acclimation period, the water temperature will be decreased at a rate of 1^{o} C/day until all shrimp are dead. Shrimp will be checked for loss of equilibrium or mortality every three hours. The mean temperature at which shrimp experience loss of equilibrium and mortality will be calculated.

The ACE method with static temperatures involves gradual changes in water temperature at environmentally realistic rates until a predetermined test temperature is reached (Selong et al, 2001). This method is used to determine the effects of prolonged exposure to low temperatures. The ACE method with static temperatures will be conducted with two test temperatures, 5^{o} C and 8^{o} C. These temperatures were chosen based on previous studies suggesting 8.5^{o} C as the temperature at which white shrimp trawl catches decline, and 6^{o} C as the critical temperature at which mortalities begin to occur, and on recorded temperatures in Charleston Harbor during severe winters (Farmer et al, 1978; Whitaker, 1984). Two trials of each base temperature will be performed, one in each environmental chamber, with 24 shrimp per trial. After the acclimation period of 10 days, temperature will be decreased at a rate of 1^{o} C/day until the test temperature is reached. Treatment temperature will be maintained for ten days, determined to be a realistic exposure time in the natural environment based on recorded water temperatures in Charleston Harbor. Once these test temperatures are reached, shrimp will be checked for loss of equilibrium or mortality every three hours.

The ACE method with fluctuating temperatures is a modification of the static ACE method to reflect temperature fluctuations naturally occurring in shallow water habitats, such as the estuarine marsh of Charleston Harbor. The ACE method with fluctuating temperatures will be performed using two base temperatures, 5^{o} C, with temperatures fluctuating from 4^{o} C-6^{o} C, and 8^{o} C, with temperatures fluctuating from 7^{o} C-9^{o} C. After the acclimation period, temperature will be decreased at a rate of 1^{o}C/day until the test temperature is reached. Temperature decreases of 1^{o} C will occur once daily over a 12-hour period, followed by temperature increases of 1^{o} C. This range of fluctuating temperatures of 1^{o} C in either direction of the base temperature was chosen because it reflects environmentally realistic daily changes in winter water temperature for Charleston Harbor. Temperature fluctuations will be achieved by reducing air temperature in the environmental chambers. Two trials of each base temperature will be performed, one in each environmental chamber, with 24 shrimp per trial. Once test temperatures are reached and temperature fluctuations have begun, shrimp will be checked for loss of equilibrium or mortality every three hours.

Anticipated Results and Benefits

The anticipated results of this research will be an empirical determination of low temperature tolerance of overwintering white shrimp in South Carolina. This will yield the temperature at which white shrimp of the size range found overwintering in Charleston Harbor begin to experience loss of equilibrium and mortality. Studies of cold tolerance can be used to develop predictive mortality estimates for marine organisms that give managers an early detection tool. Currently, the South Carolina Department of Natural Resources has the authority to delay opening the white shrimp spring fishery, or close it altogether, during severe winters.

With access to empirical data on the thermal limits of overwintering white shrimp, managers could better anticipate mortality events and close the fishery earlier as a preventative measure. Additionally, knowledge of low temperature tolerance of overwintering white shrimp may provide an opportunity to evaluate the current management regulations.

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