THERMAL TOLERANCE OF LAKE STURGEON

by

PAUL ANDREW WILKES

(Under the Direction of Douglas Peterson)

ABSTRACT

Habitat restoration has allowed management agencies to begin reintroducing lake sturgeon (*Acipenser fulvescens*) into the Coosa River of Georgia and Alabama; however, high summer temperatures may preclude establishment of a self-sustaining population. In this study, we estimated the critical thermal maxima of 200 days post-hatch (DPH) and 400 DPH lake sturgeon. The 400 DPH age group had the lowest critical thermal maxima; they lost equilibrium at 35.1°C when acclimated at 24.9°C and at 33.1°C when acclimated to 18.1°C. To assess the effects of prolonged exposure to high temperature on lake sturgeon, we subjected groups of lake sturgeon to four increasing temperature regimes of 26°C, 29°C, 32°C and 35°C. When exposed to temperatures above the $ULST_{LT}$ (31.7°C), feed consumption was reduced by 20-35% and weight gain was negligible. At temperatures near the CTM (35.1°C), feeding rate decreased to near zero. Prolonged exposure of lake sturgeon to temperatures near their CTM resulted in mortality of all individuals.

INDEX WORDS: Lake Sturgeon, Thermal Tolerance, Critical Thermal Maxima, Stress, *Acipenser fulvescens*, Mortality

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CHAPTER 1

INTRODUCTION

SYNOPSIS

The lake sturgeon (*Acipenser fulvescens*, Acipenseridae) is a large potomodromous fish that ranges from the Hudson Bay drainages of Canada to the Coosa River System in northwest Georgia. The species was once common throughout its range, but all populations were severely depleted by overfishing in the late 1800s (Harkness and Dymond 1961). The life history of lake sturgeon makes them vulnerable to overexploitation and other anthropogenic influences (Noakes et al. 1998). In recent years, several states have initiated restoration efforts including Georgia, where lake sturgeon were extirpated in the late 1900s. In 2002 the Georgia Department of Natural Resources began a restoration effort using Wisconsin broodstock to re-establish a population of lake sturgeon in the Coosa River. Initial post-stocking assessments by Bezold and Peterson (2008) suggest that this program has been successful despite summer water temperatures in the Coosa that routinely exceed 30°C. Although quantified thermal tolerance estimates for lake sturgeon do not exist, these temperatures are well in excess of the anecdotal estimate of 26°C reported by Wehrly (1995). Despite limited survival of stocked fingerlings, high water temperatures may preclude establishment of a self-sustaining population (Bezold 2007).

DESCRIPTION

The lake sturgeon (*Acipenser fulvescens*) is a large benthic fish endemic to the Central U.S., Great Lakes and the Hudson Bay drainages of Canada. The species name “fulvescens,” which means fulvous or tawny, is based on the dull brown color of individuals captured in the Great Lakes (Priegel and Wirth 1971). Like other members of the Acipenser genus, the lake sturgeon is easily distinguished from other North American fishes by its cartilaginous skeleton, elongated rostrum, heterocercal tail, and scaleless body. Lake sturgeon are covered by five latitudinal rows of hard bony plates called scutes. As juveniles, these scutes are well developed and possess a sharp apical hook. Peterson et al. (2007) noted that the prominence of the scutes becomes increasingly diminished after maturity. The authors hypothesize that the lack of biting predators in freshwater habitats occupied by lake sturgeon eliminates the need for protective body armoring in adult lake sturgeon.

Another prominent ontogenic change in lake sturgeon morphology is body coloration. Juveniles < 30 cm have distinct black dorsal saddles posterior to the pectoral fins. The rest of the body color varies from a dark brown to a light grey. Juveniles exhibit black speckling throughout the anterior portion of their bodies. In older juveniles, these black pigmentations gradually fade until adulthood, at which point most individuals are uniformly grey or grey-brown. The ventral surface usually remains white or cream-colored at all stages of development (Peterson et al. 2007). Arguably, the most unique morphological characteristic of lake sturgeon is the elongated rostrum. The ventral surface of the rostrum is dotted with ampullae that aide in the location of prey items (Harkness and Dymond 1961; Priegel and Wirth 1971). Four fleshy barbels are positioned midway between the mouth and the end of the rostrum. The large fleshy lips of the subterminal mouth are protrusible and, hence, used to facilitate suction feeding over soft substrates (Harkness and Dymond 1961; Priegel and Wirth 1971: Peterson et al. 2007).

LIFE HISTORY AND ECOLOGY

The lake sturgeon is a long-lived species that grows slowly, matures at a late age and spawns infrequently. These characteristics, however, are balanced by high fecundity once the fish reaches maturity. Depending on latitude, females typically mature at 20-25 years of age; males mature at 14-15 years (Priegel and Wirth 1971; Scott and Crossman 1973; Bruch and Binkowski 2002). Once mature, females may spawn every 3-5 years, whereas males typically spawn every 1-3 years (Bruch and Binkowski 2002). Because of their late maturation and protracted spawning periodicity, lake sturgeon populations can rely on a relatively small number of adults to produce large numbers of offspring. In fact, a single female can produce up to 425,000 eggs in a single spawning season (Bruch 1999). This life history strategy, however, relies on low mortality of adults and makes the species vulnerable to overexploitation and other anthropogenic influences.

Spawning occurs in early spring as adult lake sturgeon migrate upriver to their natal spawning grounds (Bruch and Binkowski 2002). Peak spawning occurs as water temperatures approach 11.5 - 16.5°C (Harkness and Dymond 1961; Bruch and Binkowski 2002). During each spawning bout one female will be accompanied by two or three males, which thrash about and release milt as the female deposits eggs into the water (Bruch and Binkowski 2002). The demersal, adhesive eggs are broadcast across rocky substrates with moderate to high flow at depths of 2-10 m (Scott and Crossman 1973; LeHaye et. al. 1992) Hatching typically occurs in

5-8 days at water temperatures of 16-20^{o}C (Priegel and Wirth 1971; Scott and Crossman 1973; LaHaye et al. 1992; Kempinger 1996).

After hatching, the negatively phototactic larvae hide in the interstitial spaces of the rocky substrate (Auer and Baker 2002). Within 13-19 days post hatch (DPH), however, the larvae emerge from the substrate and begin drifting with the current at night (Auer and Baker 2002). During this period of dispersal, larvae may travel up to 45 rkm within 25-40 DPH (Kempinger 1996; Auer and Baker 2002; Benson et al. 2005). Although predation mortality is high during this period, the sheer number of larvae present simply overwhelms predators allowing a substantial number to survive through the critical period (Peterson et al. 2007). The larvae transition to the juvenile stage at about 40 mm, at which point they are well-protected from most predators by their sharp bony scutes (Auer and Baker 2002).

Adult lake sturgeon are among the largest and longest lived freshwater fish in North American. On average, females attain lengths of 130 - 215 cm and weights of 25-100 kg. Males are usually smaller, averaging lengths of 100-185 cm and weights of 11-30 kg (Priegel and Wirth 1971; Peterson et al. 2007). Adults may live well over 100 years with the oldest recorded specimen estimated to be 150 years of age (Priegel and Wirth 1971). The largest lake sturgeon on record was captured in Lake Michigan in 1943 and weighed 141 kg (Van Oosten 1956).

Lake sturgeon feed primarily in lacustrine environments on invertebrate prey (Priegel and Wirth 1971; Scott and Crossman 1973). Feeding generally occurs in shallow water (< 10 m) where invertebrate prey are abundant (Harkness and Dymond 1961; Priegel and Wirth 1971). The four taste-sensing barbels anterior to the mouth are used to locate benthic prey (Harkness and Dymond 1961; Priegel and Wirth 1971; Scott and Crossman 1973). Lake sturgeon are proficient at extracting even the smallest prey items from soft substrates, and stomachs of large adults have been found with up to 60,000 lake fly larvae (Priegel and Wirth 1971).

EXPLOITATION AND RECOVERY

Although once common throughout North America, many lake sturgeon populations have been severely over-fished (Priegel and Wirth 1971). Historically, the species was found from the Hudson Bay drainages of Canada as far south as the lower Mississippi drainages (Harkness and Dymond 1961). The southernmost extent of the range included the Coosa River in Alabama and northwest Georgia (Dahlberg and Scott 1971). Because of their delayed maturation and protracted spawning periodicity, lake sturgeon populations are particularly susceptible to over fishing, but habitat degradation and habitat fragmentation (from impoundment of spawning tributaries) have also affected many populations (Priegel and Wirth 1971). Prior to the mid-1800s, lake sturgeon were routinely discarded by commercial fishermen as a nuisance species. In some instances the fish were even stacked on shore to dry and then used to fire the boilers of steamboats (Harkness and Dymond 1961; Scott and Crossman 1973). In the 1860s, however, the species became valuable when people discovered that lake sturgeon roe could be made into a fine caviar and that the smoked flesh was of excellent flavor (Harkness and Dymond 1961; Priegel and Wirth 1971; Scott and Crossman 1973). After the 1860s, commercial markets for lake sturgeon emerged quickly. Thereafter most populations were intensely exploited. Historic catch records suggest that most populations were over-exploited within just a few decades. In Lake Erie, for example, more than 5 million pounds of lake sturgeon were removed in 1885 alone (Harkness and Dymond 1961; Priegel and Wirth 1971; Carlson 1995). By the early 1900s, commercial fishing had decimated most populations (Harkness and Dymond 1961)

Although lake sturgeon are not federally listed in the U.S. or Canada, they are currently protected throughout most of their range and they are currently listed under Appendix II of the Convention on International Trade of Endangered Species (CITES). Various management strategies have been implemented throughout the US and Canada to restore stocks. Arguably, the most successfully managed stock in US waters is that of the Lake Winnebago System in Wisconsin, where lake sturgeon have been managed for over 100 years (Bruch 1999; Bruch and Binkowski 2002).

Despite increased protection, some populations of lake sturgeon have still been slow to recover; as a result, artificial propagation has been used to help spur recovery in many of these populations (Peterson et al. 2007). In the Coosa River of northwest Georgia, the Department of Natural Resources (GDNR) has been stocking lake sturgeon since 2002 as part of a long-term reintroduction program. Although the program is still being assessed, a recent study by Bezold and Peterson (2008) estimated that survival among the stocked cohorts averaged only 2%. Unpublished records from Bezold's assessment show Coosa River water temperatures routinely exceeding 32°C. Although no quantified CTM data are available, Wehrly (1995) reported an anecdotal estimate of the uppermost thermal tolerance of lake sturgeon to be around 26°C. Because of this disparity, Bezold and Peterson (2008) hypothesized that thermal stress was likely the cause of poor condition during late summer and fall.

Prior to exploitation and habitat alteration, the Coosa River lake sturgeon population may have possessed unique adaptations that enabled them to survive in the relatively warm climate of north Georgia. Although empirical data are not available, some biologists have argued that the use of northern lake sturgeon brood stock for the Coosa River reintroduction may be ill-advised because the stocked fish may not be well-adapted to the warm summer condition typical of the

Coosa System. Because GDNR typically stocks age-0 fish in the fall surviving juveniles do not encounter summer temperatures until after reaching age-1 (> 365 DPH). Although the Bezold and Peterson (2008) study shows that at least some of these stocked juveniles are surviving, the long-term success of the reintroduction program could be in jeopardy if wild age-0 juveniles are not capable of surviving typical summer water temperatures.

THERMAL TOLERANCE

Brett (1956) defined the upper and lower thermal lethal temperatures as the range of temperatures within which 50 percent of a population can survive indefinitely. Hence, thermal tolerance is defined as the range of water temperatures in which a fish can survive. The critical thermal maxima (CTM) method is an experimental protocol commonly used to quantify the upper thermal tolerances of fish species (Becker and Genoway 1979; Benfey et al. 1997; Beitinger et al. 2000). The method uses a linear heating rate to progressively increase water temperature *in vitro*, until the upper lethal temperature has been obtained. Previously, Becker and Genoway (1979) identified an optimum heating rate for CTM studies of 0.1 to 0.3^{o}C min^{-1}. Confirmatory studies by Kilgour and McCauley (1986) show that heating rates within this range are gradual enough to avoid any significant lag in internal body temperature, but are fast enough to prevent acclimation. In typical CTM studies, as upper lethal temperatures are approached, test subjects lose equilibrium before death. Hence, the CTM method assumes that death is imminent once the loss of equilibrium has occurred because *in vivo*, a fish that has lost equilibrium is unable to seek cooler water. Some authors, however, have criticized the CTM method because the variables of temperature and time are confounding, which can result in an overestimation of upper lethal temperature (Becker and Genoway 1979). Nonetheless, the CTM method is still widely recognized as the most reliable means of identifying the range of thermal tolerance for specific fish species.

Although a variety of different endpoints, including onset of muscle spasms or opercular flaring, can be used in the CTM method, loss of equilibrium (LOE) is typically used as the endpoint in CTM trials. The onset of LOE in CTM studies results from disorganization of nerve impulses that render test subjects incapable of escaping the warming water temperature. When subjected to CTM testing, fish typically reach LOE well before death occurs, which allows the researcher to revive test subjects by immediately returning them to cooler water (typically, their acclimation temperature). This approach is especially advantageous when the supply of test fish is limited, as is typically the case with rare or imperiled species. Hence, CTM testing is also widely regarded as the most efficient method of determining the maximum thermal tolerance of a fish species (Benfey et al. 1997).

Another advantage of the CTM method is that it allows the researcher to precisely identify thermal maxima (within ± 0.2°C) at different acclimation temperatures so long as all other experimental parameters are precisely controlled (Brett 1956). This advantage is especially important in comparisons of thermal tolerance among different populations of the same species. CTM testing also allows the researcher to isolate and, hence, quantify effects of acclimation temperature on thermal tolerance. Previous CTM studies have shown that thermal maximum of some taxa, such as killifish, may vary by as much as 12°C depending on acclimation temperature (Beitinger et al. 2000). This precision allows field biologists to understand and then quantify the minute, intra-specific variations in thermal tolerance among species such as lake sturgeon.

In practical application, results of CTM experiments may be used to calculate upper limits of safe tolerance (ULST), final thermal preference (FTP), and thermal growth optimum

(TGO) for specific fish populations (Jobling 1981). Because fish undergo numerous cellular changes in response to temperature variation (change in lipid concentrations and homeoviscous adaptation), thermal preference and thermal growth optima may be influenced by acclimation temperature. Hence, CTM trials can provide aquaculturists with critical information for optimizing growth of hatchery reared fish.

Although thermal tolerance of fishes has been well studied, the most accurate method of determining maximum thermal tolerances has been greatly debated. Early thermal tolerance testing methods were developed with the sole purpose of helping researchers understand the physiology of fishes and to identify cellular changes that help maintain metabolic function when body temperatures fluctuate (Fry 1947; Selong and McHahon 2001). Subsequently, these methods were altered to estimate maximum thermal tolerance of fishes in laboratory settings. Many methods of testing thermal tolerance do not accurately describe the uppermost thermal tolerance because their results are repeatable yet confounded with other variables introduced by the methodology. Several previous studies have demonstrated that maximum thermal tolerance can be quite plastic in many species depending on acclimation temperature (Cocking 1958; Wang et al. 1985; Beitinger et al. 2000; Ziegweid et al. 2008). Because thermal maxima can change with different acclimation temperatures, determining a maximum thermal tolerance can be difficult unless experiments are rigorously replicated with different acclimation temperatures.

Because wild fish have considerable plasticity in their maximum thermal tolerance to cope with seasonal variation in mean temperature, the maximum thermal tolerance is typically several degrees above the ambient mean maximum water temperature of the specific population in question (Brett 1956). Interestingly, acclimation to a higher thermal tolerance typically occurs quite rapidly (~24 hrs for most species), whereas the reverse acclimation process occurs much more slowly (Brett 1956). The difference in acclimation time to increase and decrease maximum thermal tolerance may be linked to the speed at which cellular responses occur at high versus low acclimation temperatures. Because acclimation temperature is typically important in determining thermal tolerance, CTM experiments must account for acclimation temperature of the test subjects. Furthermore, experiments with multiple acclimation temperatures also provide more insights into the ecological flexibility of a species with regard to seasonal temperature regimes. This may be particularly important for species with broad latitudinal distributions.

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CHAPTER 2

CRITICAL THERMAL MAXIMA OF JUVENILE LAKE STURGEON

SYNOPSIS

Habitat restoration has allowed management agencies to begin reintroducing lake sturgeon (*Acipenser fulvescens*) into the Coosa River of Georgia and Alabama; however, high summer temperatures may preclude establishment of a self-sustaining population. The upper level of thermal tolerance for lake sturgeon has been estimated to be 26°C, which is much lower than average summer temperatures in the Coosa River. In this study, we estimated the critical thermal maxima of 200 days post-hatch (DPH) and 400 DPH lake sturgeon. Lake sturgeon were maintained at acclimation temperatures of either 24.9°C or 18.1°C for a minimum of seven days prior to thermal tolerance testing. The repeated measures ANOVA results indicated that acclimation temperature ($F_{1,49} = 165.59$, $p < 0.0001$) and age ($F_{1,49} = 9.06$, $p = 0.0041$) significantly affected critical thermal tolerance. The 400 DPH age group had the lowest critical thermal maxima; they lost equilibrium at 35.1°C when acclimated at 24.9°C and at 33.1°C when acclimated to 18.1°C. Although the summer water temperatures of the Coosa River are much higher than previously thought, temperatures routinely remain above the upper limits of safe tolerance estimated in this study. Any increase in river temperature may significantly impair the success of the reintroduction. Future studies should examine the chronic effects of high temperature on individuals and how it affects long-term population viability.

INTRODUCTION

Although once common throughout North America, many lake sturgeon populations have been severely over-fished (Priegel and Wirth 1971; Secor et al. 2002; Peterson et al. 2007). Historically, the species was found from the Hudson Bay drainages of Canada as far south as the lower Mississippi drainages (Priegel and Wirth 1971). The southernmost extent of the range included the Coosa River in Alabama and northwest Georgia (Scott and Crossman 1973; Ono et al. 1983). Lake sturgeon populations are particularly susceptible to over-fishing because of their delayed maturation and protracted spawning periodicity. Habitat degradation and fragmentation from the impoundment of spawning tributaries have also affected many populations (Priegel and Wirth 1971). Lake sturgeon are currently protected throughout most of their range - though not federally listed in the US or Canada, they are listed under Appendix II of the Convention on International Trade of Endangered Species (CITES).

Despite increased protection, many lake sturgeon stocks, such as that of the Coosa River, have not recovered to their historic population levels (Kynard 1997; Schram et al. 1999). Lake sturgeon in the Coosa River System were extirpated during the 1970s; the last specimen was documented in 1967 (Smith-Vaniz 1968; Dahlberg and Scott 1971). Artificial propagation has been used to help spur recovery of many depressed populations of sturgeon (Peterson et al. 2007). The Georgia Department of Natural Resources (GDNR) has been stocking lake sturgeon in the Coosa River since 2002 as part of a 20 year reintroduction program. Bezold and Peterson (2008) showed that post-stocking survival of juvenile lake sturgeon averaged 2% over a three-year period and that body condition of stocked juveniles declined as water temperatures exceeded 30°C during late summer and fall (Bezold and Peterson 2008). Because lake sturgeon are primarily found in cooler climates, quantified estimates of the species' upper thermal tolerance do not exist. Wehrly (1995), however, reported substantial mortality to northern lake sturgeon when temperatures neared 26°C, leading him to estimate this temperature to be near the upper level of thermal tolerance of the species. Although Bezold and Peterson (2008) concluded that some stocked juveniles were surviving in the Coosa, high summer water temperatures may preclude re-establishment of a self-sustaining population (Wehrly 1995; Bezold 2007).

Although empirical data are not available, some biologists have argued that using lake sturgeon progeny from northern rivers for reintroduction in the Coosa River may produce juveniles that are not well-adapted to the warmer conditions typical of southern rivers. Bezold and Peterson (2008) showed that some of these stocked juveniles are surviving, however, the long-term success of the reintroduction program could be jeopardized if naturally spawned juveniles cannot survive the high ambient summer water temperatures (Bezold 2007). Although there are numerous variables that affect the survival of stocked lake sturgeon, field studies by Bezold and Peterson (2007) suggest that thermal stress may be severely affecting stocked sturgeon in the Coosa River. Because of this, thermal tolerance estimates are needed to help managers and scientists better understand the causes of the low condition and survival of lake sturgeon in this watershed (Bezold and Peterson 2008). The objective of this study was to identify critical thermal maxima (CTM) and lethal thermal maxima (LTM) of juvenile lake sturgeon at 200 and 400 days post hatch (DPH). These CTM and LTM estimates will then be used to determine the upper limits of safe tolerance, final thermal preference and thermal growth optima for the species.

MATERIALS AND METHODS

Fish culture

Lake sturgeon eggs were obtained from the Wild Rose Fish Hatchery in Wisconsin and reared at the GDNR's Summerville Fish Hatchery. Fingerlings were then transported to the Whitehall Fish Lab at the University of Georgia. Two year classes (2008 and 2009) of sturgeon were held in re-circulating culture systems supplied with de-chlorinated municipal water for the duration of the project. Fish in the 400 DPH group were fed a 2% body weight per day ration of commercially prepared salmonid diet (Rangen Inc. Franklin, Louisiana) to maintain optimal growth and survival (Hung and Lutes 1987; Moore et al. 1988). Because the younger age group of lake sturgeon would not switch to a commercial diet, they were maintained on a diet of bloodworms fed at a rate of 15% body weight per day. Despite the difference in feeding protocols, both age groups of sturgeon received 1% body weight of protein per day. The daily ration of each age group was measured and distributed throughout each tank by hand once daily. Any uneaten food was siphoned post-feeding.

Lake sturgeon were randomly separated into four 3000 L round re-circulating tanks based on age (200 DPH and 400 DPH) and acclimation temperature (18.1°C (± 0.4) and 24.9°C (± 0.1)) with one tank designated for each of the four combinations of age and acclimation temperature. Temperatures were maintained by either a chiller (Drop-in Unit, Frigid Units Inc.) or heater (Submersible 1.5kW Clepco Cleveland, Ohio). De-chlorinated municipal water was supplied to acclimation tanks at a rate of 100 mL/s. A 60 cm stand-pipe was used to maintain consistent water depth in all tanks. Water quality was maintained through 90% water recirculation through biological and particulate filters. Ammonia (± 0.02, ppm), nitrite (± 0.02, ppm), nitrates ((± 0.02, ppm), pH ((± 0.05), dissolved oxygen (± 0.02, $mg \cdot L^{-1}$), alkalinity (±

0.02, ppm) and hardness (± 0.02, ppm) were measured weekly using a LaMotte Colorimeter. Water temperature (± 0.1,°C) was measured once daily using a handheld National Institute of Standards and Technology (NIST) calibrated mercury thermometer. A continuous temperature log was also recorded using a HOBO temperature data logger (Onset data Corporation © 2005).

Water quality parameters were maintained within acceptable limits for cool water fish as described by Timmons et al. (2002; Table 1). Water temperatures in the treatment tanks were maintained within ± 0.5°C of the target temperatures. Despite the high temperatures in some of the treatment tanks the dissolved oxygen levels remained within tolerable limit for the species (Timmons et al. 2002).

Experimental Design

A total of 69 lake sturgeon was randomly divided into four treatment groups: 200 DPH at 24.9°C, 200 DPH at18.1°C, 400 DPH at 24.9°C and 400 DPH at18.1°C. Each treatment group consisted of 18 fish which were maintained in an isolated treatment tank, three of which were randomly selected as controls. After treatment (seven days of acclimation) fish from each tank were randomly selected for CTM and LTM testing until all fish were tested. Control fish remained in the testing tanks for the duration of the experiment to exclude variables other than temperature as the cause of LOE and death. Although technically pseudoreplicated, this design is considered standard for thermal tolerance experiments (Benfey et. al. 1997; Curie et. al. 1998; Beitinger et. al. 2000; Selong and McHalon 2001; Ziegweid et. al. 2008)

A two-way factorial analysis of variance (ANOVA) was used to determine differences in thermal tolerance (CTM and LTM) attributed to age and acclimation. Analyses were considered significant at the alpha = 0.05 level. The following equations from Jobling (1981) were used to identify the final thermal preferendum (FTP) and thermal growth optima (TGO) of each treatment group:

1. $$FTP = (CTM - 1643)(0.66^{-1})$$

2. $$TGO = (CTM - 13.81)(0.76^{-1})$$

Additionally, upper limits of safe tolerance (ULST) were calculated (Young and J.J. 1996; Ziegweid et al. 2008):

3. $$ULST = CTM - 5$$

Experimental setup

All thermal maxima experiments were conducted in one of two 38 L glass aquaria filled with 17.5 L of de-chlorinated municipal water. Each aquarium had two pumps and one airstone to ensure uniform temperature and aeration. An NIST calibrated mercury thermometer was mounted on the inside sidewall of the aquarium. Each aquarium was elevated on 10 cm wooden blocks so that a hot-plate (Thermolyne Nuova) could be positioned approximately 3 mm beneath the aquarium floor. All other sidewalls of the aquarium, except for the front, were insulated with 1 cm Styrofoam to minimize heat loss during experimental trials.

Experimental protocol

Juvenile lake sturgeon were maintained at their specific acclimation temperatures for at least seven days prior to each trial. Prior to the start of the trial, water from the acclimation tank was transferred to the trial aquaria. Individual lake sturgeon were then transferred from the acclimation tank to the trial tank. After one hour of acclimation, the hot plate (Thermolyne Nuevo) was activated to obtain a heating rate of 0.1°C min^{-1} (Beitinger et al. 2000). Water temperature was recorded every 15 min from the beginning of each trial and at designated endpoints (loss of equilibrium and death).

Because of their demersal nature, lake sturgeon do not always exhibit loss of equilibrium during CTM testing (Ziegweid et al. 2008). Consequently, we recorded water temperatures at both the loss of equilibrium and death. The CTM was the temperature at which loss of equilibrium occurred. Loss of equilibrium was defined as the temperature at which the fish experienced an inability to maintain dorso-ventral orientation for at least 10 consecutive seconds (Wattenpaugh and Beitinger 1985). The LTM was defined as the temperature at which death occurred, as evident from the cessation of opercula movements and a lack of response to tactile stimuli (Bettoli et al. 1985). At the conclusion of the trial each fish was weighed and measured and all equipment was rinsed and replaced for the next trial.

RESULTS

Trial results showed that both CTM and LTM were significantly affected by acclimation temperature ($F_{1,49} = 165.59$, $p < 0.0001$, $F_{1,55} = 1058.37$, $p < 0.0001$) regardless of age group (Table 2). When acclimation temperature increased was increased by 6.8°C the CTM of test fish increased 2.5°C in the 200 DPH age group and 2.2°C in the 400 DPH age group. Similarly, both CTM and LTM were significantly affected by age ($F_{1,49} = 9.06$, $p = 0.0041$, $F_{1,55} = 10.13$, $p = 0.0024$). The CTM of the 200 DPH age group was 0.1°C higher than that of the 400 DPH age when acclimated to 18.1°C and 0.6°C higher when acclimated to 24.9°C. Results showed there was not a significant interaction between age and acclimation temperature in neither the CTM nor LTM tests ($F_{1,49} = 0.00$, $p = 1.0000$, $F_{1,55} = 0.00$, $p = 1.0000$). The CTM and LTM were positively associated with acclimation temperature and negatively associated with age. Across treatment groups CTM temperatures ranged from 33.2 – 35.1°C whereas the LTM temperatures were slightly higher and ranged from 35.0 – 36.7°C. The FTP ranged from 25.4°C to 28.3°C and the TGO optima ranged from 25.5 – 28°C.

Sturgeon exhibited varying levels of activity through the CTM and LTM trials. Sturgeon activity was observed to increase with temperature until approximately 30-33°C. Once at these temperatures the fish often became lethargic and remained nearly motionless on the bottom of the experimental tank. In seven of the 400 DPH sturgeon this impaired determination of CTM because the loss of muscular control did not result in a LOE; therefore, the sturgeon remained upright even after the LTM endpoint was reached. Additionally, CTM and LTM estimates for one of the 400 DPH lake sturgeon was excluded from the analysis because an equipment failure resulted in an inconsistent heating rate during the trial.

DISCUSSION

The thermal tolerance of lake sturgeon was much higher than the previous estimate of 26°C reported by Wehrly (1995). Furthermore, the thermal tolerance of lake sturgeon was significantly affected by acclimation temperature as well as age. A 6.8°C increase in acclimation caused an increase of 2°C in the critical thermal tolerance and a 1.9°C increase in the lethal thermal tolerance. In addition, age also affected thermal tolerance decreasing 0.1-0.5°C over a 200 day age difference.

The results of this study illustrate the importance of acclimation temperature in determining thermal tolerance. Like all other fishes studied thus far, the lake sturgeon's thermal tolerance increased with acclimation temperature (Brett 1956; Becker and Genoway 1979; Bosch et al. 1988; Elliot 1991; Bennett and F.W. 1992; Benfey et al. 1997; Beitinger et al. 2000; Chung

2001). Ziegweid (2008) found that juvenile shortnose sturgeon gained 0.3°C in thermal tolerance for every 1°C increase in acclimation temperature. Our results were remarkably similar; we observed a 0.29°C increase in thermal tolerance for every 1°C increase in acclimation temperature. Theoretically, this would lead to the assumption that fish do not have an absolute upper level of thermal tolerance; however, as a fish species acclimation temperature is increased the subsequent gain of thermal tolerance decreases (Currie et al. 1998).

Summer temperatures in the Coosa and other southern rivers routinely reach 33-34°C and although these temperatures are well below the LTM for lake sturgeon they are, however, near the CTM. In the wild, temperatures at or above a species' CTM will likely be lethal because the onset of muscular spasms and loss of equilibrium (LOE) will render the fish incapable of seeking cooler water (Bennett and F.W. 1992; Beitinger et al. 2000). Additionally, while peak river temperatures are slightly lower than the CTM of lake sturgeon, they are much warmer than both the $ULST_{LT}$ and $ULST_{CT}$ at the warmer acclimation temperature. Chronic exposure to temperatures above the ULST may exacerbate the lower post-summer condition of fish noted by Bezold and Peterson (2008) in their assessment of the Coosa River lake sturgeon reintroduction program (Brett 1956).

Although this study showed that age significantly affected thermal tolerance of juvenile lake sturgeon, further studies are needed to clarify the relationship between age and thermal tolerance. Our results showed that thermal tolerance was significantly affected by age, but the actual effect on CTM was only 0.1-0.5°C. Furthermore, because weight and age of the test fish may have been confounding variables in our trials, the causal mechanism was unclear. Despite this problem, our results suggested that thermal tolerance in juvenile lake sturgeon may actually decline with age. Future studies are needed to identify thermal tolerance testing methods for larger bodied fishes so that ontogenetic changes in thermal tolerance can be identified. Resulting experiments could provide important new information regarding changes in habitat requirements associated with saltatory development.

CTM provides a relatively inexpensive method of testing thermal tolerance that is consistently repeatable; however, conventional CTM methodology has some drawbacks (Currie et al. 1998; Beitinger et al. 2000). Like similar methods, the CTM results can be confounded because variables of time and temperature cannot be easily separated (Becker and Genoway 1979; Beitinger et al. 2000). Effective use of the CTM method is also limited to juveniles, or small bodied fishes, because of the time lag associated with internal body warming as water temperatures are increased (Beitinger et al.1976; Becker and Genoway 1979). Consequently, most CTM experiments are limited to trials with YOY fish. As demonstrated in this study, however, some species may exhibit important ontogenetic changes in thermal tolerance. We suggest that future studies are needed to develop alternative methods of CTM testing that provide a more precise evaluation of thermal tolerance throughout the entire life cycle. Many of the drawbacks of the CTM methodology stem from its reliance on acute exposure to dynamic temperature changes; however, a more chronic approach to thermal tolerance testing may eliminate these shortcomings.

Studies examining chronic exposure of fish to high temperatures are rare, and of the few that have been attempted, most rely on ILT and CTM to quantify thermal tolerance. Although these methods provide a precise, easily quantifiable result, they only measure acute lethal thermal tolerance and do not simulate conditions typically encountered by wild fish (Selong and McHahon 2001). Although these studies have been useful in determining acute lethal temperature tolerances of fishes, they do not address chronic or sub-lethal effects of high temperature or the interactive effect of time and temperature. In the wild, fish kills resulting from acute high temperatures are rare – probably because thermal tolerances of most fishes are typically 5-6°C above the maximum mean temperatures encountered (Brett 1956). However,thermal tolerance may vary lattitudinally across a species range (Power and McKinley 1997; Portner 2002). Consequently, environmental thresholds developed for one population may not accurately define critical habitat for all populations, particularly for species like sturgeon, which are broadly distributed.

Future studies should examine the sub-lethal effects of chronic high temperature and how it may impair growth, condition and survival of fishes. Such studies may help broaden our understanding of the linkages between habitat and fish behavior, particularly for populations at the margins of their range.

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CHAPTER 3

SUB-LETHAL EFFECTS OF CHRONIC THERMAL STRESS ON LAKE STURGEON

SYNOPSIS

Habitat restoration has allowed management agencies to begin reintroducing lake sturgeon (*Acipenser fulvescens*) into the Coosa River of Georgia and Alabama; however, high summer temperatures may preclude the establishment of a self-sustaining population. Based on thermal tolerance testing in the previous chapter, the upper level of thermal tolerance for 400 days post-hatch lake sturgeon is estimated to be 35.1°C. This temperature is higher than summer temperatures reach in the Coosa River, indicating that conditions within the Coosa are suitable for lake sturgeon. However, summer temperatures in the Coosa often exceed 31.7°C, the lethal upper limit of safe tolerance for lake sturgeon, suggesting that sub-lethal high temperature effects may adversely affect this species within the Coosa system. The objective of this study was to estimate the sub-lethal effects of temperatures at or above the critical thermal maxima and lethal upper limits of safe tolerance of juvenile lake sturgeon. To assess the effects of prolonged exposure to high temperature on lake sturgeon, we subjected groups of lake sturgeon to four increasing temperature regimes of 26°C, 29°C, 32°C and 35°C. When exposed to temperatures above the $ULST_{LT}$ (31.7°C), feed consumption was reduced by 20-35% and weight gain was negligible. At temperatures near the CTM (35.1°C), feeding rate decreased to near zero. Prolonged exposure of lake sturgeon to temperatures near their CTM resulted in mortality of all individuals.

INTRODUCTION

The lake sturgeon (*Acipenser fulvescens*, Acipenseridae) is a large, potomodromous fish that ranges from the Hudson Bay drainages of Canada to the Coosa River System in northwest Georgia (Priegel and Wirth 1971; Scott and Crossman 1973; Becker 1983; Pflieger 1997; Hung et al. 2003). Though this species was once common throughout its range, most populations were severely depleted by overfishing in the late 1800s (Harkness and Dymond 1961). The late age at maturity and protracted spawning periodicity of lake sturgeon makes them vulnerable to overexploitation and other anthropogenic influences (Noakes et al. 1998). In the southeastern United States, lake sturgeon were historically abundant in the Coosa River system of Georgia and Alabama (Dahlberg, M.D. and D. C. Scott. 1971). However, because of over-harvest and habitat degradation, the Georgia Department of Natural Resources believes they were extirpated from Georgia in the early 1980s. In response, several states, including Georgia, have recently initiated efforts to restore populations of lake sturgeon.

In 2002, the Georgia Department of Natural Resources began a long-term lake sturgeon restoration effort in the Coosa River system in which they stocked fingerling lake sturgeon from Wisconsin broodstock. An initial post-stocking assessment by Bezold and Peterson (2008) suggests that this program has been successful despite summer water temperatures that routinely exceed 30°C. Though temperatures in the Coosa River do not exceed the critical thermal maxima (CTM; 35.1°C) of the species, they regularly exceed the upper limit of safe tolerance ($ULST_{LT}$; 31.7°C) of juvenile lake sturgeon (Wilkes Thesis, Chapter 2). In the post-stocking assessment of lake sturgeon in the Coosa River, Bezold (2007) noted a decrease in condition of lake sturgeon following the summer months. This suggests that high temperatures within the

Coosa River may have sub-lethal effects, such as reduced feeding and increased stress on lake sturgeon, which may preclude the establishment of a self-sustaining population. Previous results from CTM experiments show that acute exposure to temperatures above the $ULST_{LT}$ are non-lethal; however, possible sub-lethal effects are not addressed in Chapter 2 of this document. Thus, studies examining these sub-lethal effects represent an important piece of information needed for managing lake sturgeon populations at the southern extent of their range.

The most common method of measuring thermal tolerance has been the CTM method because of its relative simplicity and relevance to environmental conditions (Brett 1956; Becker and Genoway 1979; Currie et al. 1998). Albeit useful, the CTM method has received criticism because the variables of temperature and time are confounding and the point temperature obtained is usually several degrees higher than that of other thermal tolerance testing methods (Beitinger et al. 2000). These criticisms lead to the development of the chronic lethal methodology (CLM) and the acclimated chronic exposure (ACE) method (Selong and McHahon 2001). These methods use a slower heating rate of 1-2°C·hr^{-1} which allows fish to continually acclimate to rising temperatures. The temperature is increased until it reaches a predetermined set point at which point it is held constant for a specified length of time (typically 60 days) (Zale 1984; Elliot and Elliot 1995; Selong et al., 2001). While at the final constant temperature, fish mortality is recorded (Beitinger et al. 2000). Although these methods incorporate a realistic heating rate, they require a sample size of > 200 individuals, which is often not feasible in the case of threatened or endangered species (Zale 1984; Elliot and Elliot 1995; Selong et al., 2001).

The goal of this study was to investigate the sub-lethal effects of prolonged exposure to thermal stress in juvenile lake sturgeon by exposing them to incremental increases in temperature. The specific objective was to quantify changes in growth and condition of juvenile lake sturgeon exposed to stepwise increases in water temperature from 6°C below the ULST, to 3°C above the ULST over an eight week exposure period. Because pre-established testing methods for such an experiment have not been developed previously, our secondary objective was to evaluate the use of dynamic incremental temperature increase methodology for estimating the sub-lethal effects chronic thermal stress in juvenile sturgeons.

MATERIALS AND METHODS

Fish Culture

Lake sturgeon eggs were obtained from the Wild Rose Fish Hatchery in Wisconsin and reared at the GDNR's Summerville Fish Hatchery. Fingerlings were then transported to the Whitehall Fish Lab at the University of Georgia where they were maintained in re-circulating systems supplied with de-chlorinated municipal water. Prior to the experiment, six groups of lake sturgeon were maintained in 3000 L circular tanks. Total water volume was maintained at approximately 2000 L using a 60 cm stand-pipe with a 10% daily exchange of fresh de-chlorinated municipal water. Temperatures were maintained at 20-25°C prior to the start of the experiments. Water was filtered, aerated, and re-circulated to each tank at 100 $mL \cdot s^{-1}$ to establish a turnover rate of 0.18 $exchanges \cdot h^{-1}$.

Experimental setup

To accomplish the objectives, we conducted an experiment exposing sturgeon to incremental increases in temperature. Sturgeon were held at temperatures of 26°C, 29°C, 32°C and 35°C to compare differences in growth, condition, and feeding behavior. A total of 30 lake sturgeon was used – 15 were exposed to dynamic increases in temperature and 15 were held at a constant temperature. The experiment was conducted at the Whitehall Fisheries Research Lab and lasted a total of 63 days.

A completely randomized repeated measures design was used to evaluate the effects of chronic exposure to high temperature on juvenile lake sturgeon (Von Ende 1993). A total of 30 lake sturgeon (~500 days post hatch) was tagged with passive integrated transponders (PIT) and randomly distributed among three randomly assigned to treatment and control groups - to obtain a final density of five fish/tank. Randomization was accomplished by netting six sturgeon individually from a group of 50 sturgeon and placing each in any one of the six treatment tanks. This process was then repeated until five sturgeon had been placed in each treatment tank. The temperature of each treatment tank was maintained within ± 0.7°C of target temperatures by using individually controlled submersible heaters (Clepco Cleveland, Ohio) set to 26, 29, 32 or 35°C. Water quality was monitored weekly with a LaMotte Colorimeter to ensure all parameters were maintained within optimal ranges for juvenile lake sturgeon as described by Timmons et al. (2002). Water temperature (± 0.1°C) was checked at least once daily using a calibrated mercury thermometer. A continuous temperature logger (HOBO, Onset data Corporation © 2005) was used to automatically record water temperature throughout the duration of the experiment.

Experimental protocol

Juvenile lake sturgeon were acclimated to 26°C for seven days prior to the start of the experiment. At the beginning of the experiment, water temperatures in the all tanks were maintained at 26°C then the treatment tanks were increased by 3°C, at a rate of $0.25°C \cdot hr^{-1}$ to reach four different temperature regimes. Sturgeon were maintained at each temperature for two weeks, after which each fish was anesthetized, measured and weighed.

Lake sturgeon were fed a commercially prepared salmonid diet (Rangen Inc. Franklin, Louisianna) at a rate of 2% body weight per day (bwd) (Hung and Lutes 1987; Moore et al. 1988). The feed rate was held constant over the entire experiment, based on the mean body weight of experimental fish at the start of the experiment. Fish were fed once daily. To reduce the chance of experimenter error, tanks were fed in different order each day. After 10 min, uneaten feed in each tank was siphoned and filtered through a 4-mm screen. The reclaimed feed was placed on an aluminum foil tray and dried overnight (14-16 h) at 75°C in a drying oven. Feed consumption was then calculated by subtracting the dried weight of uneaten feed from the daily ration of feed provided to each tank. Preliminary trials showed no significance difference between the initial weight of feed and the weight of feed that had been submerged in water and subsequently dried.

Weight and condition of individual fish in each tank (including both replicates and control groups) were measured every two weeks. To ensure accuracy and precision of individual weight measurements, fish were anesthetized in a buffered solution of 50 $mg \cdot L^{-1}$ tricaine methanesulfonate. After an induction time of five minutes, each individual was removed from the anesthesia, indentified by PIT tag, and measured for total length (± 0.1 cm) and weight (± 0.1 g) before being returned to its experimental tank. At the conclusion of the study, mean percent weight change was calculated for each experimental group. The mean condition of all individuals in each experimental tank was measured using Fulton’s condition factor:

1. $$K = (W \times L^{-3}) \times 10^{a}$$

Where K is condition, W is weight (g), TL is length (mm), and a is a scaling constant (Ricker 1975).

Visual observations of treatment and control groups were made daily so that any dead fish could be removed. Behavioral indications of thermal stress such as erratic swimming, inability to maintain dorso-ventral orientation, and muscular spasms (Becker and Genoway 1979; Ziegweid 2008), were also noted and recorded during these observations.

Statistical Analysis

Mean condition and percent weight change in each control and treatment tank were calculated by averaging the data from the five individual fish within each of the three replicate control and treatment tanks. Mean values of all response variables were calculated for each two week period of the experiment. These values were then analyzed using repeated measures analysis of variance (ANOVA) with Greenhouse-Geisser adjustments to the degrees of freedom (Von Ende, 1993). In growth experiments such as this, using repeated measures ANOVA, a significant treatment effect is indicated by a significant interaction between time and treatment (Von Ende, 1993).

RESULTS

Feed Consumption

The percentage of feed consumed declined in treatment groups, particularly at temperatures at or above the $ULST_{LT}$ (Table 2). Results of ANOVA indicated that feed consumption was significantly affected by time ($F_{1,4} = 140.18$, $p < 0.0001$) and the interaction of time and temperature ($F_{1,4} = 147.24$, $p < 0.0001$). Although feed consumption remained at 94-99% in the control group, it declined by 19-43% at temperatures above the $ULST_{LT}$ (31.7°C) and by 43-81% at temperatures near the CTM (35.1°C; Figure 10).

Growth and Condition

Mean condition of lake sturgeon increased in all experimental groups; however, the rate of increase was lower in treatment groups (Table 2). At the beginning of the experiment mean condition for all fish was 0.30 (SE = 0.01, N = 30). In the control group, condition increased by 12% in the first two weeks; similarly, the treatment groups condition increased by 13%. In contrast, during weeks 5-6 the control group's condition increased by 8% at 26°C compared to only 3% in the treatment groups at 32°C. While the condition in both the treatment and control group increased throughout the experiment the condition of the treatment groups increased at a slower rate than the control groups (Figure 2). The ANOVA results showed that condition was significantly affected time ($F_{1,4} = 77.31$, $p < 0.0001$) and the interaction between time and temperature ($F_{1,4} = 6.01$, $p = 0.0038$).

Weight of the control fish increased steadily throughout the experiment; however, mean weight of treatment fish initially increased, but then declined (Table 2). At the start of the experiment, mean total length of lake sturgeon was 46.7 cm (SE = 0.7, N = 30) with a mean weight of 340.9 g (SE = 16.8, N=30). Not surprisingly, time significantly affected weights of fish ($F_{1,4} = 96.62$, $p < 0.0001$); however, ANOVA results also showed a significant interaction between time and temperature in the groups ($F_{1,4} = 13.78$, $p = 0.0003$; Figure 3).

Stress and Mortality

The treatment groups exhibited behavioral anomalies indicative of thermal stress as water temperatures reached the $ULST_{LT}$ and perished when temperatures neared the CTM. In contrast none of the control fish exhibited signs of thermal stress or perished. As lake sturgeon in the treatment groups neared their lethal tolerance limits (Day 42), most displayed two distinct states of thermal stress. Initially, the sturgeon swam erratically in an attempt to maintain dorso-ventral orientation; however, as time progressed, they lost equilibrium and subsequently could not swim upright. Some lake sturgeon remained in this incapacitated state for 2-5 days and often managed to right themselves to feed. In contrast, fish in the control group did not exhibit visual signs of stress during the same time period. Lake sturgeon in the treatment groups began to perish on day 44 of the experiment when they were exposed to a temperature near their CTM (35.1°C). None of the individuals in groups one or two survived after four days of exposure; in group three none survived after 13 days of exposure. Conversely, none of the fish in the control groups perished.

DISCUSSION

The results of this study show that the use of dynamic incremental increases in water temperature can be used to effectively quantify sub-lethal effects of chronic exposure to temperatures above the $ULST_{LT}$ for sturgeons and, potentially, other rare fishes for which traditional CTM evaluations are not possible or practical. Selong and McHahon (2001) concur that most current thermal tolerance testing methods lack natural heating rates and fail to account for exposure time when quantifying thermal tolerance. In contrast, our dynamic incremental increases in water temperature occurred a rate of $0.25°C \cdot hr^{-1}$, which is slower than other testing methods (Currie et. al. 1998; Beitinger et. al. 2000; Chung 2001). Additionally, the incremental temperature increase in our experiment allowed us to estimate the effect of exposure time on thermal tolerance.

Furthermore, the incremental heating rate used in this study eliminated potential biases associated with differential heating of water and fish – a major drawback of traditional CTM methods that use rapid continuous heating. Becker and Genoway (1979) emphasized that this potential bias limits thermal tolerance studies to larval or fingerling life stages. Unfortunately, the results presented in Chapter 2 largely support the previous findings of Young and Cech (1996) who show that thermal tolerance of some fishes declines in older life stages. Consequently, evaluations of the thermal tolerances in older fishes are notably absent from the literature. Concerns about the bias of rapid heating rates towards larger-bodied fish can be addressed by using a slower heating rate (Becker and Genoway 1979). In this study a relatively slow heating rate of $0.25°C \cdot hr^{-1}$ was used to minimize any potential bias caused by differential warming of test fish and tank water.

Another limitation of conventional CTM testing methods stems from their reliance on lethal endpoints which may not be practical for threatened or endangered species (Becker and Genoway 1979; Currie et. al. 1998; Beitinger et. al. 2000; Ziegweid et al. 2007). The results of this study showed sub-lethal effects were clearly evident at temperatures between the $ULST_{LT}$ and the CTM – long before the lethal endpoint was reached. Consequently, we suggest that future studies assessing sub-lethal effects of thermal stress need not include lethal endpoints provided that a repeated measures design is used to identify significant treatment effects over time. In any repeated measures growth experiment, time (as an independent variable) is expected to significantly affect treatment groups and is therefore not an indication of a treatment affect. A true treatment effect occurs only when control and treatment groups react differently over time. Hence, a significance treatment effect is indicated by a significant time and treatment interaction rather than a main effect of treatment (Von Ende 1993). In this study the repeated measures of our control groups were clearly not independent and our data did not meet the assumptions of sphericity of covariances. However, by using the Greenhouse-Geisser adjustments to the degrees of freedom (Von Ende 1993) we accounted for these two violations.

As such, the results of this study have important implications for studies of thermal tolerance in fish. Although conventional CTM methods are useful in defining lethal tolerance limits for different populations and species (Becker and Genoway 1979), the protocol used in this experiment provides a new methodology for assessing sub-lethal effects of chronic thermal stress while quantifying ontogenic shifts in thermal tolerance.

From an ecological viewpoint, our results show that juvenile lake sturgeon exposed to prolonged periods of water temperatures at or above the $ULST_{LT}$ are likely to consume less food, lose weight, and experience diminished condition and possibly, higher mortality. In all three replicate treatments, juvenile lake sturgeon began to lose weight as temperatures exceeded the $ULST_{LT}$ (31.7°C). Furthermore, the rate of weight loss continued to accelerate as temperatures neared the CTM (35.1°C). In contrast to the results of the acute CTM trials discussed in Chapter 2, lake sturgeon in this experiment died after prolonged exposure to their CTM (35.1°C), and many fish exhibited behavioral indications of thermal stress after only one week of exposure to temperatures above the $ULST_{LT}$ (31.7°C). The significant interaction of time and temperature evident from the ANOVA analysis suggests that the longer the fish were exposed to temperatures above the $ULST_{LT,}$ the more severe these effects became.

Not only do the findings from this study demonstrate the inadequacy of traditional CTM testing methods, they also have important implications for the restoration of lake sturgeon populations at the southern margin of their range. In recent years several southern states, including Missouri, Tennessee, Kentucky and Georgia, have initiated long-term reintroductions in an effort to reestablish lake sturgeon populations in their native habitats (Peterson et. al. 2007). Ultimately, the success of these reintroductions will depend on the reproductive success of stocked individuals (Schram et. al. 1999). Although many environmental variables can affect the spawning success of lake sturgeon, adult condition is among the most important (Harkness and Dymond 1961; Priegel and Wirth 1971). Studies of other Acipenserids have shown that both fecundity and egg size increase as a function of female size and condition (Van Eenennaam 1996; Van Eenennaam and Doroshov 1998). Consequently, any reduction in growth or condition of stocked sturgeon resulting from chronic thermal stress could significantly hinder restoration efforts.

With regard to juvenile lake sturgeon, further studies are needed to better understand how chronic exposure to water temperatures above the $ULST_{LT}$ may ultimately affect long-term growth and survival in the wild. The thermal tolerance thresholds identified in this study suggest a range of upper tolerance levels for lake sturgeon held under laboratory conditions. However, growth, condition and, ultimately, survival of wild lake sturgeon populations are dependent on a number of other habitat variables in addition to temperature. As such, future studies are needed to better understand how chronic exposure to water temperatures above the $ULST_{LT}$ may ultimately affect growth, survival and reproduction of lake sturgeon in the wild.

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Table 1. - Lethal thermal maxima (LTM), lethal upper limits of safe tolerance ($ULST_{LT}$; + SE), critical thermal maxima (CTM), critical upper limits of safe tolerance ($ULST_{CT}$; + SE), final thermal preferendum (FTP), and thermal growth optima (TGO) of juvenile lake sturgeon. Fish from two different age groups and were acclimated to one of two temperatures prior to thermal tolerance testing. Both the CTM and LTM differed significantly across ages ($p = 0.0041$, $p = 0.0024$) and acclimation temperatures ($p < 0.0001$, $p < 0.0001$).

Temp (°C)	Age	N	LTM (°C)	$ULST_{LT}$	N	CTM (°C)	$ULST_{CT}$	FTP	TGO
18.1	200 DPH	15	35.0 (.1)	30.0	15	33.2 (.2)	28.2	25.4	25.5
18.1	400 DPH	15	34.8 (.1)	29.8	13	33.1 (.2)	28.1	25.3	25.4
24.9	200 DPH	15	36.8 (.1)	31.8	15	35.7 (.1)	30.7	29.2	28.8
24.9	400 DPH	14	36.7 (.1)	31.7	9	35.1 (.2)	30.1	28.3	28.0

Table 2. - Treatment groups (T; N = 3) were exposed to temperatures of 26, 29, 32 and 35°C for two wks. Control groups (C; N = 3), shown for comparison, were continually maintained at 26°C. Mean and standard error of weight increase, condition and feeding rate are reported for each two week period.

	Day 14		Day 28		Day 42		Day 56	
	C (26°C)	T (26°C)	C (26°C)	T (29°C)	C (26°C)	T (32°C)	C (26°C)	T (35°C)
Weight Increase (%)	26.9(1.2)	23.7(0.5)	18.6(0.6)	11.3(0.7)	14.7(0.5)	0.8(1.4)	14.6(1.2)	-3.7(2.3)
Condition (g·mm^{-3})	0.35(0.01)	0.33(0.01)	0.37(0.01)	0.34(0.01)	0.40(0.01)	0.35(0.01)	0.4(0.01)	0.35(0.01)
Feeding Rate (%)	92.4(1.2)	87.2(1.4)	98.0(0.5)	90.0(0.5)	98.6(0.5)	65.0(3.1)	97.0(1.1)	16.0(4.9)

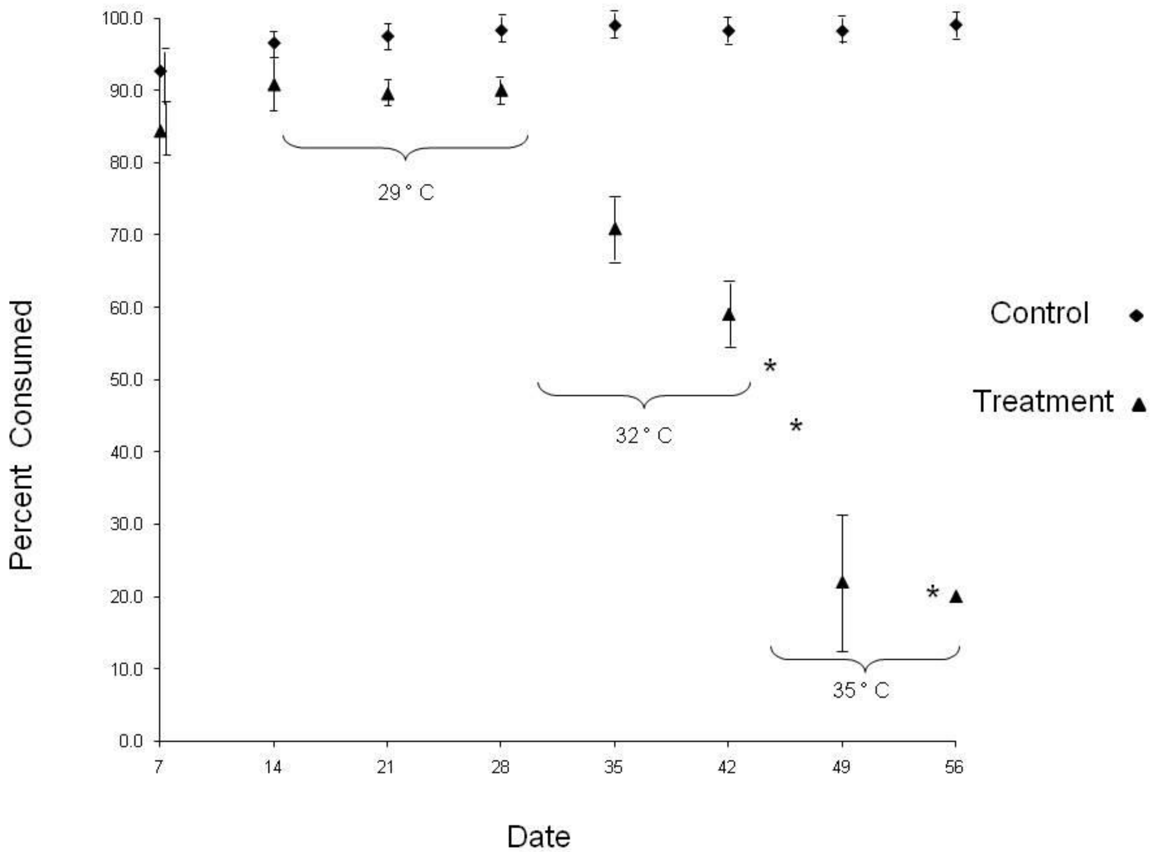

Figure 1. - Changes in mean (+ SE) weekly feed rate of juvenile lake sturgeon held at a constant temperature of 26°C (N=3) versus an incremental increase to 35°C (N=3). Brackets indicate incremental temperature increases in the treatment groups. Asterisks indicate 100% mortality within each replicate treatment group.

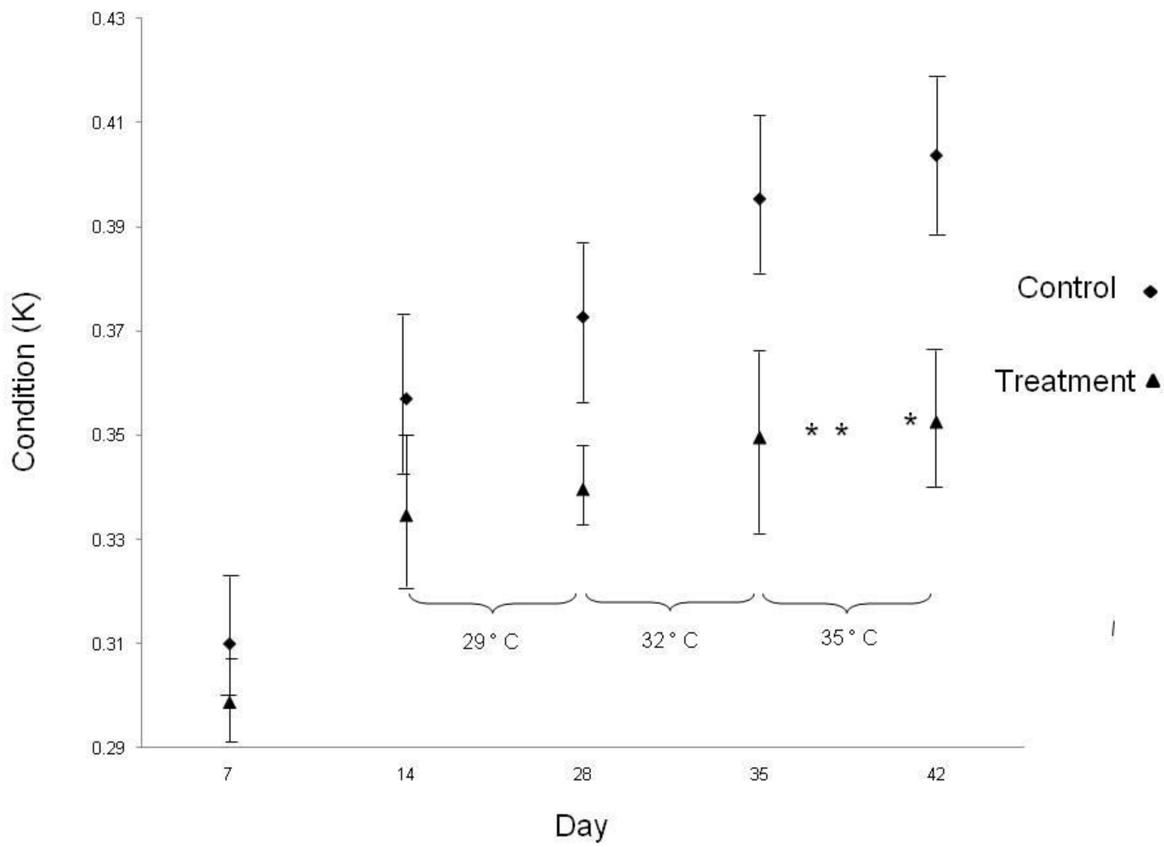

Figure 2. - Changes in mean (+ SE) condition of juvenile lake sturgeon held at a constant temperature of 26°C (N=3) versus an incremental increase to 35°C (N=3). Brackets indicate incremental temperature increases in the treatment groups. Asterisks indicate 100% mortality within each replicate treatment group, at which time final condition was calculated. Unless otherwise indicated, temperatures were maintained at 26°C.

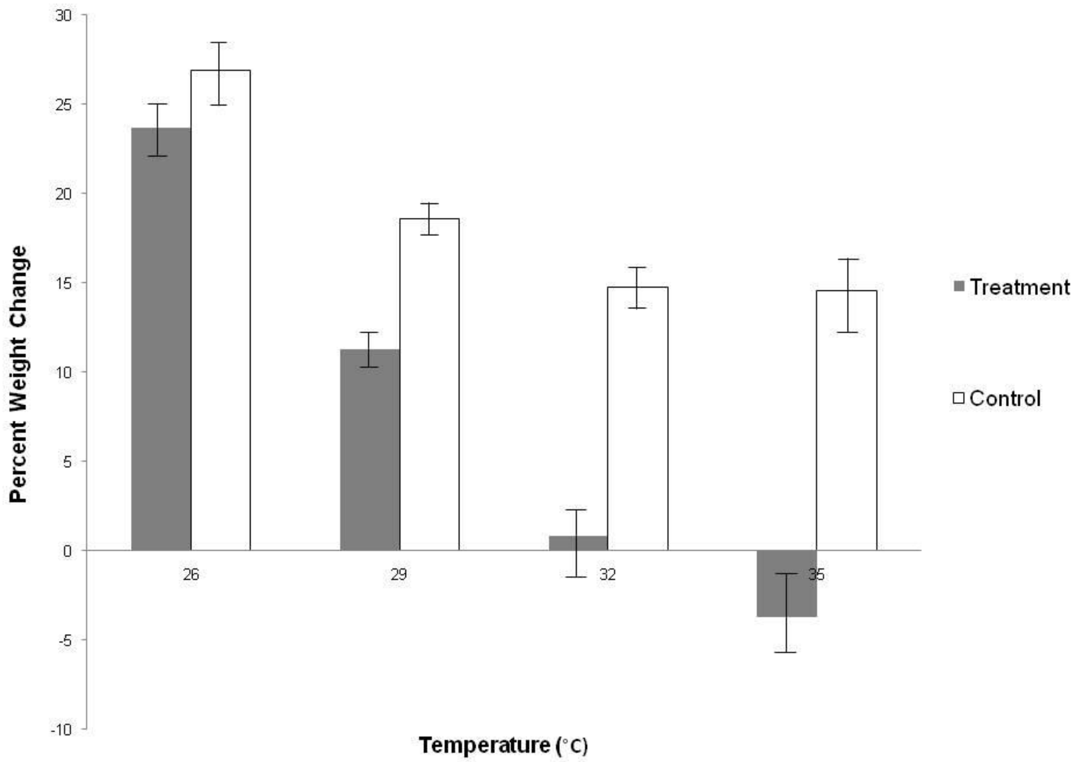

Figure 3. - Changes in mean (+ SE) weight change of juvenile lake sturgeon held at a constant of 26 °C (N=3) and incrementally increasing temperatues (35°C [N=3]). Control groups were maintained at 26°C for the entirety of the experiment. The final weight gain percentage estimate was obtained by weighing the fish immediately after death and does not represent a full two week period.