ONTOGENETIC CHANGES IN SALINITY AND TEMPERATURE TOLERANCES OF YOUNG-OF-THE-YEAR SHORTNOSE STURGEON, *Acipenser brevirostrum*

by

Jeffrey Roy Ziegeweid

(Under the direction of Cecil A. Jennings)

ABSTRACT

Degraded water quality may affect recruitment of juveniles to populations of shortnose sturgeon, *Acipenser brevirostrum*, in the southeastern United States. However, field data for young-of-the-year (YOY) shortnose sturgeon are limited. Therefore, little information exists about the physiological capabilities of YOY shortnose sturgeon. I conducted LC_{50}, factorial, and thermal maxima experiments to examine ontogenetic changes in salinity and temperature tolerances of YOY shortnose sturgeon. In LC_{50} experiments, salinity and temperature tolerances increased with increasing size. Results of factorial experiments were used to generate a predictive survival model based on salinity (ppt), temperature (°C), and weight (g). Plasma osmolalities and hematocrit values of survivors indicated significant sublethal effects of salinity and temperature ($p < 0.0001$). Thermal maxima experiments demonstrated that acclimation temperature significantly affected temperature tolerances ($p < 0.0001$). Results can be used to identify critical YOY habitats and mitigate further disturbances to rivers in the southeastern United States.

INDEX WORDS: Fish, Shortnose sturgeon, *Acipenser brevirostrum*, YOY, Ontogenetic, Salinity, Temperature, Weight, Body size, Endangered species, Survival, Mortality, LC_{50}, Thermal maximum, Plasma osmolality, Hematocrit

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Populations of shortnose sturgeon, *Acipenser brevirostrum*, in the southeastern United States have been extirpated or severely depleted in recent years (Collins et al. 2000). Efforts to manage remaining populations of shortnose sturgeon have been difficult because of its complex life history and amphidromous lifestyle. In the southeastern United States, degraded water quality of nursery habitats is one of many factors that are believed responsible for a decline in recruitment of juveniles, which negatively affects shortnose sturgeon populations (Smith and Collins 1996 Collins et al. 2000). Further, a lack of field data on young-of-the-year (YOY) shortnose sturgeon has resulted in a poor understanding of physiological habitat requirements of YOY individuals in rivers of the southeastern United States.

Salinity and temperature are two environmental variables that concern managers of sturgeon populations in southeastern rivers. Historical data suggests that in the past 30 years, salinities in several Georgia estuaries have increased, possibly as a result of reductions in river discharges (Alber and Sheldon 1999a,b). Reductions in river discharges are attributed to hydrologic alterations and removal of water for agricultural, industrial, and domestic purposes (Baxter 1977, Dynesius and Nilsson 1994, Lydeard and Mayden 1995, Poff et al. 1997, Bunn and Arthington 2002, Pringle 2003). Furthermore, bioenergetics models of the sympatric Atlantic sturgeon, *Acipenser oxyrinchus* demonstrate that juveniles undergo a three-way habitat squeeze (dissolved oxygen, salinity, and temperature) in summer conditions, with temperature being the most important factor limiting suitable habitat (Niklitschek and Secor 2005). Therefore,

understanding the physiological limitations of sturgeons in their early life stages may allow managers to develop strategies to improve recruitment of sturgeon in southeastern rivers.

In the two papers presented in this thesis, I used artificially propagated fish to investigate the ontogenetic changes in salinity and temperature tolerances of YOY shortnose sturgeon. In the first paper, I used the results of dose-response experiments to estimate salinity and temperature LC_{50} values for fish ranging from 56 to 128 mm in total length (TL). Results of the LC_{50} experiments were used to design a factorial experiment that examined the interactive effects of salinity and temperature on YOY survival. Data collected in 2005 were used to design further factorial experiments that were conducted in 2006. I used combinations of four salinities and three temperatures to further examine the interactive effects of salinity and temperature on the survival of YOY shortnose sturgeon. Survival data were used to construct a predictive survival model based on salinity, temperature, and fish weight. Physiological conditions of surviving fish were evaluated using hematocrit and plasma osmolality values.

In the second paper, I evaluated and expanded upon the temperature data of the first paper. I used thermal maximum experiments conducted with artificially propagated fish to examine how temperature tolerances of YOY shortnose sturgeon change with changes in acclimation temperature (Becker and Genoway 1979, Young and Cech 1996). Furthermore, I then used thermal maximum data to estimate upper limits of safe temperature (Bridges 1971, Young and Cech 1996), thermal preferences, and optimum growth temperatures for YOY shortnose sturgeon (Jobling 1981, Young and Cech 1996). Upon completion of the study, total length, weight, and hematocrit levels of the fish at each acclimation temperature were measured to evaluate the physiological condition of the fish. Results of both papers will assist natural resource agencies in identifying and maintaining critical habitat of YOY shortnose sturgeon in southeastern rivers.

BACKGROUND

Shortnose sturgeon are among the most primitive and ancient of the bony fishes and have maintained several ancestral characteristics for over 150 million years (Kieffer et al. 2001). Such characteristics include protective scutes, a largely cartilaginous skeleton (Vladykov and Greely 1963), a physostomous swim bladder, a notochord, a spiral valve intestine, rostral chemosensory barbels, and a shark-like heterocercal tail (Scott and Crossman 1973, Dadswell et al. 1984).

Harvesting of shortnose sturgeon led to dramatic population declines across the entire range of the species, from the Saint John River, Canada, to the St. Johns River, Florida (Vladykov and Greely 1963). Shortnose sturgeon were harvested for their flesh and caviar beginning in the mid-1800s (Collins and Smith 1993). A commercial fishery for shortnose sturgeon began in the 1870s, and by the early twentieth century, overfishing had drastically reduced shortnose sturgeon populations (Dadswell et al.1984). Population declines led to the listing of shortnose sturgeon as federally endangered under the Endangered Species Act in 1973 (Kynard 1997). However, the damming of spawning rivers, pollution, habitat loss, dredging of nursery areas, diminished water quality, commercial bycatch, and poaching all contribute to the continued decline of shortnose sturgeon populations (Collins et al. 1996; Collins et al. 2000; Collins et al. 2003).

PHYSIOLOGY AND ECOLOGY

Although shortnose sturgeon reach similar lengths (120 cm) and weights (24 kg) across their geographic range, there are distinct differences between populations north and south of Chesapeake Bay (Kynard 1997). In northern populations, shortnose sturgeon mature later and live longer than shortnose sturgeon of southern populations (Kynard 1997). The specific reason for these differences is unknown, but differences are generally attributed to a combination of

genetic divergence, climatic differences, and differences in habitat and food availabilities (Kynard 1997). Southern populations exhibit greater genetic divergence among rivers compared to northern populations (Wirgin et al. 2005).

Shortnose sturgeon are amphidromous. They spawn in freshwater and spend the majority of the year in fresh or brackish waters of their natal river estuaries (Kynard 1997). Shortnose sturgeon in southern populations typically begin upriver spawning migrations in mid-February, although only a portion of the population participates in the upriver migration (Collins and Smith 1993). Shortnose sturgeon spawn in the fast water (about 82 cm/s) of channel curves (Hall et al. 1991), at depths of approximately 2.1-13 m (Collins et al. 2002). Cobble or other hard substrates are required to provide attachment surfaces for eggs (Dadswell et al. 1984).

Spawning periods of shortnose sturgeon vary with latitude (Dadswell et al. 1984, Gilbert 1989). Reproductively mature shortnose sturgeon in colder northern rivers spawn later than their counterparts in warmer southern rivers, and in northern populations, shortnose sturgeon also occupy freshwater habitats for longer periods than in southern populations. In northern populations, hatched YOY remain on or near the spawning grounds for about one month following the spawning period (Kynard 1997), but little is known about the habitat selection of YOY sturgeon in southern rivers (Kynard 1997). However, juvenile shortnose sturgeon > 1 year old typically remain in the freshwater/brackish water interface upriver of the estuary (Hall et al. 1991).

Sturgeon are demersal, benthic omnivores that forage for insects, crustaceans, and small fishes along the sediment/water interface. Juveniles are non-selective feeders, whereas adults are selective feeders (Kynard 1997). Sturgeon also exhibit a high degree of solitary foraging behavior during feeding (Carlson and Simpson 1987). Foraging habitat use is regulated by a

size-dependent dominance hierarchy, which could be a behavioral mechanism developed to control density and emigration relative to resource abundance (Kynard 1997).

HABITAT AND POPULATION MODIFICATIONS

Habitat degradation and by-catch continue to occur for all sturgeon populations on the Atlantic coast. In North Carolina, expansion of the hog-farm industry has accelerated the decline in water quality (Collins et al. 2000). In other rivers along the Atlantic coast, alterations of rivers, such as the construction of dams and the dredging of channels for commercial navigation, alter the natural flow regime and diminish water quality. Sturgeon bycatch mortalities from commercial shrimp and shad fisheries further add to the problems caused by habitat degradation (Collins et al. 1996). Many of the alterations for commercial navigation occur in the brackish estuary, an important nursery habitat for juvenile shortnose sturgeon (Hall et al. 1991).

Loss of nursery habitats has resulted in poor recruitment of juveniles in several populations of shortnose sturgeon in the southeastern United States (Smith and Collins 1996). However, in an effort to improve recruitment in the Savannah River, the U.S. Fish and Wildlife Service and the South Carolina Wildlife and Marine Resources Department implemented a shortnose sturgeon stocking program in 1984 (Smith and Collins 1996). Thousands of hatchery-reared shortnose sturgeon were stocked throughout the Savannah River from 1985-1992. Age of stocked fish ranged from 41 to 1,698 days post hatch (dph). Although some of the stocked fish remained and survived in the Savannah River, some appeared in other systems, including the Edisto, Ogeechee, and Cooper rivers (Smith et al. 2002). Stocked fish also appeared in Winyah Bay, South Carolina, which is about 280 km north of the Savannah River (Smith et al. 2002).

Recapture of stocked fish indicates that they have matured and may be augmenting spawning populations (Smith and Collins 1996). However, recruitment of juveniles has not increased, which suggests a recruitment bottleneck (Collins et al. 2002). Such a bottleneck may result from several factors, including degraded water quality, loss of suitable habitat, or reduced prey availability.

YOUNG-OF-THE-YEAR

Little is known about the behavior, movements, and habitat use of YOY shortnose sturgeon, particularly in southeastern river systems. Following yolk-sac absorption, larvae (~ 20 mm TL) drift downstream for about two days. This behavior may act to disperse larvae from spawning grounds and help them find suitable cover (Richmond and Kynard 1995, Bain 1997). Larvae complete the metamorphosis into juveniles at about 31.5 mm TL (Gilbert 1989). Thereafter, YOY use deep water areas and forage in channel habitats with mud or sand substrate (Pottle and Dadswell 1979, Carlson and Simpson 1987). However, these studies primarily involved northern populations of shortnose sturgeon, and behavioral and physiological differences exist between northern and southern populations of shortnose sturgeon (Kynard 1997).

Little is known about habitat selection of YOY in southern river systems, especially with regard to salinity and temperature. Studies of YOY in southern populations have been restricted to laboratory investigations of dissolved oxygen and salinity (Jenkins et al. 1993; Campbell and Goodman 2004). Field data concerning YOY are limited.

SALINITY

The amphidromous life history of shortnose sturgeon makes salinity a variable of major concern for shortnose sturgeon managers in the southeastern United States. Anthropogenic activities, such as dam operation, dredging, and water diversion for industrial and agricultural purposes reduce river discharges and allow further upstream saltwater intrusion, (Baxter 1977, Dynesius and Nilsson 1994, Lydeard and Mayden 1995, Poff et al. 1997, Alber and Sheldon 1999a,b, Bunn and Arthington 2002, Pringle 2003). Saltwater intrusion may physiologically limit available habitat of shortnose sturgeon in the southeastern United States. For example, in a recent study of the Altamaha River, Georgia, shortnose sturgeon were not captured below the fresh-saltwater interface when water temperatures exceeded 27 °C (DeVries 2006). Furthermore, summertime upstream movements of tagged fish into deep freshwater habitats coincided with the progressive intrusion of the salt wedge (DeVries 2006).

Previous studies involving salinity and sturgeon have demonstrated similar results among sturgeon species. Survival of white sturgeon, *Acipenser transmontanus*, shortnose sturgeon, *Acipenser brevirostrum*, and Adriatic sturgeon, *Acipenser naccarii*, juveniles in acute salinity exposures increased with increasing size, and salinity tolerances were similar among species at similar sizes (McEnroe and Cech 1985, Jenkins et al. 1993, Cataldi et al. 1999). Studies of chronic salinity exposure demonstrate reductions in growth, feed conversion efficiency, and ability to survive stressful disturbances at high salinity for juvenile Adriatic and shortnose sturgeons (McKenzie et al. 1999, Jarvis et al. 2001). These results suggest that shortnose sturgeon may enter brackish water for favorable foraging habitats and more tolerable thermal regimes (Jarvis et al. 2001).

TEMPERATURE

Temperature strongly affects the behavior and may define critical habitat of sturgeon. Temperature fluctuations trigger spawning activity, affect the development of fertilized eggs (Hardy and Litvak 2004), and influence the seasonal movements of shortnose sturgeon between freshwater and brackish habitats (Hall et al. 1991, Collins and Smith 1993, Kynard 1997, Collins et al. 2002, DeVries 2006). However, the direct effects of temperature on sturgeon survival are rarely examined (Mayfield and Cech 2004). Typically, effects of temperature on sturgeon survival are examined in conjunction with other water quality parameters.

Temperature and dissolved oxygen are negatively correlated, and for this reason, combined effects of high temperatures and hypoxia are examined. For example, hypoxia and high temperatures resulted in increased mortality, reduced growth rates, and reduced swimming activity in white sturgeon (Cech et al. 1984, Crocker and Cech 1997). Juvenile Atlantic sturgeon, *Acipenser oxyrinchus*, exposed to hypoxia and high temperatures had decreased respiration rates, and a 6 °C increase in temperature (20 to 26 °C) resulted in a 65.5% decrease in survival (from 78 to 12.5%) in hypoxic conditions (Secor and Gunderson 1998).

Direct effects of temperature have been most thoroughly examined in green sturgeon, *Acipenser medirostris* (Mayfield and Cech 2004, Allen et al. 2006). Larval green sturgeon grew better at higher temperatures but were more susceptible to handling mortality (Allen et al. 2006). The effects of temperature on several bioenergetics variables were measured for juvenile green sturgeon (Mayfield and Cech 2004), and Mayfield and Cech (2004) also used thermal preference data to estimate lethal temperatures for green sturgeon (Jobling 1981). However, similar thermal tolerance data does not currently exist for shortnose sturgeon.

DISSOLVED OXYGEN

Dissolved oxygen concentration can significantly affect the physiological well-being and habitat availability of shortnose sturgeon in southern rivers. Although dissolved oxygen concentrations are high (6-8 mg/L O_2) during fall, winter, and spring seasons, decreasing flow and increasing water temperatures during summer months can drastically lower dissolved oxygen concentrations in southeastern rivers (Collins et al. 2000). Such degraded water quality can significantly affect the survival and growth of fish populations, particularly juveniles. Previous studies have demonstrated that significant mortalities occur when dissolved oxygen concentrations fall below 3.0 mg/L (Jenkins et al. 1993, Campbell and Goodman 2004). Results were similar for fish of varying sizes exposed to various temperatures and salinities (Jenkins et al. 1993, Campbell and Goodman 2004).

In general, the ancient physiological characteristics of sturgeon may result in reduced efficiency of respiration, especially when compared to other teleosts (Secor and Niklitschek 2001). Such traits include less efficient gill ventilation, low cardiac performance, and lower affinity of hemoglobin for oxygen (Secor and Niklitschek 2001). During periods of low dissolved oxygen, sturgeon may experience reductions in growth, food consumption, and routine metabolism. These effects are further exacerbated by increasing temperatures and may become lethal, particularly to juveniles (Secor and Niklitschek 2001).

BLOOD ANALYSIS

Blood variables, such as hematocrit and plasma osmolality, are used to assess physiological responses to environmental stress (Handy and Depledge 1999). Hematocrit measures the percentage of red blood cells in the blood. Red blood cells contain hemoglobin and deliver

oxygen throughout the body. Environmental stress, such as changes in temperature or pH, may alter the oxygen requirements of fish and affect the oxygen affinity of the red blood cells. Changes in oxygen requirements or in the oxygen affinity of the red blood cells may alter the production of red blood cells (Riggs 1970). Therefore, hematocrit levels can be used as biomarkers to assess physiological responses to environmental stress (Handy and Depledge 1999).

Plasma osmolality is a measure of the ions in blood. Fish regulate plasma osmolality based on their surrounding environment (Moyle and Cech 1988). Freshwater environments contain low ion concentrations relative to the internal organs of fish, so fish in freshwater minimize plasma osmolality to reduce the osmotic gradient between their bodies and the surrounding environment. Increases in salinity increase the ion concentration of the water, and if the ion concentration of the water is higher than the internal ion concentration of a fish, a fish will increase its plasma osmolality to minimize the osmotic gradient between its body and the surrounding water (Riggs 1970). Therefore, plasma osmolality can be used to assess physiological responses of fish to changing salinity concentrations.

Blood analyses have been used to assess the physiological responses of sturgeons to changing abiotic factors such as temperature and salinity. In chronic exposures, a return of plasma osmolalities to freshwater levels demonstrated acclimation to increased salinities for both Adriatic and shortnose sturgeons (McKenzie et al. 1999, McKenzie et al. 2001, Martinez-Alvarez et al. 2002, Jarvis and Ballantyne 2003). However, studies involving Siberian sturgeon, *Acipenser baeri*, and lake sturgeon, *Acipenser fulvescens*, demonstrated increasing plasma osmolalities with increasing salinity (LeBreton and Beamish 1998, Rodriguez et al. 2002). Furthermore, shortly after initial exposure, plasma osmolalities were similar among sturgeon

species for fish of similar size that were exposed to similar salinities (LeBreton and Beamish 1998, McKenzie et al. 1999, McKenzie et al. 2001, Martinez-Alvarez et al. 2002, Jarvis and Ballantyne 2003).

HYDROLOGIC ALTERATIONS

BACKGROUND

Anthropogenic activities, such as the operation of dams, have severely altered the natural flow regimes of most rivers and negatively affect riverine ecosystems. Negative effects of dam operation include the dampening of flow pulses necessary for many benthic invertebrates to complete their life cycles and upstream retention of nutrients and detritus necessary for downstream productivity (Baxter 1977, Dynesius and Nilsson 1994, Poff et al. 1997, Bunn and Arthington 2002). Other negative effects of dams include sedimentation (Baxter 1977, Lydeard and Mayden 1995, Poff et al. 1997), loss of silica to coastal food webs (Pringle 2003), alteration of thermal regimes (Baxter 1977, Poff et al. 1997, Bunn and Arthington 2002, Pringle 2003), increased bioaccumulation of contaminants (Pringle 2003), and creation of physical barriers to migratory fish species (Baxter 1977, Bunn and Arthington 2002, Pringle 2003).

In addition to dams, other anthropogenic disturbances further alter flow regimes and damage riverine ecosystems. Dredging can destroy benthic habitats and increase sedimentation. Thermal effluents and water withdrawls for waste treatment, agriculture, and drinking water contribute to changes in the thermal regimes of many rivers (Baxter 1977, Poff et al. 1997, Bunn and Arthington 2002, Pringle 2003). Water removal, dredging, and the construction of dams also can reduce discharges (Baxter 1977), further increasing upstream saltwater intrusion in coastal rivers (Baxter 1977, Alber and Sheldon 1999a,b), changing thermal regimes (Baxter 1977, Poff

et al. 1997, Bunn and Arthington 2002, Pringle 2003), and reducing dissolved oxygen concentrations (Baxter 1977, Bunn and Arthington 2002). Industrial effluents may increase the biological oxygen demand, which reduces available oxygen for aquatic biota (Baxter 1977). All of these factors alter both the composition and the timing of emergence for aquatic biota at all levels of the aquatic food web, damaging native species and allowing for easier colonization by invasive species (Lydeard and Mayden 1995, Bunn and Arthington 2002, Pringle 2003).

Historical water quality data indicates that salinities in several Georgia estuaries have substantially increased since 1972, possibly resulting from decreases in surface water discharge (Alber and Sheldon 1999a,b). Human alterations to channel morphology may further alter salinity gradients in many estuaries. Increases in surface water withdrawal may decrease discharge, contributing to increased salinities in estuaries (Alber and Sheldon 1999 a,b).

EFFECTS ON STURGEON

The complex migratory life histories and primitive nature of sturgeons probably make them susceptible to changes in the timing of both biotic and abiotic conditions. Bioenergetics modeling of juvenile Altantic sturgeon in Chespeake Bay suggests a 3-way habitat squeeze summer conditions, which is created by low dissolved oxygen, high salinities, and high temperatures, with temperature being the key factor defining habitat availability. In fact, a 1°C bay-wide temperature increase resulted in a 65% reduction in suitable habitat for juvenile Atlantic sturgeon (Niklitschek and Secor 2005). In Chesapeake Bay, there have been historical losses of thermal refugia, and most thermal refuges for juveniles were found down-estuary in brackish water. However, large fractions of thermal refuges are unsuitable because of dissolved oxygen and salinity thresholds (Niklitschek and Secor 2005). Similar habitat use patterns were

observed in the Hudson River, with most Atlantic sturgeon juveniles using brackish areas with lower temperatures and higher dissolved oxygen concentrations than upstream areas (Haley 1999).

Several studies have examined the effects of hydrologic alterations on white sturgeon (Sullivan et al. 2003, Geist et al. 2005, Snyder and Minshall 2005). In the Brownlee Reservoir, Idaho, decreases in suitable habitat caused by impoundment have resulted in crowding of white sturgeon in suitable habitats (Sullivan et al. 2003). Some of the habitat loss is attributed to thermal stratification within the reservoir, which causes anoxic benthic conditions. In the Snake River, water temperature has the greatest effect on movement, oxygen consumption, and swimming speed of white sturgeon, which are often found at locations with low, stable bottom velocities (Geist et al. 2005). Finally, on the Koontenai River, Idaho, upstream retention of nutrients is resulting in food limitation downstream of the dam. This food limitation is causing white sturgeon population declines in the Koontenai River (Snyder and Minshall 2005).

Recent studies have examined the distribution of gulf sturgeon (*Acipenser oxyrinchus desotoi*) in the Suwannee River in relation to prey availability. Prey of juvenile gulf sturgeon are significantly more abundant in the saline estuary than above the salt wedge, and based on search patterns, prey density and prey patch size may be more important than prey biomass for juvenile gulf sturgeon (Brooks and Sulak 2005). Distribution of gulf sturgeon also varies seasonally in relation to prey abundance. In the fall, gulf sturgeon are associated with cold, sandy, saline areas containing high abundance of benthic prey. However, in the spring, gulf sturgeon were concentrated in warm, less saline water with an abundance of brachiopods before beginning their upriver migrations in to freshwater (Harris et al. 2005).

Studies examining the effects of hydrologic alterations, such as the previously mentioned studies of white sturgeon, do not currently exist for shortnose sturgeon. However, the need for such models is evident. Loss of suitable freshwater habitat and decreases in benthic fauna could mean that juvenile shortnose sturgeon are now reaching brackish estuaries at earlier ages and/or at smaller sizes than have been previously anticipated (Kynard 1997). Furthermore, increasing salinities farther upriver could make current nursery habitats of YOY shortnose sturgeon unsuitable, thus forcing them into other suboptimal habitats and making them more susceptible to predation, disease, and competition (Collins et al. 1996). Therefore, establishing physiological bounds of salinity and temperature for several size classes of YOY shortnose sturgeon is important.

OBJECTIVES

The primary goal of this study was to identify physiological limits of YOY shortnose sturgeon based on salinity and temperature thresholds. I accomplished the primary goal through the fulfillment of five specific objectives.

1) I confirmed current salinity threshold information for YOY shortnose sturgeon (Jenkins et al., 1993).
2) I established acute lethal temperature thresholds for YOY shortnose sturgeon.
3) I determined the combined effects of salinity and temperature on survival of YOY shortnose sturgeon.
4) I quantified changes in salinity and temperature tolerances with increasing size.
5) I documented the physiological responses of YOY sturgeon to sub-lethal thermal and osmoregulatory stress by measuring hematocrit and plasma osmolality of surviving fish.

CHAPTER 2

SURVIVAL OF JUVENILE SHORTNOSE STURGEON IN RELATION TO SALINITY, TEMPERATURE, AND BODY SIZE[1]

[1] Ziegeweid, J.R., C.A. Jennings, D.L. Peterson, and M.C. Black. To be submitted to *Transactions of the American Fisheries Society*.

ABSTRACT

Hydrologic alterations of rivers in the southeastern United States may degrade habitats of young-of-the-year (YOY) shortnose sturgeon and reduce recruitment. However, little is known about habitat use of YOY. In this study, data from two years of experiments were used to examine ontogenetic changes in salinity and temperature tolerance for YOY shortnose sturgeon. Results of first year experiments were used to estimate LC_{50} values for temperature and salinity, ranging from 28.2-30.7 °C and 14.8-20.9 ppt for fish 56-128 mm TL. Factorial experiments demonstrated that salinity and temperature significantly interact to affect YOY survival ($p < 0.0001$). Salinities ranged from 0-24 ppt, temperatures ranged from 23-31 °C, and fish weights ranged from 0.4-42.8 g. Results were used to generate a predictive survival model based on salinity, temperature, weight, and all variable interactions. Hematocrit and plasma osmolality values were determined from blood samples of surviving fish. Hematocrit levels varied significantly with temperature and age ($p < 0.0001$), and plasma osmolalities varied significantly with salinity and age ($p < 0.0001$). Results of this study may be used to identify suitable habitats for YOY shortnose sturgeon and to mitigate future modifications to rivers in the southeastern United States.

INTRODUCTION

Populations of shortnose sturgeon, *Acipenser brevirostrum*, are severely depleted throughout the species' native range, from the Saint John River in New Brunswick, Canada to the St. Johns River, Florida (Vladykov and Greeley 1963). Commercial fishing in the late 19th century decimated most shortnose sturgeon populations (Dadswell et al. 1984) and led to federal protection of the species under the Endangered Species Act (Kynard 1997). However, many

populations remain severely depleted (Collins et al. 2000), partly because the complex life history, including amphidromy, make shortnose sturgeon difficult to manage. Degradation of both habitats and water quality is believed to contribute to reduced juvenile recruitment (Smith and Collins 1996, Collins et al. 2000). However, a lack of field data has limited knowledge of habitat requirements for young-of-the-year (YOY) shortnose sturgeon (Kynard 1997).

Anthropogenic modifications to river and estuarine habitats continue to degrade water quality and alter the timing of many ecological processes (Baxter 1977, Dynesius and Nilsson 1994, Poff et al. 1997, Bunn and Arthington 2002). Hydrologic alterations, such as the construction of dams, dredging, thermal pollution, and water removal for waste treatment, agriculture, and drinking water all reduce river discharges (Baxter 1977), alter thermal regimes (Baxter 1977, Lydeard and Mayden 1995, Poff et al. 1997, Bunn and Arthington 2002, Pringle 2003), and allow further upstream saltwater intrusion in coastal rivers (Baxter 1977, Alber and Sheldon 1999a,b), particularly during reduced summer flows. Historical salinity data indicates that salinities in several Georgia estuaries have increased substantially since 1972, possibly resulting from decreases in surface water discharge and human modifications of channel morphology (Alber and Sheldon 1999a,b). Furthermore, summer water temperatures in Georgia rivers often exceed 31 °C (DeVries 2006). Changes in salinity and temperature regimes may limit available habitat and cause a recruitment bottleneck for many shortnose sturgeon populations in the southeastern United States (Collins et al. 2002). Similarly, bioenergetics models for juvenile Atlantic sturgeon, *Acipenser oxyrinchus*, demonstrate that summer salinities, temperatures, and dissolved oxygen levels in Chesapeake Bay can limit habitat use (Niklitschek and Secor 2005).

Previous studies have found increases in salinity tolerance of juvenile white sturgeon, *Acipenser transmontanu*s, shortnose sturgeon, and Adriatic sturgeon, *Acipenser naccarii*, with

increasing size (McEnroe and Cech 1985, Jenkins et al. 1993, Cataldi et al. 1999). These studies demonstrated similar salinity tolerances among species, at similar sizes and temperatures. However, salinity tolerance experiments done with sturgeon (McEnroe and Cech 1985, Jenkins et al. 1993, Cataldi et al. 1999) have not examined the interactive effects of temperature on juvenile sturgeon survival. In contrast, factorial experiments of salinity and temperature have been used to identify suitable habitat for the anadromous striped bass, *Morone saxatilis* (Otwell and Merriner 1975), the estuarine weakfish, *Cynoscion regalis* (Lankford and Targett 1994), and *Sesarma cinereum*, a larval marine invertebrate (Costlow et al. 1960).

Temperature governs many behaviors and activities of sturgeons; however, few studies have examined the effects of temperature on sturgeon populations. Studies of green sturgeon, *Acipenser medirostris*, bioenergetics suggest that temperature may be used as an effective management criteria for evaluating optimal environmental conditions and protecting fish (Mayfield and Cech 2004, Allen et al. 2006). For example, thermal preference data obtained in the bioenergetics study were used to estimate lethal temperature thresholds of green sturgeon (Jobling 1981, Mayfield and Cech 2004). Other studies of shortnose sturgeon demonstrated that temperature changes trigger spawning activity, affect egg development, and influence the seasonal movements of shortnose sturgeon (Hall et al. 1991, Collins and Smith 1993, Kynard 1997, Collins et al. 2002, Hardy and Litvak 2004, DeVries 2006). However, thermal tolerances of shortnose sturgeon have not been investigated.

Changes to surrounding environments, such as increases in temperature and salinity, can cause sturgeon to alter both the ionic concentrations and the percentage of red blood cells in their blood (Moyle and Cech 1988). Environmental stress, such as increasing temperatures, can change both the oxygen requirements of fish and the oxygen affinity of the red blood cells (Riggs

1970). Fish can cope with this stress by altering their hematocrit- the percentage of red blood cells in the blood. In addition, euryhaline fish species, such as sturgeon, cope with changing salinities by altering their plasma osmolality- a measure of the ions in blood. Therefore, hematocrit and plasma osmolality can be used as biomarkers of physiological responses to salinity and temperature stress for juvenile shortnose sturgeon (Handy and Depledge 1999).

Hematocrit and plasma osmolality values have been used to demonstrate physiological changes associated with increasing salinity for several sturgeon species (LeBreton and Beamish 1998, McKenzie et al. 1999, Martinez-Alvarez et al. 2002, Rodriguez et al. 2002, Jarvis and Ballantyne 2003). Studies of lake sturgeon, *Acipenser fulvescens* (LeBreton and Beamish 1998) and Siberian sturgeon, *Acipenser baeri* (Rodriguez et al. 2002), demonstrate increasing plasma osmolality with increasing salinity. However, hematocrit values of Siberian sturgeon were unaffected by salinity increases (Rodriguez et al. 2002). In chronic salinity exposure experiments using both Adriatic and shortnose sturgeons, plasma osmolalities of fish in brackish conditions returned to values similar to those of fish in freshwater, which demonstrated physiological acclimation to brackish conditions (McKenzie et al. 1999, Martinez-Alvarez et al. 2002, Jarvis and Ballantyne 2003). Osmolalities were similar across sturgeon species at similar sizes and salinities (LeBreton and Beamish 1998, McKenzie et al. 1999, Martinez-Alvarez et al. 2002, Rodriguez et al. 2002, Jarvis and Ballantyne 2003).

In this study, dose-response experiments were used to examine salinity and temperature tolerances of hatchery-reared YOY shortnose sturgeon. Salinity and temperature LC_{50} experiments were conducted in 2005, and results of the LC_{50} experiments were used to design an experiment that examined the interactive effects of salinity and temperature on YOY survival. Data collected in 2005 were used to design further experiments. Experiments conducted in 2006

used combinations of four salinities and three temperatures to further examine the interactive effects of salinity and temperature on the survival of YOY shortnose sturgeon. Survival data were used to construct a predictive survival model based on salinity, temperature, and fish weight. Physiological changes associated with the salinity and temperature combinations were assessed for surviving fish by measuring hematocrit and plasma osmolality concentrations from collected blood samples.

METHODS

FISH CULTURE

Young-of-the-year shortnose sturgeon were artificially propagated from captive brood stock for use in salinity and temperature experiments in 2005 and 2006. Progeny were the offspring of three males and one female. Fish were raised in four 550-L cylindrical flow-through tanks. Tanks received unchlorinated spring water through a directional spray bar. The spray bar created a mild current in the tank, and the direction of the spray bar was changed every other week. Suitable hardness, alkalinity, and pH levels were maintained using a chemical injection system (Hickson et al. 2001). Water temperature remained a relatively stable 19 °C, and tanks were oxygenated with airstones attached to a central blower system. The flow of incoming water and thus the current in each tank was increased as the fish grew and ranged from 60 to 120 mL per second.

Diets and rations of the YOY shortnose sturgeon changed with growth and development. Initially, fish were fed *ad libitum* rations of brine shrimp, but fish were completely transitioned onto Rangen Soft-Moist® commercial pellet feed (44% protein, 18% fat, < 5% fiber, < 8% ash, ~23% moisture) by two months of age. Starting at this age, fish received a 3.0% body weight

per day ration allocated over four daily feedings. Daily rations were based on the relative growth rate (RGR) of the fish and weight data obtained from tank biomass estimates that were performed every two to three weeks (Brian Hickson, USFWS, personal communication).

$$RGR = [\ln(\text{final weight}) - \ln(\text{initial weight})]/\text{days} \quad (1)$$

As the fish grew, pellet size was increased and ranged from 0.8-1.6 mm.

Water quality was carefully monitored in the holding tanks. Dissolved oxygen and temperature were measured daily with a YSI-85® multi-parameter meter. Other water quality variables, such as pH, hardness, and alkalinity were measured weekly with colorimetric test kits (Aquarium Pharmaceuticals, Inc.). Tanks were siphoned at least once daily to remove accumulated waste and scrubbed at least once per week to remove algal growth on tank surfaces. A 14-hour light:10-hour dark photoperiod was maintained throughout the study.

EXPERIMENTAL PROTOCOL

In 2005, three salinity LC_{50} experiments, three temperature LC_{50} experiments, and one factorial experiment were conducted with YOY shortnose sturgeon. All exposures lasted 48 hours. Salinity LC_{50} experiments were conducted at 66, 86, and 107 days post hatch (dph), temperature LC_{50} experiments were conducted at 70, 94, and 112 dph, and the factorial experiment was conducted at 101 dph. The number of treatments and replicates varied slightly among experiments (Table 2.1).

All experiments were conducted in 38-L static aquaria equipped with AquaClear Mini® biofilters to maintain suitable water quality and oxygenate the water. Fish were starved for at least 24 hours prior to conducting experiments to further maintain water quality. Because the

Table 2.1. Distribution of treatments for experiments conducted with YOY shortnose sturgeon in 2005. Salinity and factorial treatments are expressed in parts per thousand (ppt), and temperature treatments are expressed in degrees Celsius (°C). Standard errors for all treatments were ≤ 0.1 ($n \geq 12$) and were not listed in the table.

Trial	Replicates	Treatments				
		1	2	3	4	5
Salinity 1, 23 °C	5	0.3	5.2	10.1	15.2	20.1
Salinity 2, 23 °C	5	0.2	5.1	10.3	15.2	20.3
Salinity 3, 23 °C	5	0.2	14.9	17.9	21.0	24.0
Temperature 1	4	19.3	22.9	26.5	30.1	---*
Temperature 2	5	27.0	28.5	30.0	31.5	33.0
Temperature 3	5	27.0	28.4	30.0	31.5	33.0
Factorial 23.1 °C	3	0.2	8.3	16.2	24.1	---*
Factorial 28.6 °C	3	0.2	8.5	16.6	24.2	---*

* indicates that these trials did not contain a fifth treatment

building was not temperature controlled, 550-L cylindrical tanks were used as water baths to maintain desired temperatures in experimental tanks, with two 38-L tanks in each water bath.

Water quality variables of experimental tanks were measured in similar manners to the water quality variables of the holding tanks. In all experiments, water quality variables were checked frequently to ensure that they were maintained at levels that would not confound the mortality results. The number of fish per tank was reduced from 10 to eight after 94 dph to further maintain water quality. The frequency of enumerating and recording tank mortalities varied slightly among experiments. Mortalities were removed at 12 hour intervals in the first salinity and temperature experiments and at four hour intervals in the second and third salinity and temperature experiments. Death was measured based on cessation of opercular movements and non-response to tactile stimuli. At the conclusion of each experiment, survivors were removed from the tanks, total length measurements were recorded, and fish were placed in a post-trial holding tank.

2005 TREATMENT ACCLIMATION

In all experiments, each tank received an equal number of fish from each holding tank. All fish were used without replacement. For salinity and factorial experiments, fish were removed from the holding tanks and randomly distributed to the experimental aquaria, which were kept at the same temperature as the holding tanks. Following distribution to the experimental aquaria, the aquarium temperatures were increased to the temperature for that treatment at a rate of 0.1°C per minute. Once the experimental temperature was reached (Table 2.1), the fish were given about three hours to adjust to the new temperature. Next, pre-measured amounts of Instant

Ocean® marine salt were added to the tank incrementally over a two hour period to reach desired treatment salinities.

The protocol for acclimating fish to temperature treatments was different from the protocol used in salinity and factorial experiments. Prior to beginning the temperature experiments, the aquaria were heated to the desired temperatures. Fish were randomly distributed to 9.5-L re-sealable plastic bags kept inside foam coolers to maintain water temperature. Following distribution, the bags were removed from the coolers, sealed, and placed into the experimental aquaria. The fish acclimated in the bag for 40 minutes before release into the experimental aquaria. Release of the fish from the bags to the experimental aquaria signaled the starting time for the temperature experiments.

In addition to the experimental aquaria, fish were randomly distributed to 10 sub-sample buckets prior to beginning all experiments. The total lengths of the fish in each sub-sample were compared with one-way ANOVA and Tukey's Honestly Significant Difference tests. If significant differences were not found among the sub-samples, fish were assumed to be randomly distributed to the experimental aquaria and the experiment continued as planned (Jennings 1996). Sub-samples were used to estimate the average length of the fish in the experiment, thus preventing the experimental fish from experiencing additional handling stress. However, sub-sampling was not performed in the first salinity experiment, so the post-trial length of the fish was used to estimate the size of the fish in the experiment.

2006 EXPERIMENTAL SETUP

Factorial experiments were conducted using 38-L glass aquaria. Experimental treatments consisted of combinations of four salinities (0.2, 8.5, 16.5, and 24.3 ppt) and three temperatures

(23.3, 27.2, and 31.1°C), for a total of 12 experimental treatment combinations. Each treatment combination had four replicates for a total of 48 tanks. Treatment tanks were placed inside cylindrical fiberglass tanks (550 L), which served as water baths to help maintain temperatures prior to starting the trial. Water baths were necessary since the building was not temperature controlled.

Within water baths, treatments were blocked according to temperature, but each 38-L tank was an experimental unit (Figure 2.1). Tanks were heated with 250 watt Won® titanium heaters. Four-way diffuser manifolds supplied oxygen to each 38-L aquarium. Each manifold was connected to a central blower system with 178 cm of 0.5 cm diameter tubing. Four airstones were connected to each manifold with a 122 cm piece of 0.5 cm diameter tubing. Thus one manifold aerated each of the four aquarium tanks inside each water bath tank. The airstones provided sufficient oxygen to each tank and also mixed the water to ensure that it heated evenly. Each tank was filled with about 30-L of water, and salinity treatments were randomly distributed within temperature blocks, which were also randomly distributed (Figure 2.1).

2006 EXPERIMENTAL PROTOCOL

Prior to their distribution to the experimental tanks, fish were starved for 24-48 hours. Following the starvation period, fish were removed from the holding tanks with a 15-cm, soft-mesh net and randomly distributed to the experimental tanks. Each experimental tank received an equal number of fish from each of the four holding tanks. In addition to the 48 experimental tanks, fish were distributed to 10 plastic buckets for sub-sampling similar to that of experiments conducted in 2005. However, in addition to total lengths (mm), individual weights (g) were

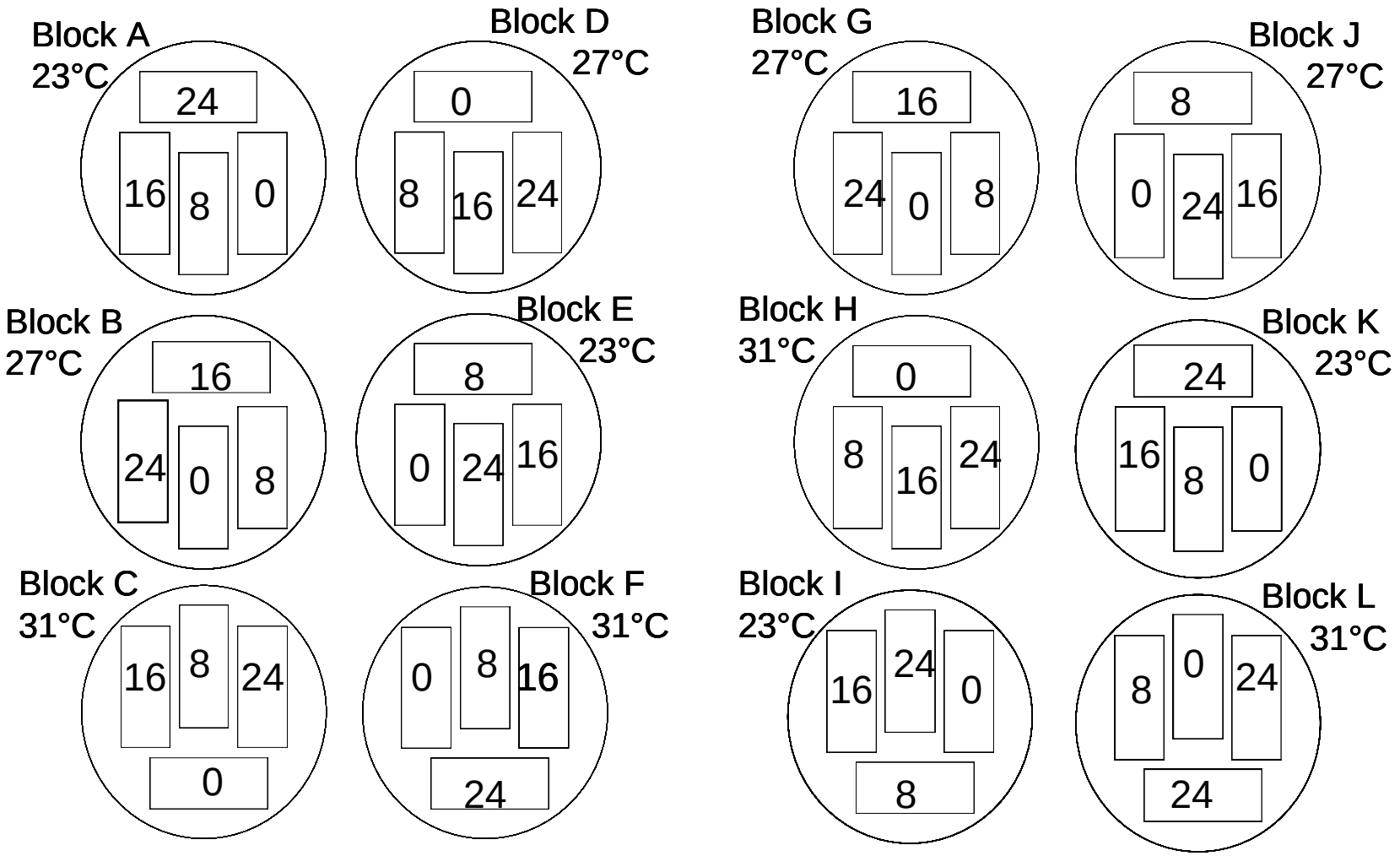

Figure 2.1. Schematic diagram of experimental setup for factorial experiments conducted with YOY shortnose sturgeon in 2006. The circles represent water bath tanks, while the rectangles represent individual 38 L tanks. Numbers inside the tank represent the salinity treatment (ppt).

recorded for sub-sample fish. Weight measurements were used to estimate holding tank biomass and growth rate for feeding ration adjustments.

Following distribution, fish were kept at conditions similar to the holding tanks for 12 hours to adjust to the new tanks and recover from handling stress. Following this recovery period, the water baths were drained, and the experimental tanks were heated at a rate of 0.08 °C/min until the desired experimental temperature was reached. The tanks were heated in a staggered fashion to ensure that all the tanks reached their experimental temperatures at approximately the same time. Tank heaters set to the highest experimental temperature were plugged in first, and tank heaters set to the lowest temperature were plugged in last. This was accomplished over three hours.

Once the tanks reached their experimental temperatures, pre-measured amounts of Instant Ocean® marine salt were added to the tanks in increments of 293 g (~ 8 ppt). Tanks containing 24 ppt treatments received three salt increments, 16 ppt treatments received two salt increments, and 8 ppt treatments received one salt increment. Salt was added in a staggered fashion so that all tanks reached their final experimental salinities at about the same time. Addition of the salt increments was done in one hour.

Four experimental trials were conducted throughout the summer (Table 2.2). As the fish grew throughout the summer, the number of fish per tank in each experiment was reduced to maintain suitable density and water quality, from 10 fish per tank initially to six fish per tank in the last experiment. Exposures to experimental treatments lasted 48 hours. Length and wet weights were recorded for all mortalities, which were removed every four hours.

Water quality was checked at regular intervals throughout each experiment. Oxygen saturation (%), dissolved oxygen (mg/L), temperature (°C), and salinity (ppt) were measured

Table 2.2. Growth data of YOY shortnose sturgeon for each trial conducted with in 2006. Length and weight are presented as averages, and standard error are given in parentheses. Ages are given in days post hatch (dph). Relative growth rates (RGR) are based on pre-trial sub-sample weight measurements. The RGR for trial one represents the RGR following transition to 100% commercial feed for 15 days prior to starting the trial.

Trial	Date	Age (dph)	N	TL (mm)	Weight (g)	RGR (%)
1	5/16/06	69	100	69 (1)	1.4 (0.0)	6.4
2	6/12/06	96	100	114 (1)	5.1 (0.1)	4.8
3	7/5/06	119	80	155 (1)	12.1 (0.3)	3.9
4	7/30/06	144	60	187 (2)	21.3 (0.8)	2.3

with a YSI-85® multi-parameter meter. Ammonia, nitrite, nitrate, pH, alkalinity, and hardness levels were measured using colorimetric test kits (Aquarium Pharmaceuticals, Inc.). Hardness was only measured in the freshwater treatments. Twenty-five percent water exchanges were used to maintain acceptable total ammonia concentrations (< 1 ppm) throughout the trials (Brian Hickson, USFWS, personal communication). Following a water exchange, water quality variables were checked to ensure that treatment conditions were not affected.

At the end of the experiments, surviving fish were removed from the tanks, measured for total length (mm) and wet weight (g), and sacrificed for blood collection and analysis. Fish were anesthetized in 75 mg/L of MS-222 (Hickson et al. 2001), and the caudal peduncle was severed with a scalpel. Blood was collected in 75 μL heparinized capillary tubes, and the tubes were sealed and centrifuged at 11,500 rpm for 10 minutes in an IEC Microcapillary Centrifuge, Model MB®. Hematocrit levels (%) were measured with a microhematocrit reader. Finally, blood plasma was removed and frozen for later analysis.

Plasma osmolalities were measured with a Wescor Vapor Pressure Osmometer Model 5520®. A three-point standard curve was used to ensure the accuracy of osmometer readings. Standards used were 100, 290, and 1000 mOsm, and each standard was analyzed in triplicate prior to analysis. Plasma samples were not analyzed until triplicate standard values were within 3.0 mOsm of the known values. During sample analysis, standards were analyzed after every 10-12 samples to check osmometer calibration.

Plasma osmolalities and hematocrit levels of holding tank fish, as well as osmolalities of tank water samples, were measured for further comparisons to blood variables of experimental fish. Water samples were collected from each experimental tank in the second, third, and fourth experiments. Osmolalities of water samples were analyzed in duplicate following plasma sample

analysis. Between the third and fourth experiments, 72 fish were removed from the four holding tanks at random, and total length and weight measurements were recorded. Fish were anesthetized, blood was collected, and hematocrit and plasma osmolality values of holding tank fish were measured in the same manner as the experimental fish.

STATISTICAL ANALYSES

Salinity LC_{50} estimates from experiments conducted in 2005 were generated with the maximum likelihood estimation (MLE) procedure in SAS v8 (SAS Institute, Inc.). Maximum likelihood estimates generate slopes that allow the mode of toxic action to be compared across the three salinity experiments. This approach makes MLE more powerful than non-parametric LC_{50} estimates. Normal, logistic, and Weibull distributions were compared with Pearson χ^2 goodness-of-fit estimates according to procedures outlined in Newman (1995), but the Weibull distribution gave the best fit over all three experiments and was selected over the other distributions.

In the first experiment, one mortality occurred in the 5.1 ppt treatment that kept the data from properly fitting the distribution of the MLE. However, the other fish in the 5.1 ppt treatment did not appear to be stressed, and other mortalities did not occur in this treatment. Furthermore, mortality monotonically increased with increasing salinity both with and without the single data point. The lack of convergence appeared to be a statistical anomaly, so following consultation (Dr. Michael C. Newman, Virginia Institute of Marine Science, personal communication), the single data point was removed from the analysis and the MLE were generated.

Because partial mortalities did not occur in the first and third experiments, the 2005 temperature LC_{50} estimates were generated with the binomial method (Newman 1995):

$$LC_{50} = [(A)(B)]^{0.5} \quad (2)$$

A = highest treatment with 0% mortality, B = lowest treatment with 100% mortality

95% Confidence Interval = interval between A and B

For factorial experiments, LC_{50} estimates were generated by using the non-parametric Trimmed-Spearman Karber (TSK) method (Hamilton et al. 1977). The binomial and TSK LC_{50} values were determined with an USEPA shareware program that was developed for the CT DEP Toxicity Program (2/28/90).

The data from interaction experiments conducted in 2005 and 2006 were not analyzed in the same manner. The interactive effects of salinity and temperature on the survival of 101-day old shortnose sturgeon (2005) were examined with a two-way ANOVA. The survival data from experiments conducted in 2006 were analyzed with logistic regression and tank as a random effect variable. Incorporating tank as a random effect allowed the examining of mortality bias among tanks and incorporating an error term into the model. The final model included salinity, temperature, weight, and all possible interactions of salinity, temperature, and weight as predictors of the binomially distributed survival variable. The two-way ANOVA was performed in SAS JMP v5.1 (SAS Institute, Inc.), and the logistic regression modeling was performed in SAS v8 (SAS Institute, Inc.).

Following examination of residuals among treatments and some exploratory ANOVAs, plasma osmolality and hematocrit data were grouped for ease of interpretation. Plasma osmolality values were compared among salinity treatments and ages, whereas hematocrit values were compared among temperature treatments and ages. Plasma osmolalities of holding-tank fish were compared to plasma osmolalities of fish from 0.2 and 8.5 ppt treatments (23.3°C) in experiments three and four, whereas hematocrit values of holding-tank fish were compared to those of fish in experiments three and four at 0.2 ppt and 23.3°C. Plasma osmolality and

hematocrit data for experiments three and four were pooled among similar treatments. Hematocrit data were compared with a pooled t-test, and all other blood data were evaluated with one-way ANOVA and Tukey's HSD tests. All statistical analyses of blood data were performed in SAS JMP v5.1 (SAS Institute, Inc.).

RESULTS

WATER QUALITY

Water quality of the holding tanks were similar between years of the study. Pooled averages of pH, hardness, and alkalinity are 6.9, 100.6 ppm, and 63.4 ppm, respectively. An average temperature of 18.8°C was observed in both years of the study. Dissolved oxygen (D.O.) concentrations were similar, with an overall mean of 7.4 mg/L.

Some variation in water quality was observed among survival experiments, but suitable water quality was maintained throughout all experiments. Alkalinity and pH increased with increasing salinity and ranged from 64.8 to 185.3 ppm and 7.4 to 8.4, respectively. Hardness was measured only for freshwater treatments, and mean values ranged from 80.9 to 195.4 ppm. Mean D.O. concentrations were adequate in all treatments (Campbell and Goodman 2004) and ranged from 6.0 to 7.9 mg/L. Total ammonia levels were low enough to avoid toxicity, ranging from 0.3 to 0.9 ppm (Fontenot et al. 1998). Nitrite and nitrate were rarely detected. When detected, nitrite concentrations did not exceed 0.25 ppm, and nitrate concentrations did not exceed 10 ppm.

2005 EXPERIMENTS

Increases in salinity LC_{50} values demonstrate that salinity tolerances of YOY shortnose sturgeon increase with increasing size. Salinity LC_{50} values ranged from 14.8 to 20.9 ppt

(Table 2.3), and the 95% confidence intervals indicate that the increase in salinity tolerance was significant for all trials. Confidence intervals were relatively small, and the p-values associated with the Pearson χ^2 statistics were all less than 0.01, which indicated that the models fit the data well. Equations were derived to estimate the probability of mortality at a given salinity:

56 mm TL: Probability of mortality = 7.14*[ln(ppt)] - 19.613 (3)

93 mm TL: Probability of mortality = 15.94*[ln(ppt)] - 47.391 (4)

128 mm TL: Probability of mortality = 20.60*[ln(ppt)] - 63.001 (5)

The steepness of the slopes (Figure 2.2) indicates that there is an abrupt salinity threshold for surviving acute exposures, and the threshold point becomes more abrupt with increasing size.

Increasing temperature LC_{50} values with increasing size demonstrate that temperature tolerance also increases with size. However, the wide confidence intervals from the LC_{50} experiments suggest that the increases were not statistically significant (Table 2.3). Although the changes in LC_{50} values were not significant, the width of the confidence intervals did decrease with size (Table 2.3). Furthermore, mortality at 30 °C decreased with increasing size interval: 100% in trial one, 26% (SE = 12%, n = 5) in trial two, and 0% in trial three, which demonstrates an increase in thermal tolerance (Figure 2.3).

Results of the two-way ANOVA indicate that salinity and temperature significantly interact to affect survival for juveniles at 101 dph ($R^2 = 0.99$, $p < 0.0001$). Furthermore, all effects (salinity, df = 3; temperature, df = 1; interaction, df = 3) were highly significant ($p < 0.0001$). Differences in mortality were not observed between temperatures for 0.2, 8.4, and 24.2 ppt salinity treatments (Figure 2.4). At 16.4 ppt, mortality did not occur at 23.1 °C, but 58% (SE = 5, n = 3) mortality occurred at 28.6 °C.

Table 2.3. Forty-eight hour LC_{50} estimates for YOY shortnose sturgeon at various salinities and temperatures based on experiments conducted in 2005 and 2006. Length estimates are based on pre-trial sub-samples, except for the initial salinity trial in 2005, when post-trial lengths were used. Ages are expressed as days post hatch (dph).

Variable	Year	Age (dph)	N	Length (SE) (mm)	LC_{50}	95% CI (Low, High)
Salinity 23.0°C	2005	66	247	56 (1)	14.8 ppt	(14.0, 15.6)
Salinity 23.3°C	2006	69	100	70 (1)	16.4 ppt	(15.1, 17.8)
Salinity 27.2°C	2006	69	100	70 (1)	12.0 ppt	(11.5, 12.5)
Salinity 23.2°C	2005	86	100	93 (1)	19.1 ppt	(18.2, 19.6)
Salinity 27.2°C	2006	96	100	114 (1)	16.2 ppt	(14.9, 17.6)
Salinity 28.6°C	2005	101	80	115 (1)	14.2 ppt	(12.6, 15.8)
Salinity 23.2°C	2005	107	80	128 (1)	20.9 ppt	(20.4, 21.5)
Salinity 27.2°C	2006	119	80	155 (1)	18.7 ppt	(17.6, 19.9)
Salinity 27.2°C	2006	144	60	187 (2)	19.6 ppt	(18.8, 20.5)
Temperature	2005	70	100	68 (1)	28.2 °C	(26.5, 30.0)
Temperature	2005	94	100	106 (1)	30.3 °C	(28.5, 31.5)
Temperature	2005	112	80	128 (1)	30.7 °C	(30.0, 31.5)

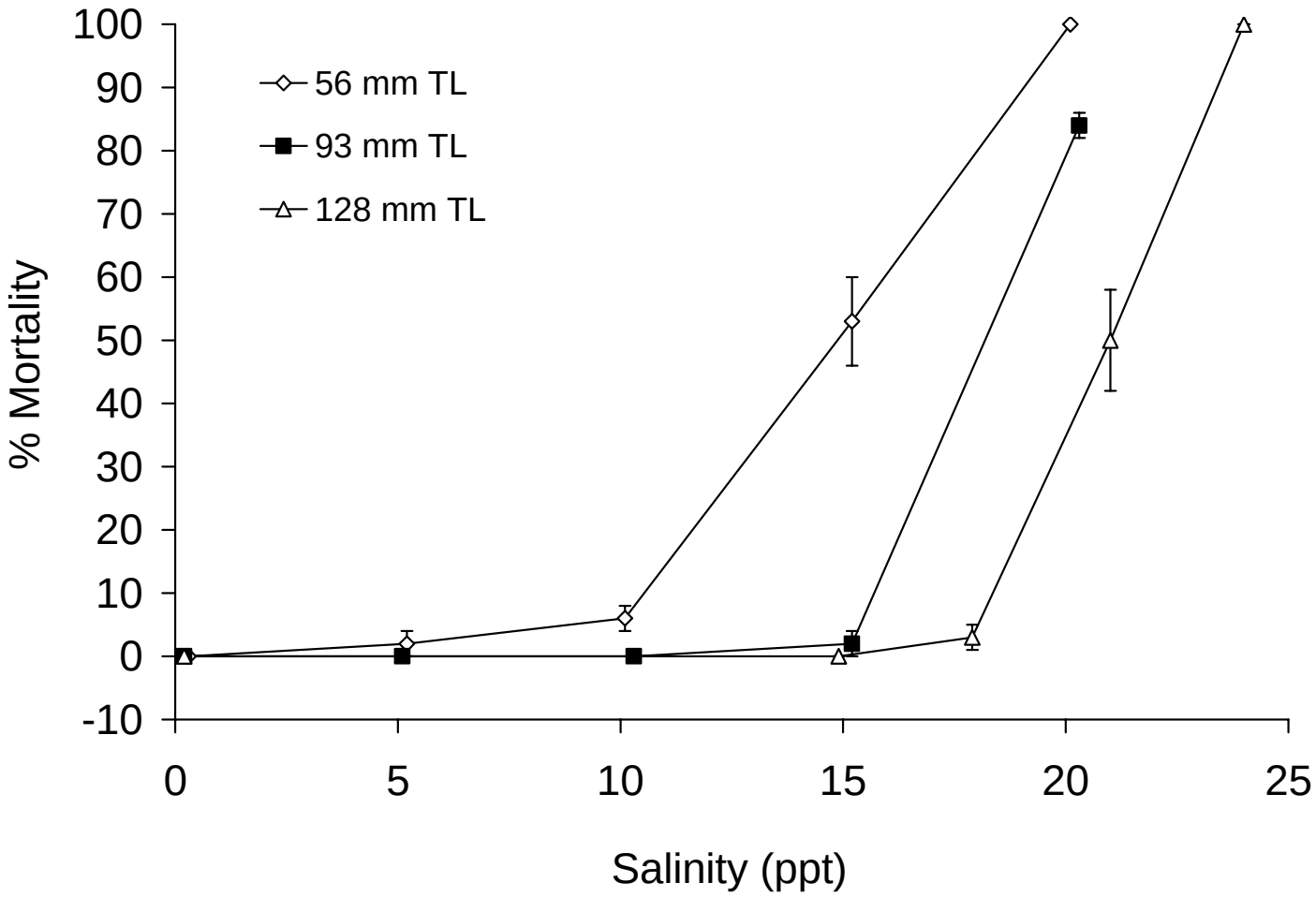

Figure 2.2. Percent mortality of YOY shortnose sturgeon at various salinities (ppt) for experiments conducted in 2005. Error bars represent standard errors (n = 5). Data presented here were used to estimate the salinity LC_{50} values presented in Table 2.3.

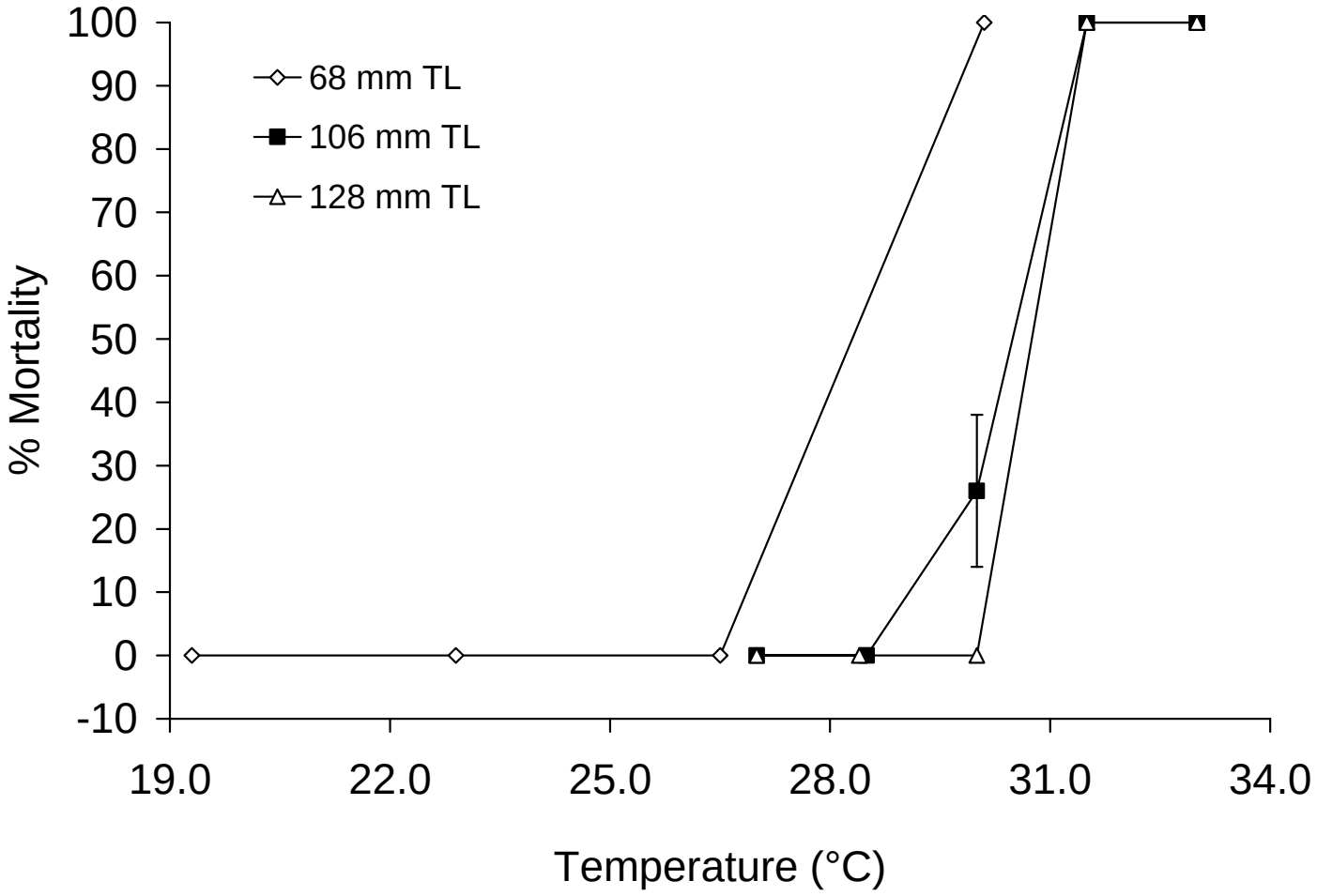

Figure 2.3. Percent mortality of YOY shortnose sturgeon at various temperatures (°C) for experiments conducted in 2005. The error bar represents standard error (n = 5). Data presented here were used to estimate the temperature LC_{50} values presented in Table 2.3.

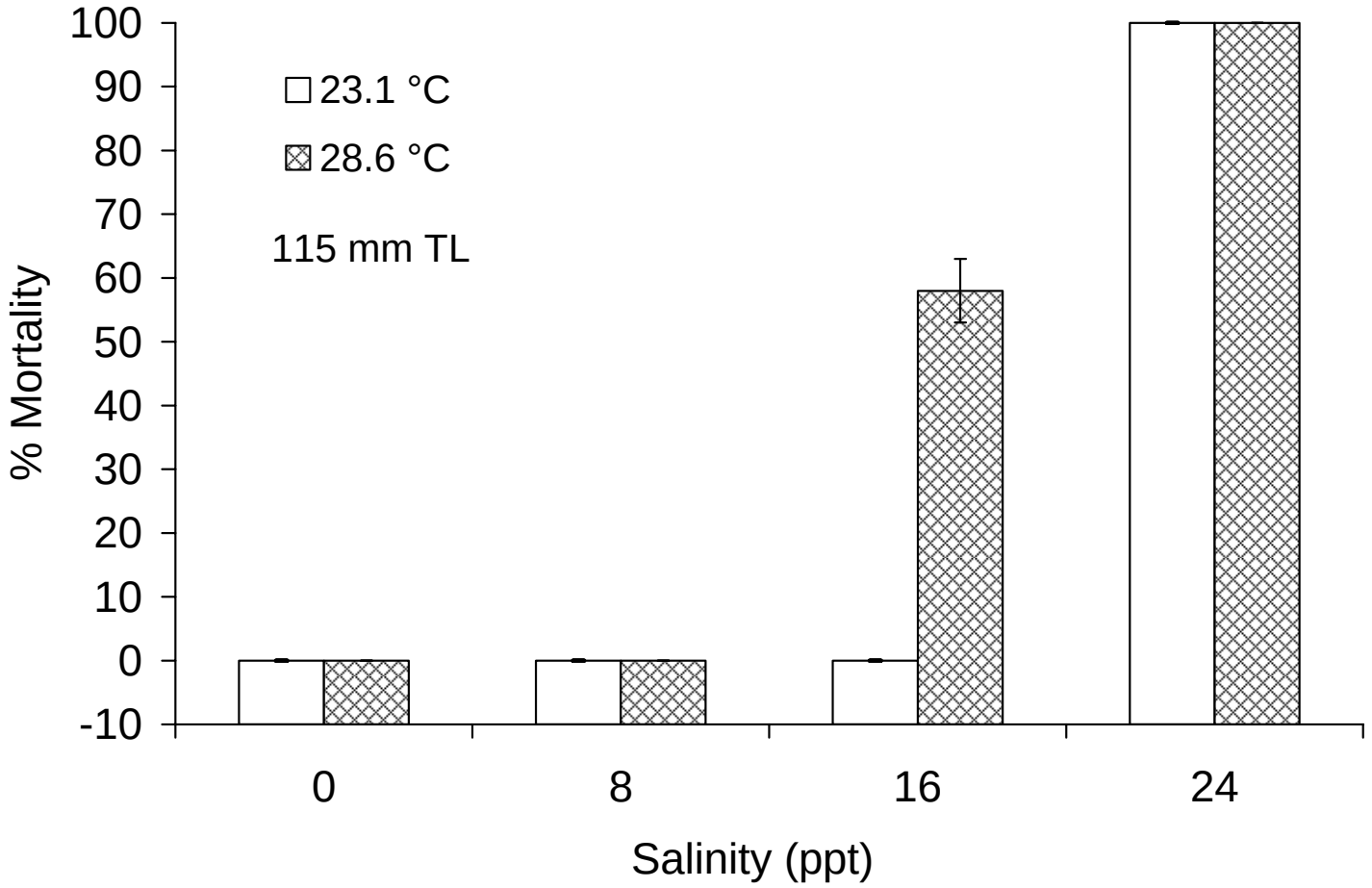

Figure 2.4. Percent mortality of YOY shortnose sturgeon for the factorial experiment conducted in 2005. The error bar represents standard error (n = 3).

Interestingly, the salinity LC_{50} value calculated at the 28.6 °C temperature treatment is similar to the salinity LC_{50} value from the first salinity experiment conducted with fish 35 days younger at a temperature of 23 °C (Table 2.3).

The distribution of experimental fish did not seem to affect the mortality results in any of the experiments. Differences among sub-samples in all of the trials were not significant ($\alpha = 0.05$). Most of the mortalities in temperature experiments occurred within the first four hours, whereas most of the mortality in salinity experiments occurred within the first 12 hours. Among all experiments, only three mortalities occurred after 24 hours.

2006 EXPERIMENTS

Mortality of YOY shortnose sturgeon in various combinations of salinity and temperature generally decreased as size increased (Figure 2.5), and sub-sampling indicated that the distribution of fish did not affect the mortality results ($p > 0.05$). The decrease in mortality with increasing size is illustrated further by the results of the survival model (Table 2.4), which contained seven model parameters. All variables and interactions were highly significant ($p \leq 0.0003$). To examine the random effect of treatment tank, each tank in each experiment was assigned a unique tank identification number. This resulted in a total of 192 tanks (48 tanks per experiment, four experiments) and 191 degrees of freedom. The negative coefficients for salinity and temperature indicate a decrease in survival probability with increasing salinity and temperature, and the positive coefficient for weight demonstrated that increases in weight significantly increase survival probability.

The parameter estimates for salinity, temperature, and weight can be transformed into odds ratios to further evaluate the relative risks of fish exposed to different salinities and temperatures

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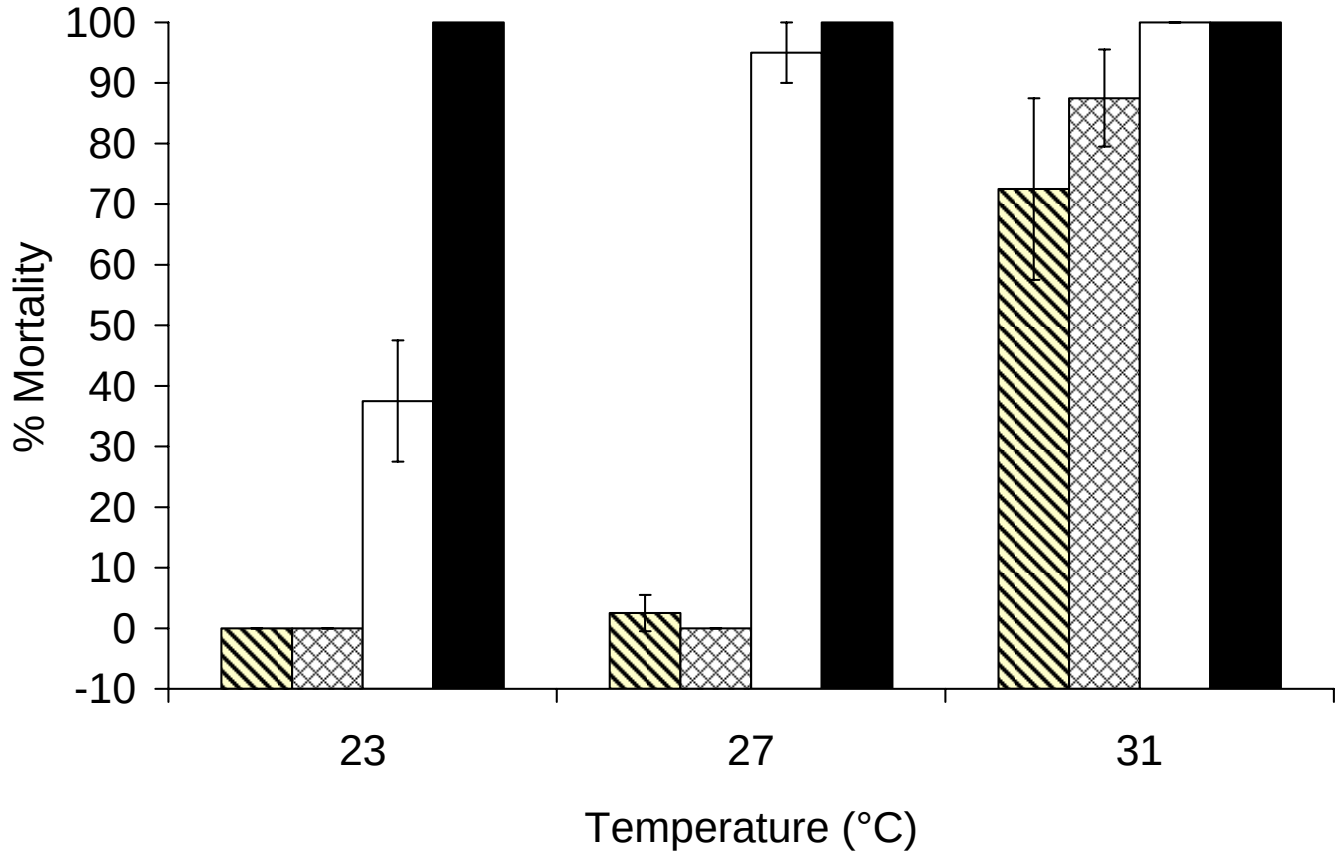
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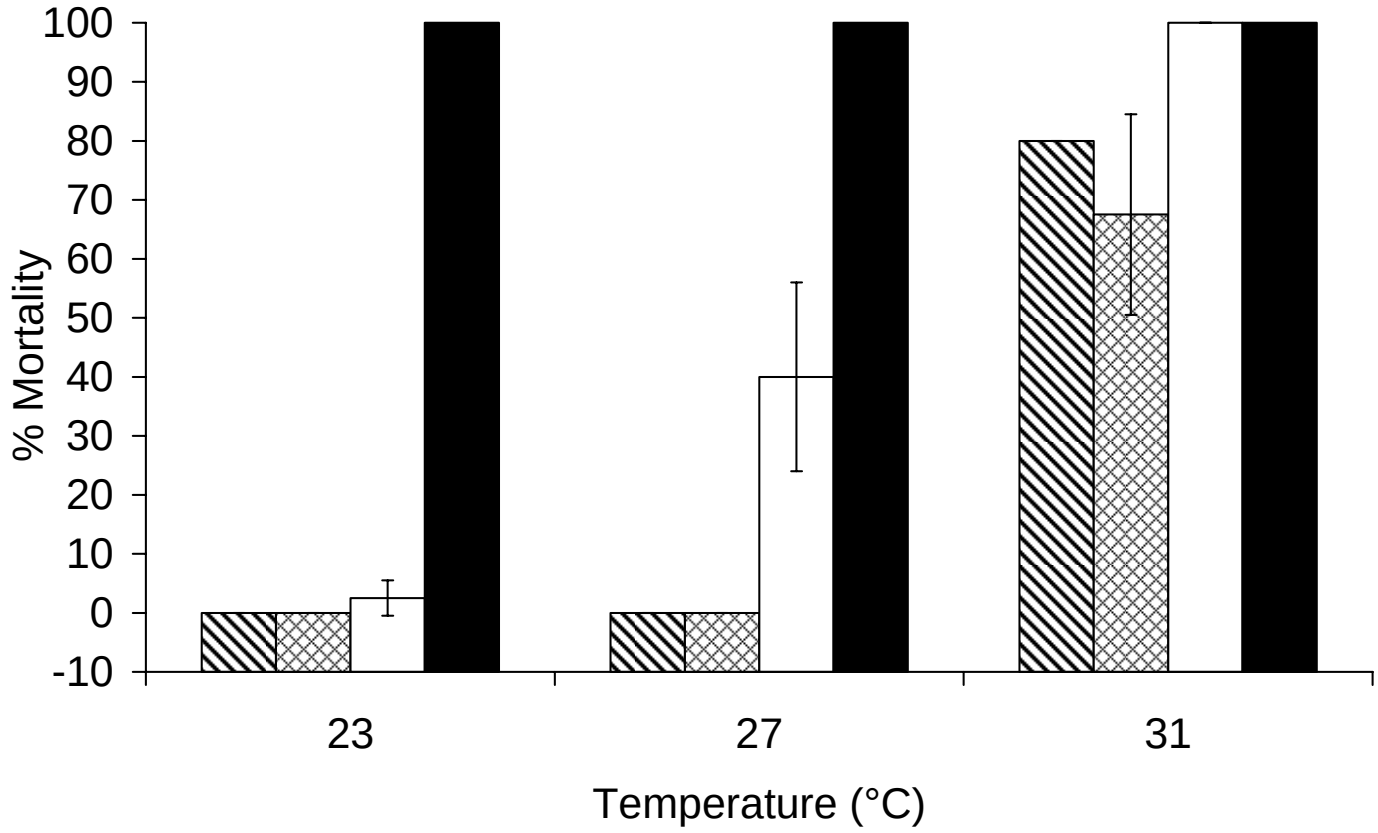
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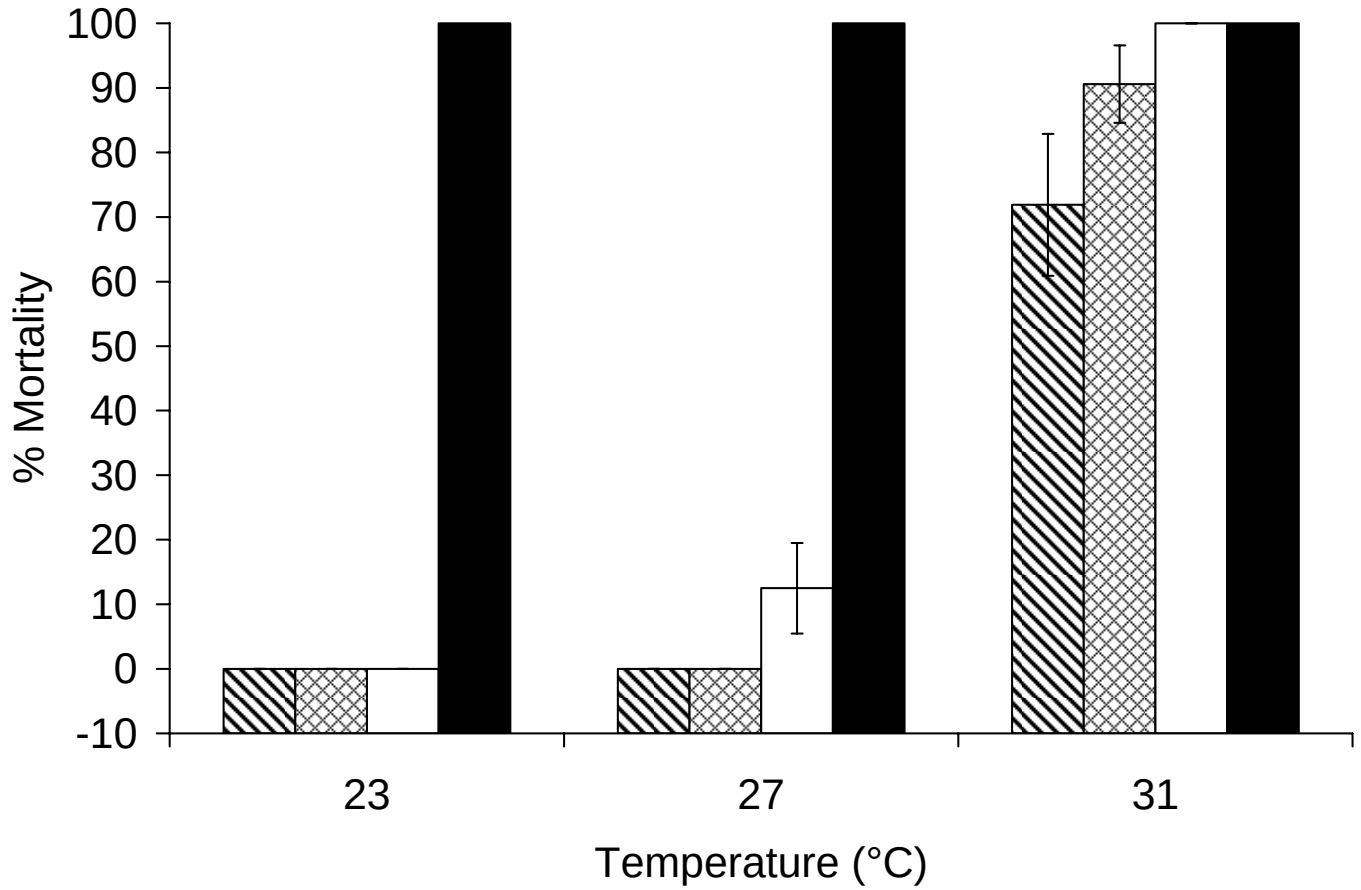
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Temperature (°C)

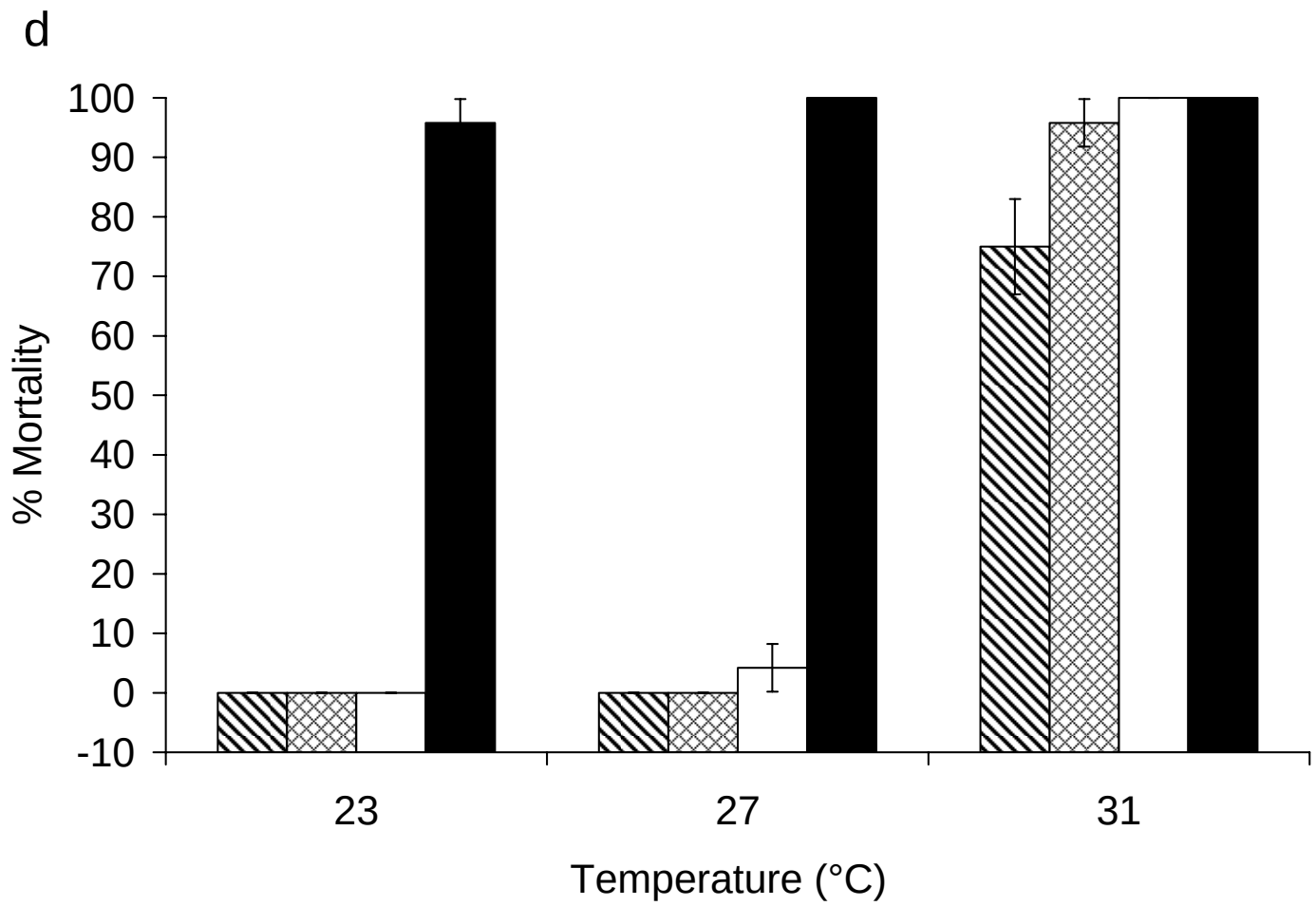

Figure 2.5. Percent mortality for YOY shortnose sturgeon in 12 different combinations of salinity (ppt) and temperature (°C) at 69 (a), 96 (b), 119 (c), and 144 (d) days post hatch (dph). Error bars represent standard errors (n = 4). Bars at each temperature represent salinity treatments. Diagonal lines represent 0.2 ppt, crossed lines represent 8.5 ppt, white bars represent 16.4 ppt, and black bars represent 24.3 ppt.

Table 2.4. Parameter estimates for predictor variables included in the survival model for YOY shortnose sturgeon based on data collected in 2006. The error term refers to the variation associated with individual tanks. Tank was the random effect variable in the logistic regression model.

Parameter	Estimate (SE)	df	t-value	p-value	95% CI (Lower, Upper)
Intercept	72.110 (15.80)	191	4.57	< 0.0001	(40.954, 103.270)
Salinity	-3.718 (0.925)	191	-4.02	< 0.0001	(-5.543, -1.894)
Temperature	-2.332 (0.512)	191	-4.56	< 0.0001	(-3.342, -1.322)
Weight	11.306 (2.553)	191	4.43	< 0.0001	(6.271, 16.342)
S*W	-0.407 (0.105)	191	-3.89	0.0001	(-0.613, -0.201)
S*T	0.112 (0.030)	191	3.69	0.0003	(0.052, 0.172)
T*W	-0.366 (0.082)	191	-4.44	< 0.0001	(-0.528, -0.203)
S*T*W	0.013 (0.003)	191	3.86	0.0002	(0.006, 0.020)
Error	1.237 (0.289)	191	4.28	< 0.0001	(0.667, 1.807)

(Dr. James T. Peterson, GA Coop. Fish and Wildlife Research Unit, University of Georgia, personal communication). Odds ratios are calculated with the following formulas:

$$\text{For negative coefficients: Odds Ratio} = 1/[e^{(\text{estimate}*\text{relevant unit change})}] \quad (6)$$

$$\text{For positive coefficients: Odds Ratio} = e^{(\text{estimate}*\text{relevant unit change})} \quad (7)$$

Using the negative coefficient formula, for every 1.5 ppt salinity increase, a given YOY sturgeon is 264 times less likely to survive, and for every 2.4°C temperature increase, a given YOY shortnose sturgeon is 269 times less likely to survive. Using the positive coefficient formula, for every 0.5 g weight increase, a given YOY shortnose sturgeon is 285 times more likely to survive. However, these same odds ratio transformations cannot be applied to interaction terms (Dr. James T. Peterson, GA Coop. Fish and Wildlife Research Unit, University of Georgia, personal communication).

The error term associated with the random effect also was significant, which indicates that there is some effect of tank on mortality, although the magnitude of the error term is relatively small and not large enough to significantly affect the reliability of the model (Dr. James T. Peterson, GA Coop. Fish and Wildlife Research Unit, University of Georgia, personal communication). The model, which is based on the parameter estimates given in Table 2.4, can be used to predict survival probabilities within the range of the experimental data (0.1-25.3 ppt, 22.9-31.6°C, and 0.4-42.8 g) by using the following equations:

$$P(\text{survival}) = 1/[1 + e^{(-H)}] \quad (8)$$

$$\text{where } H = 72.110 - (3.718)S - (2.332)T + (11.306)W - (0.407)S*W + (0.112)S*T - (0.366)T*W + (0.013)S*T*W \quad (9)$$

and S = salinity (ppt), T = temperature (°C), W = weight (g)]

An example of how this equation can be used to predict survival probabilities for fish of differing sizes in different salinities and temperatures is given (Figure 2.6).

The similarities of the methodology between the two years of experiments facilitated easy comparisons of mortality among salinities, temperatures, and size ranges. The salinity LC_{50} estimates from experiments conducted in 2006 were similar to estimates obtained from experiments conducted in 2005 for similar temperatures and fish sizes (Table 2.3). Estimates were similar despite differences in treatment concentrations and methods of calculating LC_{50} values.

Hematocrit levels were significantly affected by experimental treatments. Hematocrit levels were significantly different among temperatures and ages ($p < 0.0001$). Hematocrit levels did not differ significantly between 23.3 °C and 27.2 °C at any age interval, but at 31.1 °C, levels were significantly lower and were not significantly different among ages (Figure 2.7). Dark coloration of fish plasma at 31.1 °C indicated that hemolysis was occurring in fish exposed to this temperature. In fact, for some fish at 31.1 °C, hematocrit levels were as low as 7%. Finally, hematocrit levels increased with increasing size of the fish (Figure 2.7).

Plasma osmolalities were significantly affected by experimental treatments. Plasma osmolalities were significantly different among salinities and ages ($p < 0.0001$). At 69 dph, the overlap of the confidence intervals indicates that the fish were less able to regulate their plasma osmolalities (Figure 2.8). However, at increasing age intervals, the separation of osmolalities among salinity treatments increased, whereas the width of the confidence intervals decreased. At 144 dph, plasma osmolality significantly increased with each increasing salinity treatment ($p < 0.0001$), and the difference was especially pronounced among fish in the 16.4 ppt (302 mOsm) and 24.1 ppt (342 mOsm) treatments.

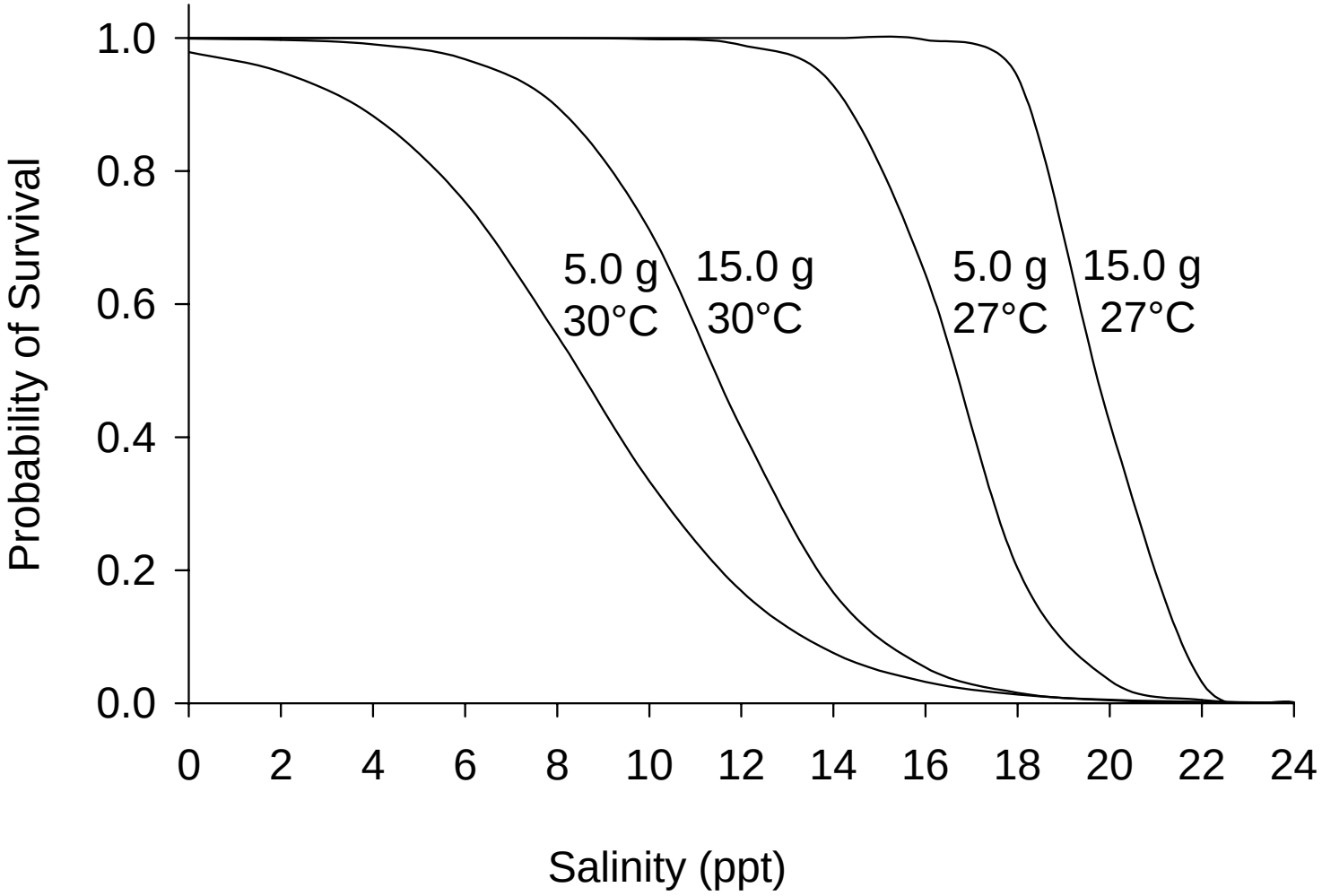

Figure 2.6. Predicted survival probabilities for 5-g and 15-g shortnose sturgeon at 27 and 30 °C at salinities ranging from 0 to 24 ppt. Predictions are based on the equation generated from parameter estimates given in Table 3.4.

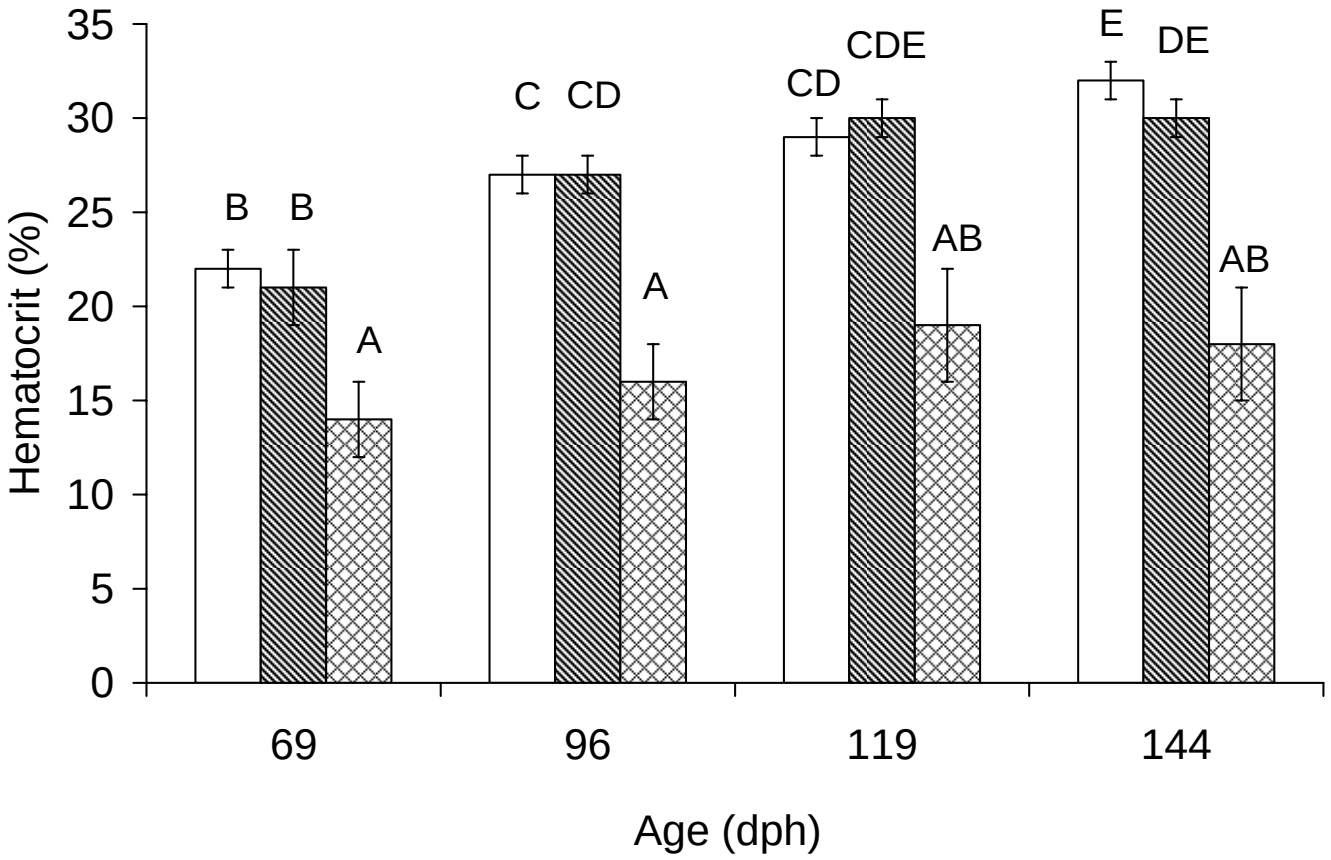

Figure 2.7. Hematocrit values (%) for surviving YOY shortnose sturgeon at each temperature for trials conducted in 2006. Hematocrit values are pooled across all salinity treatments, and similar letters indicate that differences are not significant at $\alpha = 0.05$. Error bars represent standard errors ($9 \leq n \leq 57$). Within each age interval, each bar represents a different temperature. White bars represent 23.3 °C, diagonal lines represent 27.2 °C, and crossed lines represent 31.1 °C.

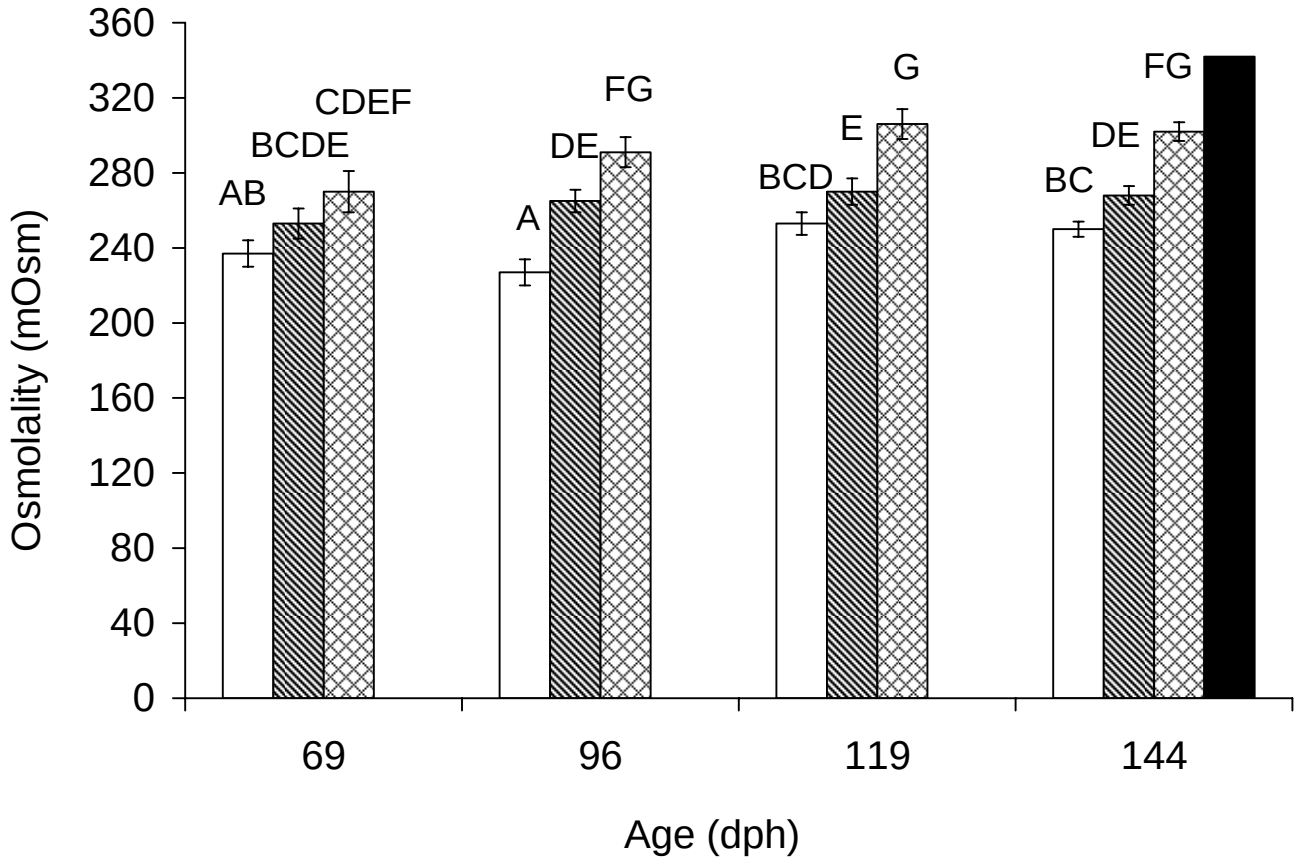

Figure 2.8. Plasma osmolalities (mOsm) for surviving fish at each salinity for trials conducted in 2006. Osmolalities are pooled across temperatures, and similar letters indicate that differences are not significant at $\alpha = 0.05$. White bars represent 0.2 ppt (30 mOsm), diagonal lines represent 8.5 ppt (246 mOsm), crossed lines represent 16.4 ppt (485 mOsm), and black bars represent 24.1 ppt (706 mOsm). Error bars represent standard errors ($13 \leq n \leq 52$). The bar for the 24.1 ppt treatment does not have error bars and was not included in the analysis because the value is based on a blood sample from a single fish. Only treatments with surviving fish used for blood analysis were used to estimate the listed water salinities and osmolalities.

Comparison of blood variables from holding-tank fish (18.8°C) to blood variables of experimental fish (ages 119 and 144 dph, temperature 23.3°C) produced some interesting results. Hematocrit levels were significantly higher in fish from the holding tanks than experimental fish in freshwater ($p < 0.0001$). The mean plasma osmolality for fish from the holding tanks (259 ± 2 mOsm) was not significantly different from mean plasma osmolalities of experimental fish at both 0.2 ppt (251 ± 2 mOsm) and 8.5 ppt (266 ± 1 mOsm), although the osmolalities of experimental fish at 0.2 and 8.5 ppt were significantly different from each other ($p = 0.0038$).

DISCUSSION

SALINITY TOLERANCE

In both years of the study, salinity tolerances of YOY shortnose sturgeon increased with size. In the first experiments conducted in 2005 and 2006, the age, size, and percent mortalities were nearly identical to those of shortnose sturgeon in a previous study (Jenkins et al. 1993). Furthermore, salinity tolerances and changes in salinity tolerances also were similar to those of white and Adriatic sturgeon juveniles of similar size and age (McEnroe and Cech 1985, Cataldi et al. 1999). These results further support the idea that salinity tolerances of juvenile sturgeons are closely related to body size. In addition, the method of determining salinity LC_{50} values for experiments conducted in 2005 was similar to the method used to determine LC_{50} values for Adriatic sturgeon (Cataldi et al. 1999). The slopes of the LC_{50} curves for both species demonstrate that there is an abrupt salinity threshold and that this threshold increases with size.

Microscopic examinations of the gills, kidney, and intestine have been used to document increased development of osmoregulatory organs with increasing size for Adriatic sturgeon (Cataldi et al. 1999). At 150 dph, all of the organs had developed into those of adult Adriatic

sturgeon. Furthermore, salinity tolerances of juvenile Adriatic sturgeon appeared to be closely linked to body size and gill surface to body volume ratios (Cataldi et al. 1999). Smaller fish have higher gill surface to body volume ratios than larger fish, thus they are subjected to higher osmotic imbalances from passive ion exchanges (Muir 1969, Cataldi et al. 1999). The increase in tolerance with increasing body size may be the result of a hormonally-induced maturation event, such as an increase in chloride cells in the gills (McEnroe and Cech 1985). Although the physiological mechanisms for developing salinity tolerance may not be fully understood, the results of this study and previous studies (McEnroe and Cech 1985, Cataldi et al. 1999) suggest that the mechanisms are similar among sturgeon species.

TEMPERATURE TOLERANCE

Temperature tolerance increased with size, although the wide confidence intervals of the LC_{50} estimates do not suggest a significant increase in tolerance. However, because partial mortality did not occur in the first and third temperature experiments, the temperature threshold may be abrupt and narrow. If so, then the LC_{50} method used here may not be the ideal method to evaluate temperature tolerance.

The LC_{50} method used in this study was selected over classical thermal tolerance methods, such as direct transfer or slow-heating methods for several reasons. First, the method was chosen for similarity to the salinity tolerance tests that were also conducted. Next, along with the LC_{50} method, tempering was used to reduce both the handling stress associated with direct transfer methods and the partial acclimation problem of slow-heating experiments, which often over-estimates acute temperature tolerances. The use of tempering in thermal tolerance

experiments was suggested by Kilgour and McCauley (1986), but literature accounts of use of this technique are non-existent.

The temperature LC_{50} estimates of this study provide thermal tolerance information that did not previously exist for YOY shortnose sturgeon. However, temperature tolerances of YOY shortnose sturgeon need to be investigated further for several reasons. First, thermal tolerances of fish are dependent upon acclimation temperature, previous thermal history, and heating rate (Becker and Genoway 1979, Jobling 1981). Fish used for LC_{50} experiments were raised at a very stable temperature of about 19 °C, and so the effects of acclimation temperature on temperature tolerance could not be investigated. Future investigations of shortnose sturgeon temperature tolerance should include classical temperature tolerance methods and fish acclimated to a variety of temperatures to determine the degree to which those factors influence thermal tolerance.

Comparing the results of this study to results obtained with classical temperature tolerance methods would provide much additional information. First, lethal temperature data collected with either the direct transfer or the slow-heating method can be transformed to predict results produced with the other method (Kilgour and McCauley 1986). Furthermore, regression equations can be used to estimate relationships among lethal temperature, preferred temperature, and optimum growth temperature (Jobling 1981). These equations were used to predict preferred temperatures and optimum growth temperatures of splittail from lethal temperature data (Young and Cech 1996). Similarly, Mayfield and Cech (2004) used thermal preference data obtained with thermal gradient tanks to predict lethal temperatures of juvenile green sturgeon. Such techniques could be used to further evaluate the temperature tolerances of juvenile shortnose sturgeon.

SALINITY AND TEMPERATURE INTERACTION

Previous studies of juvenile sturgeons have not examined the interactive effects of salinity and temperature on sturgeon survival. Other salinity tolerance studies involving sturgeon have examined changes in tolerance with increasing size, but these studies have been conducted at a single, moderate temperature (McEnroe and Cech 1985, Jenkins et al. 1993, Cataldi et al. 1999). In this study, interaction experiments demonstrated that salinity and temperature significantly interact to affect YOY shortnose sturgeon survival. Survival decreased with increasing salinities and temperatures. Factorial designs, such as those used in this study, have been used to determine suitable habitat areas of the anadromous striped bass, *Morone saxatilis* (Otwell and Merriner 1975), the estuarine weakfish, *Cynoscion regalis* (Lankford and Targett 1994), and *Sesarma cinereum*, a larval marine invertebrate (Costlow et al. 1960).

Increases in salinity and temperature tolerances with increasing size were observed among the four experiments conducted in 2006. However, changes in salinity and temperature tolerances were most effectively illustrated by the 27.2 °C, 16.5 ppt treatment combination. Interestingly, at 31 °C, mortality was not significantly different at 0.2 and 8.5 ppt for all experiments. This suggests that shortnose sturgeon may be able to survive in moderate salinities up to their freshwater temperature limit. Furthermore, at stressfully high temperatures, juvenile sturgeon may be able to move into isosmotic salinities to reduce osmoregulatory costs and conserve energy reserves.

BLOOD ANALYSIS

Blood analysis revealed significant sub-lethal effects of salinity and temperature on YOY shortnose sturgeon. Hematocrit levels significantly increased with fish size, demonstrating increasing oxygen requirements with rapid growth and corresponding body sizes. Hematocrit levels were not significantly different between 23 and 27 °C at any age/size interval. At 31 °C, low hematocrit levels and dark plasma indicate that hemolysis was occurring in the blood of surviving fish. Hemolysis demonstrated that YOY shortnose sturgeon at this temperature were under substantial stress. Significant differences were not observed in hematocrit levels of fish at different salinities within each temperature treatment, indicating that osmoregulatory changes did not alter the oxygen requirements of the fish.

Plasma osmolalities of surviving fish also were affected by experimental treatments. Plasma osmolalities increased with increasing salinity, with mean values ranging from 237 to 342 mOsm. At 69 dph (70 mm TL, 1.4 g weight), the confidence intervals around mean osmolality values were wide among all salinity treatments, and the osmolalities of fish at 16.4 ppt were low compared to those of older fish. These data suggest that at smaller sizes, YOY shortnose sturgeon are less able to regulate essential ions in their blood plasma. However, at larger size intervals, plasma osmolalities of fish at 16.4 ppt increased, variation in plasma osmolality values decreased, and survival at a salinity of 16.4 ppt increased.

The ability to regulate plasma osmolality may be a physiological mechanism that dictates the survival of YOY shortnose sturgeon at increasing salinities. Regulatory abilities may improve with development of osmoregulatory organs, or abilities may be linked to reductions in gill surface to body volume ratios, which reduce the passive flow of ions and may allow the fish sufficient time to regulate blood plasma (Muir 1969, Cataldi et al. 1999). Finally, increased

ability to regulate blood plasma may result from increases in the number of chloride cells in the gills (McEnroe and Cech 1985).

The elevated plasma osmolality (~ 340 mOsm) of the surviving YOY shortnose sturgeon at 24.1 ppt (706 mOsm) in this study suggests that shortnose sturgeon may have to elevate their plasma osmolality within a certain range (~ 50%) of the osmolality of the surrounding medium to maintain an adequate rate of ion flux and survive in a hypertonic saltwater environment. Similar results were observed for 150 day old Adriatic sturgeon, when only partial survival occurred in environments in which water osmolalities were over twice the plasma osmolalities of the fish (Cataldi et al. 1999).

Several studies have used sturgeon hematocrit and plasma osmolality values as indicators of physiological changes associated with increases in salinity (LeBreton and Beamish 1998, McKenzie et al. 1999, Martinez-Alvarez et al. 2002, Rodriguez et al. 2002, Jarvis and Ballantyne 2003). Studies of juvenile lake and Siberian sturgeons have demonstrated increases in plasma osmolalities with increasing salinity (LeBreton and Beamish 1998, Rodriguez et al. 2002). However, as observed in this study, hematocrit values of Siberian sturgeon were unaffected by increasing salinities (Rodriguez et al. 2002). In chronic salinity exposure experiments, the return of plasma osmolalities to freshwater levels demonstrated acclimation to brackish conditions for both juvenile Adriatic sturgeon (McKenzie et al. 1999, Martinez-Alvarez et al. 2002) and 16-month old shortnose sturgeon (Jarvis and Ballantyne 2003). Plasma osmolality values for sturgeons in this study and other studies were highly similar at similar salinities and sizes (LeBreton and Beamish 1998, McKenzie et al. 1999, Martinez-Alvarez et al. 2002, Rodriguez et al. 2002, Jarvis and Ballantyne 2003). However, an osmolality of 451 mOsm for lake sturgeon at 25 ppt (LeBreton and Beamish 1998) was significantly higher than the 342 mOsm plasma

osmolality observed for shortnose sturgeon at 24 ppt in this study. The observed differences may be related to the size and age of the fish used in the studies. The lake sturgeon were six years old (LeBreton and Beamish 1998) and thus would have larger gill surface areas with a higher number of chloride cells (McEnroe and Cech 1985).

BODY SIZE

Although other studies have demonstrated increasing salinity tolerance with increasing body size among sturgeons (McEnroe and Cech 1985, Jenkins et al. 1993, Cataldi et al. 1999), this study goes further by quantifying the changes in both salinity and temperature tolerances with increasing body size. The logistic regression model generated from data collected in 2006 suggests that slight changes in body weight (< 0.5 g) can significantly affect the probability of survival. In addition, the model demonstrates the significant interaction among weight, salinity, and temperature on the survival of YOY shortnose sturgeon. Both of these concepts were discussed by Miller et al. (1988), who used a comprehensive literature review to make inferences about factors that most significantly affect larval recruitment.

The substantial increase in survival probability with increasing weight may seem exorbitant, but the increase is actually sensible. Smaller fish have limited energy reserves, shorter reactive distances, limited swimming abilities, and limited search images (Hunter 1981, Blaxter 1986, Webb and Weihs 1986). Body size determines vulnerability to predators through differential encounter rates and predator gape limitation (Zaret 1980, Blaxter 1986). Body size also determines mouth gape, which limits the prey available to juveniles (Hunter 1981). Finally, body size can change the threshold effect of abiotic factors such as salinity and temperature, as was observed in this study.

LIMITATIONS

Although the results of this study are an important step in identifying physiological limitations and potential habitats of YOY shortnose sturgeon, the models used to explain the results do have limitations. First, the experiments were conducted in a hatchery setting using YOY that were artificially propagated from captive broodstock. The stability of the laboratory conditions also likely affected the tolerance results. Next, the experiments were conducted using dose-response methods to determine tolerance results. The relationships between treatment concentration and mortality were used to estimate lethal thresholds, which were presented as LC_{50} values. Although the LC_{50} method has become a widely accepted estimator of environmental tolerances, the LC_{50} method was developed for statistical and operational convenience and should not be used to document acceptable levels for variables of interest (Newman 1995).

Finally, the separation of salinity treatments, temperature treatments, and the average size of the fish in each experiment may have influenced the survival probabilities generated from the logistic regression model. Therefore, the equation should serve as an estimator for wild populations, but the results of both the logistic regression model and the LC_{50} experiments should be used as relative guides, not absolute thresholds. Alderice (1985) outlined a three-step process to study the affects of environmental variables on larval fish populations: (1) experimental definition of an organism's physiological capacities, (2) inferential determination of how those capacities are used in the natural environment, and (3) judgement of how these relations affect the distribution and abundance of the natural population. The results of this study should be regarded as fulfilling step one, while providing a framework to pursue fulfillment of steps two and three.

IMPLICATIONS

This study establishes physiological habitat bounds of salinity and temperature for YOY shortnose sturgeon. Such habitat bounds could be used to identify potential habitats of YOY shortnose sturgeon. Similar experimental data was used to identify suitable habitat for juvenile Atlantic sturgeon in Chesapeake Bay (Niklitschek and Secor 2005). In summer flow conditions, juvenile Atlantic sturgeon were subjected to a 3-way habitat squeeze from high temperatures, low dissolved oxygen concentrations, and increased salinities, with temperature being the key factor structuring habitat availability. In fact, a 1 °C bay-wide temperature increase resulted in a 65% reduction in suitable habitat (Niklitschek and Secor 2005). The survival probability equation generated from data collected in 2006 allows such information to be easily incorporated into models similar to those of Niklitschek and Secor (2005).

The information presented in this study can be used in other ways as well. First, it provides a framework for future studies to investigate such factors as acclimation temperature, rate of acclimation to experimental conditions, and effects of chronic salinity and temperature exposures. Effects of chronic salinity exposures on shortnose sturgeon were previously examined by Jarvis et al. (2001). Using a low feeding rate (0.75% per day) in chronic exposure tests, growth of 16-month old sturgeon was reduced with increasing salinity (Jarvis et al. 2001). However, the study was directed towards aquaculture and may not be directly applicable to wild populations because of differences in food availability and feeding rates.

Identification of physiologically suitable habitat will lead to field investigations to identify critical habitats in southeastern rivers. Once critical habitats are identified, the survival models can be used to evaluate risks to populations associated with future anthropogenic modifications to river morphology. This may lead to the development of regulations that restrict both the

number and scale of future modifications that could further degrade habitats of YOY shortnose sturgeon. Mitigation of hydrologic alterations is important not only for YOY of shortnose sturgeon, but also other fish and invertebrate larvae. Flow regulation has been shown to reduce the abundance of larval fish, alter taxonomic composition, and disrupt microhabitat relations (Scheidegger and Bain 1995).

Several studies of white sturgeon have demonstrated that hydrologic alterations negatively affect sturgeon populations (Sullivan et al. 2003, Geist et al. 2005, Snyder and Minshall 2005). In reservoirs, thermal stratification creates anoxic benthic conditions that limit suitable habitat and cause crowding of white sturgeon in remaining habitats (Sullivan et al. 2003). Nutrient retention by dams causes downstream food limitation and contributes to declines in white sturgeon populations (Snyder and Minshall 2005). In rivers with highly altered flow regimes, water temperature has the greatest effect on movement, oxygen consumption, and swimming speed of white sturgeon (Geist et al. 2005). These are just a few examples of how hydrologic alterations negatively affect sturgeon populations.

Although the availability of shortnose sturgeon prey has not been examined, previous studies have examined prey availability for Gulf sturgeon, *Acipenser oxyrinchus desotoi*, in the Suwannee River. Seasonal differences in habitat use in relation to environmental variables and benthic prey have been observed (Harris et al. 2005). Furthermore, prey items were significantly more abundant in the saline estuary than above the salt wedge (Brooks and Sulak 2005). Also, based on search patterns, prey density and prey-patch size may be more important than prey biomass for juvenile Gulf sturgeon (Brooks and Sulak 2005). Similar investigations should be conducted for shortnose sturgeon in southeastern rivers. If the emergence of prey does not match the migration patterns of YOY shortnose sturgeon, juvenile shortnose sturgeon may be

undersized and malnourished. In this regard, the salinity and temperature tolerance information presented in this study could be increasingly important for defining suitable rearing habitat.

CONCLUSIONS

This study provides acute salinity and temperature threshold information for YOY shortnose sturgeon ranging from 66-144 dph and 56-187 mm TL. The presented survival model allows probabilities of survival to be determined based on salinity (ppt), temperature (°C), and fish body weight (g). Such acute tolerance information can be used in habitat and population models and to structure chronic exposure experiments. Models and laboratory results should be used to structure field studies that identify critical YOY habitats, prey abundances, and growth rates of YOY shortnose sturgeon. Finally, field and laboratory data should then be used to guide mitigations of further anthropogenic disturbances to southeastern river systems.

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CHAPTER 3

THERMAL MAXIMA FOR JUVENILE SHORTNOSE STURGEON ACCLIMATED TO DIFFERENT TEMPERATURES[2]

[2] Ziegeweid, J.R., C.A. Jennings, and D.L. Peterson. To be submitted to *Environmental Biology of Fishes*.

ABSTRACT

Populations of shortnose sturgeon, *Acipenser brevirostrum*, in the southeastern United States continue to suffer from poor juvenile recruitment. High water temperatures, which are exacerbated by anthropogenic river modifications, are thought to affect recruitment by limiting available summer habitat. However, information regarding temperature thresholds of shortnose sturgeon is limited. This study used the thermal maximum method and a heating rate of 0.1 °C per minute to determine critical and lethal thermal maxima for young-of-the-year (YOY) shortnose sturgeon acclimated to temperatures of 19.5 and 24.1 °C. Fish were 0.6 to 35.0 g in weight and 64 to 140 days post hatch (dph) in age. Critical thermal maxima significantly increased with an increase in acclimation temperature ($p < 0.0001$). Lethal thermal maxima were significantly affected by acclimation temperature, the $\log_{10}$(fish weight), and the interaction between $\log_{10}$(fish weight) and acclimation temperature ($p < 0.0001$). Thermal maxima were used to estimate upper limits of safe temperature, thermal preferences, and optimal growth temperatures of YOY shortnose sturgeon. Upper limits of safe temperature were similar to previous temperature tolerance information and indicate that summer temperatures in southeastern rivers may be lethal to YOY shortnose sturgeon if suitable thermal refuge cannot be found.

INTRODUCTION

Populations of shortnose sturgeon, *Acipenser brevirostrum*, are severely depleted throughout the sturgeon's native range, from the Saint John River in New Brunswick, Canada to the St. Johns River, Florida (Vladykov and Greeley 1963). Commercial fishing in the late 19th century drastically reduced shortnose populations (Dadswell et al. 1984) and led to the protection of

shortnose sturgeon under the Endangered Species Act (Kynard 1997). However, the complex life history of shortnose sturgeon makes them a difficult species to manage, and as a result, many populations remain severely depleted. Degradations of habitat and water quality are believed to contribute to reduced juvenile recruitment (Smith and Collins 1996, Collins et al. 2000). However, a lack of field data has resulted in a poor understanding of habitat requirements for young-of-the-year (YOY) shortnose sturgeon (Kynard 1997).

Hydrologic alterations, such as the construction of dams, dredging, and water removal for waste treatment, agriculture, and drinking water all reduce river discharges and alter thermal regimes of rivers (Baxter 1977, Poff et al. 1997, Lydeard and Mayden 1995, Bunn and Arthington 2002, Pringle 2003). Thermal effluents cause further increases in water temperatures (Baxter 1977, Pringle 2003). River discharges also decrease with warm weather and reduced precipitation in summer. As a result, summer water temperatures in Georgia rivers often exceed 31 °C (DeVries 2006). Such high water temperatures may be extremely stressful and potentially lethal to YOY shortnose sturgeon.

Studies that examine the effects of temperature on sturgeon populations are limited. High temperature and hypoxia have been shown to reduce growth rates, increase mortality, and reduce swimming activities of white sturgeon, *Acipenser transmontanus* (Cech et al. 1984, Crocker and Cech 1997). Respiration and survival rates decreased for juvenile Atlantic sturgeon, *Acipenser oxyrinchus*, exposed to hypoxia and high temperatures (Secor and Gunderson 1998). The effects of temperature on green sturgeon, *Acipenser medirostris,* growth and bioenergetics have also been examined (Mayfield and Cech 2004, Allen et al. 2006). Mayfield and Cech (2004) also used thermal preference data to estimate lethal temperatures for green sturgeon. Studies of shortnose sturgeon have examined effects of temperature on spawning activity, egg

development, and seasonal movements (Hall et al. 1991, Collins and Smith 1993, Kynard 1997, Collins et al. 2002, Hardy and Litvak 2004, DeVries 2006).

Despite the threats that summer water temperatures pose to sturgeon populations, little work has been done to examine temperature thresholds for shortnose sturgeon. Laboratory experiments to determine LC_{50} temperatures for YOY shortnose sturgeon ranging from 68 to 128 mm TL have been conducted, and the results are presented in Chapter 2 of this thesis. However, the LC_{50} method has not used historically to determine lethal temperatures. Furthermore, fish used in LC_{50} experiments were acclimated to 19 °C, and acclimation temperature has been shown to affect thermal tolerance (Becker and Genoway 1979, Jobling 1981, Kilgour and McCauley 1986, Young and Cech 1996). Therefore, shortnose sturgeon thermal tolerances need to be further examined.

In this study, thermal maximum experiments were conducted with artificially propagated fish to examine how temperature tolerances of YOY shortnose sturgeon change with changes in acclimation temperature (Becker and Genoway 1979, Young and Cech 1996). Furthermore, thermal maximum data were used to estimate upper limits of safe temperature (Bridges 1971, Young and Cech 1996), final thermal preferences, and optimum growth temperatures for YOY shortnose sturgeon (Jobling 1981, Young and Cech 1996). Upon completion of experiments, total length, weight, and hematocrit levels of the fish at each acclimation temperature were measured to evaluate physiological condition of the fish in each treatment.

METHODS

FISH CULTURE

Hatchery-produced young-of-the-year (YOY) shortnose sturgeon of the 2006 year class were raised at the Warm Springs National Fish Hatchery for thermal maximum experiments. Fish were raised in two 120-L cylindrical, flow-through tanks. The tanks were supplied with un-chlorinated spring water via PVC pipes elevated about six inches above the tank. From this height, the falling water sufficiently mixed and oxygenated the water inside the tank. Suitable hardness, alkalinity, and pH were maintained in the source water with a chemical injection system (Hickson et al. 2001). Fish were fed a 3.0% body weight per day ration of Rangen® Soft-Moist commercial pellet feed (44% protein, 18% fat, < 5% fiber, < 8% ash, ~23% moisture). Tanks were siphoned at least once daily to remove accumulated waste and scrubbed at least once per week to remove algal growth on tank surfaces. A 14-hour light:10-hour dark photoperiod was maintained throughout the study.

Water in the two holding tanks was kept at two different experimental temperatures. Water in the first tank was maintained at 19.5 °C, with an approximate flow rate of 85 mL/s. The second tank was equipped with a 250-watt Won® titanium aquarium heater and kept at a flow rate of 10 mL/s to maintain an average temperature of 24.1 °C. This tank was further equipped with an airstone (connected to a central blower system) to help maintain suitable oxygen levels at lower flow and a higher temperature. Dissolved oxygen and temperature were checked daily with a YSI-85® multi-parameter meter, with other water quality variables, such as hardness, alkalinity, and pH were checked weekly with colorimetric test kits (Aquarium Pharmaceuticals, Inc.).

EXPERIMENTAL SETUP

Thermal maximum experiments were conducted in two 38-L glass aquaria. Each aquarium was wrapped in a layer of clear plastic and a layer of 0.5 inch Styrofoam to minimize heat loss during experimental trials. Each aquarium was equipped with a 250-watt Won® titanium aquarium heater, and water in each aquarium was circulated and oxygenated with an AquaClear Mini® biofiltration unit. Aquaria were elevated on 10-cm cinder blocks placed inside a 550-L cylindrical flow-through tank, which was used as a water bath to maintain specific water temperatures. One of the 38-L aquaria was maintained at a temperature of 19.9 °C, and the other aquarium was maintained at 24.7 °C with the aquarium heater. Both aquarium tanks were within 0.6 °C of the acclimation holding tanks.

EXPERIMENTAL PROTOCOL

Juvenile sturgeon were kept at their acclimation temperature for at least seven days and starved for 24 hours prior to use in thermal maximum experiments. One fish from each holding tank was placed in the corresponding experimental aquarium. Fish were given 15 hours to acclimate to the new tank conditions and recover from handling stress.

At the start of each trial, the water bath was drained, and both heaters were turned on. Water in the tanks was heated at a consistent rate of 0.1 °C/min (Becker and Genoway 1979, Young and Cech 1996). Temperatures were constantly monitored in each tank with two YSI® meters, models 85 and 58. One meter was used in each tank so that experiments could be conducted for each acclimation temperature simultaneously. Prior to beginning thermal maximum testing, the two meters were compared against each other and consistently read within 0.1 °C of each other.

The starting temperature of each tank was recorded, and temperatures were recorded every 10 minutes and at designated endpoints (loss of equilibrium and death).

Because shortnose sturgeon are demersal, both loss of equilibrium (LOE) and death were designated as endpoints. Fish were considered to have lost equilibrium when they were unable to right themselves within 10 seconds of first losing equilibrium. Fish were declared dead when opercular movements ceased and the fish did not respond to tactile stimulus. In accordance with Becker and Genoway (1979), LOE endpoints are referred to as critical thermal maxima (CT_{max}), while death endpoints are lethal thermal maxima (LT_{max}).

Upon completion of each trial, mortalities were removed from the tank, total length and weight were recorded, and tank water quality measurements were recorded. Dissolved oxygen concentrations were measured with a YSI-85® multi-parameter meter, and hardness, alkalinity, pH, ammonia, nitrite, and nitrate levels were determined by using colorimetric test kits (Aquarium Pharmaceuticals, Inc.). Finally, the exchange rate of the biofiltration unit was estimated by using a 1.0-L beaker to collect and measure the water leaving the unit for a period of 10 seconds.

Once the trials for fish at both acclimation treatments were completed, the equipment was turned off, the aquaria were cleaned with a mild disinfectant, rinsed, and refilled, and the equipment was reset in preparation for the next trial. Trials were repeated throughout the summer as the fish grew and developed. A total of 32 fish, ranging in age from 64 to 140 dph, were tested at each acclimation temperature. Weights ranged from 0.6 to 35.0 g, while total lengths ranged from 54-215 mm.

BLOOD ANALYSIS

At the conclusion of all thermal maximum experiments, remaining fish in the holding tanks were removed, anesthetized in 75 mg/L MS-222 (Hickson et al. 2001), and measured for total length (mm) and wet weight (g). Blood was collected in heparinized 75-µL microcapillary tubes through caudal puncture. The tubes were sealed and centrifuged at 11,500 rpm for 10 minutes in an International Equipment Company (IEC) microcapillary centrifuge, model MB®. Hematocrit values were determined with a microhematocrit reader.

STATISTICAL ANALYSIS

The normality of the distributions and the equality of the variances for the LOE and mortality data were examined using Shapiro-Wilkes and F_{max} tests, respectively (Sokal and Rohlf 1981). Results indicated that the data were normally distributed and that variances were not significantly different between treatments ($\alpha = 0.05$). In addition, sample sizes were relatively large ($n \geq 29$). Therefore, parametric statistical tests were used to analyze the data. Lethal thermal maximum data were compared using ANCOVA, with acclimation temperature as the categorical predictor variable and the $\log_{10}$(fish weight) as the continuous predictor variable. Weight data were linearized with log transformations because the rapid weight gain of YOY sturgeon skewed the distribution of the data. Upper limits of safe temperature (ULST) were determined by subtracting a safety factor of 5 °C from the lethal and critical thermal maxima data (Bridges 1971, Young and Cech 1996). Final thermal preferences (FTP) and thermal growth optima (TGO) were estimated from CTmax data with regressions modified from Jobling (1981):

$$FTP = (CT_{max} - 16.43)/0.66 \quad (10)$$

$$TGO = (CT_{max} - 13.81)/0.76 \quad (11)$$

Critical thermal maxima, holding tank water quality, experimental water quality, and post-experiment length, weight, and hematocrit data were compared between the two acclimation groups with two-sample t-tests ($\alpha = 0.05$).

RESULTS

WATER QUALITY

Water quality variables were maintained at adequate levels in the holding tanks. Temperatures were significantly different between holding tanks ($p < 0.0001$), with mean temperatures of 19.5 °C and 24.1 °C. Dissolved oxygen concentrations were significantly higher for the low acclimation temperatures ($p < 0.0001$), but the concentrations were adequate for both the low (8.3 mg/L) and high (7.4 mg/L) acclimation temperature treatments (Jenkins et al. 1993, Campbell and Goodman 2004). Mean hardness, alkalinity, and pH values for the holding tanks were 102.9 ppm (SE = 4.2, n = 60), 64.7 ppm (SE = 2.4, n = 52), and 6.9 (SE = 0.0, n = 60), respectively.

For thermal maxima trials, water quality variables were similar between experimental tanks for each acclimation temperature (Table 3.1). Furthermore, water quality variables in experimental tanks were maintained at levels that would not cause additional stress to the experimental fish (Table 3.1) (Piper et al. 1989). Ammonia, nitrite, and nitrate were rarely detected, and concentrations did not exceed 0.25 ppm, 0.25 ppm, and 5.0 ppm, respectively.

FISH CONDITION

Total length ($p = 0.6847$) and weight ($p = 0.5703$) of experimental fish were not significantly different between acclimation temperature treatments.

Table 3.1 Water quality variables for experimental tanks used to conduct thermal maximum experiments with YOY shortnose sturgeon. Water quality variables were compared between acclimation temperatures using two-sample t-tests, and values were based on experiments conducted with 32 fish at each acclimation temperature.

Variable	19.5 °C		24.1 °C		*p*-value
	Mean	SE	Mean	SE	
Dissolved Oxygen (mg/L)	6.6	0.1	6.3	0.1	< 0.0001
Hardness (ppm)	101.2	6.7	100.7	6.7	0.9530
Alkalinity (ppm)	62.7	2.8	63.8	2.9	0.7814
Ammonia (ppm)	0.0	0.1	0.0	0.1	1.0000
Nitrite (ppm)	0.0	0.1	0.0	0.1	1.0000
Nitrate (ppm)	0.0	0.0	0.2	0.2	0.3212
Exchange rate (mL/sec)	62.5	1.2	64.5	1.1	0.8916
pH	7.6	0.0	7.6	0.0	0.2895

The mean total length and weight of fish used in thermal maxima trials were 135 mm and 10.7 g, respectively. Furthermore, total lengths ($p = 0.8809$) and weights ($p = 0.8638$) of remaining holding tank fish were not significantly different between acclimation temperatures, with averages of 182 mm and 21.1 g. Finally, hematocrit levels of remaining holding tank fish were not significantly different between acclimation temperatures ($p = 0.9896$), demonstrating that oxygen requirements and general physiological condition were not affected by acclimation temperature.

THERMAL MAXIMA

The mean CT_{max} for fish acclimated to 24.1 °C was significantly ($p < 0.0001$) higher (35.1 °C) than the mean CT_{max} for the fish acclimated to 19.5 °C (33.7 °C) (Table 3.2). For LT_{max} data, the effects of acclimation temperature and the $\log_{10}$(fish weight), as well as the interaction between acclimation temperature and $\log_{10}$(fish weight), were significant ($R^2 = 0.87$, $p < 0.0001$), and the following equations describe the relationships of the predictor variables:

$$\text{Low Temperature (19.5°C) } LT_{max} = 35.19 + 0.30[\log_{10}(\text{fish weight})] \quad (12)$$

$$\text{High Temperature (24.1°C) } LT_{max} = 35.82 + 0.49[\log_{10}(\text{fish weight})] \quad (13)$$

The distribution of LT_{max} data for fish at each acclimation temperature is shown in Figure 3.1. Mean LT_{max} values were 36.1 °C and 34.8 °C for YOY shortnose sturgeon acclimated to temperatures of 24.1 °C and 19.5 °C, respectively (Table 3.2). Mean LT_{max} and CT_{max} values are given in Table 3.2. The sample size for the CT_{max} estimates is different for fish acclimated to 19.5 °C because three of the fish in the low temperature trials died without first losing equilibrium. Estimated final thermal preferences and thermal growth optima were 26 °C for the low acclimation temperature fish and 28 °C for the high temperature fish (Table 3.2).

Table 3.2. Lethal thermal maxima (LT_{max}), upper limits of safe temperature (ULST), critical thermal maxima ($CT_{max)}$, final thermal preferences (FTP), and thermal growth optima (TGO) for YOY shortnose sturgeon. Fish were acclimated to two different temperatures, and ages ranged from 64 to 140 days post hatch (dph). The LT_{max} and CT_{max} were significantly higher at the higher acclimation temperature. Standard errors are listed in parentheses.

Temp (°C)	N_{LT}	LT_{max} (°C)	$ULST_{LT}$	N_{CT}	CT_{max} (°C)	$ULST_{CT}$	FTP	TGO
19.5	32	34.8 (0.1)	29.8	29	33.7 (0.3)	28.7	26.2	26.2
24.1	32	36.1 (0.1)	31.1	32	35.1 (0.2)	30.1	28.3	28.0

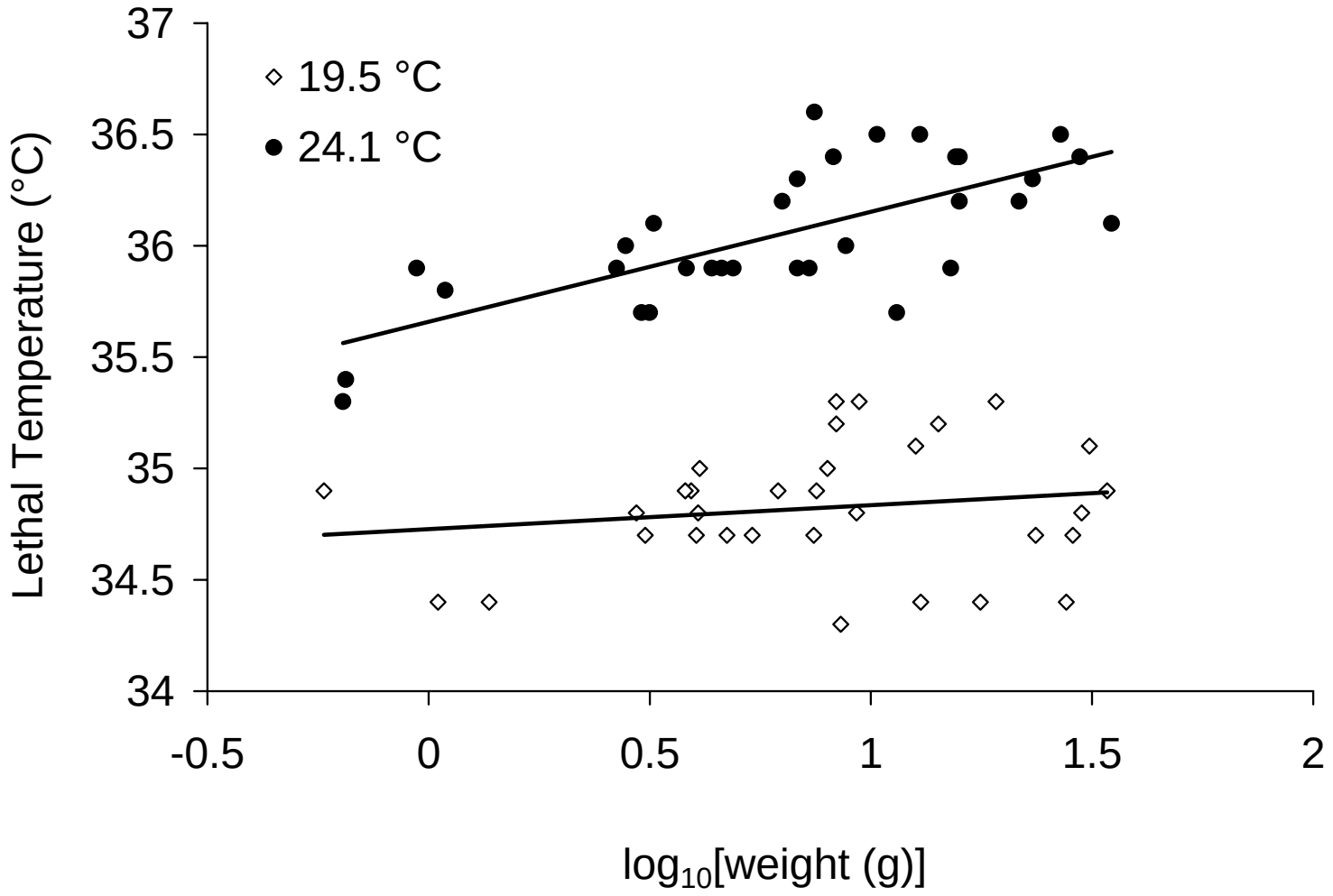

Figure 3.1. Lethal thermal maxima distribution for YOY shortnose sturgeon raised at two different acclimation temperatures.

Estimated upper limits of safe temperature ranged from 28.7 °C to 31.1 °C across critical and lethal thermal maximum data (Table 3.2).

DISCUSSION

Because shortnose sturgeon are demersal, assigning them a LOE criteria can be difficult. In this study, fish exhibited LOE in several different ways. Three fish at the low acclimation temperature died without losing equilibrium. Some fish tilted sideways but were still able to swim, whereas others turned completely upside down and were unable to swim. Similar problems in defining a LOE endpoint were noted for green sturgeon (Mayfield and Cech 2004). Therefore, in addition to recording a LOE endpoint (CT_{max}), lethal temperatures also were recorded as more definitive endpoint (LT_{max}).

Thermal tolerances of YOY shortnose sturgeon were significantly affected by acclimation temperature and body weight. A 4.6 °C increase in acclimation temperature resulted in a 1.4 °C increase in mean CT_{max}. A 4.6 °C increase in acclimation temperature also resulted in a 1.3 °C increase in mean LT_{max}, indicating that death occurred at a relatively constant interval following LOE. In addition to acclimation temperature, LT_{max} values also were significantly affected by $\log_{10}$(fish weight) and by the interaction of $\log_{10}$(fish weight) and acclimation temperature. The LT_{max} increased with increasing weight, although the magnitude of this increase was greater for fish acclimated to the higher temperature (24.1 °C).

Fish exhibited similar behaviors with increasing temperature regardless of acclimation temperature. As temperatures increased, fish activity increased. About 5-6 °C prior to the lethal endpoint, fish began frantically swimming around the tank, presumably looking for an escape

route. As fish began to lose equilibrium, their activity level decreased dramatically, and at about 0.3 °C before the lethal endpoint, most fish were completely incapacitated.

The results of this study were similar to results of LC_{50} experiments given in Chapter 2 for fish acclimated to a temperature of 18.8 °C. In the three LC_{50} experiments of Chapter 2, the highest temperatures at which mortalities did not occur increased with size and were 26.5 °C, 28.5 °C, and 30.0 °C. The lowest temperatures at which mortalities did occur were 30.1 °C, 30.0°C, and 31.5 °C, respectively. The averages of these described values create an interval of (28.3 °C, 30.5 °C) that can approximate an upper limit of safe temperature (ULST) for YOY shortnose sturgeon. Using the estimated interval for an ULST, the data from Chapter 2 can be compared to the data from this study. Upper limits of safe temperature based on LOE data in this study were 28.7 °C and 30.1 °C for fish acclimated to 19.5 °C and 24.1 °C, respectively. Upper limits of safe temperature based on mortality data in this study were 29.8 °C and 31.1 °C for fish acclimated to 19.5 °C and 24.1 °C, respectively. Upper limits of safe temperature between studies were highly similar despite differences in acclimation temperatures, measured endpoints, and methodologies.

This study also illustrates the importance of acclimation temperature in evaluating thermal tolerance, which was not evaluated in Chapter 2 of this thesis. Thermal tolerance of YOY shortnose sturgeon increased with increasing acclimation temperature, which is similar to results of the euryhaline splittail, *Pogonichthys macrolepidotus* (Young and Cech 1996). However, the increase in thermal tolerance was not as pronounced in shortnose sturgeon. In this study, a 4.6 °C increase in acclimation temperature only resulted in a 1.3 °C increase in lethal temperature, whereas a comparable increase in splittail acclimation temperature (5 °C) resulted in an 8 °C increase in thermal tolerance (Young and Cech 1996).

Although the thermal maximum method can be used to produce consistent, cost-effective results, the method has limitations. Final tolerances are influenced by acclimation temperature, thermal history, and heating rate (Becker and Genoway 1979, Jobling 1981, Kilgour and McCauley 1986), and the results of this study already demonstrated that acclimation temperature significantly affects thermal tolerance. A heating rate that is too fast will result in thermal shock, whereas a heating rate that is too slow may result in partial acclimation of the fish, biasing the tolerance limit upward. The heating rate should be just fast enough to allow deep-body temperatures to parallel test temperatures without a significant time lag (Becker and Genoway 1979). The time lag for deep-body temperatures depends on the size of the test fish, but a heating rate of < 1 °C per minute typically will not result in a significant time lag (Becker and Genoway 1979).

Concerns about heating rates in thermal maximum experiments can be addressed by conducting studies with a variety of heating rates. Studies of juvenile coho salmon (*Oncorhynchus kisutch*) and pumpkinseed sunfish (*Lepomis gibbosus*) heated at a variety of rates ranging from 0.017 to 1.0 °C per minute, suggest an optimum heating rate of 0.3 °C per minute (Becker and Genoway 1979); however, a heating rate of 0.1 °C per minute did not significantly bias the thermal maxima upward (Becker and Genoway 1979). Because coho salmon and pumpkinseed sunfish differ substantially in life history and body shape, a heating rate ranging from 0.1 °C per minute to 0.3 °C per minute should be adequate for most small fish. Therefore, we assumed that the heating rate of 0.1 °C per minute used in this study did not affect the thermal tolerances of YOY shortnose sturgeon.

Published equations were used to estimate final thermal preferences (FTP) and thermal growth optima (TGO) for YOY shortnose sturgeon (Jobling 1981, Young and Cech 1996). The

FTP estimates were similar to TGO estimates of fish acclimated to the same temperature, with values of approximately 26 °C and 28 °C for fish acclimated to 19.5 °C and 24.1 °C, respectively. The FTP and TGO estimates were close to the estimated upper limits of safe temperatures of 28.7 °C and 30.1 °C for fish acclimated to 19.5 °C and 24.1 °C, respectively. Similar results were observed for YOY splittail (Young and Cech 1996). Warmer temperatures may increase YOY metabolism and conversion of prey into somatic growth, as long as the cost of maintenance metabolism does not significantly increase (Brown 1957).

Temperature optima of many fishes are highest during early life history stages and decrease with ontogeny (Brett and Groves 1979, Kitchell 1979, Lankford and Targett 1994). For example, CT_{max}, ULST, FTP, and TGO decreased with increasing age for splittails acclimated to 17 °C (Young and Cech 1996). In addition, juvenile green sturgeon grew better at 24 °C compared to 19 °C (Allen et al. 2006), even though a previous study had both estimated an upper temperature limit of 27 °C and reported significant transport-related mortality for fish acclimated to 25 °C (Mayfield and Cech 2004). Similar trends in thermal tolerance are likely for shortnose sturgeon, although thermal maximum experiments with older juveniles are needed to confirm this relationship.

The thermal tolerance results of this study have important implications. Summer water temperatures in Georgia rivers frequently exceed 31 °C (DeVries 2006), which would be potentially lethal according to the results presented in this study. Furthermore, at high temperatures, food availability may not be able to keep up with the metabolic needs of YOY shortnose sturgeon. Therefore, the identification of thermal refugia in southeastern rivers should be an important study component for management plans that seek to improve juvenile recruitment. In addition to numbers of thermal refuges, both the size of thermal refuges and the

distance between thermal refuges should be examined. If YOY sturgeon are forced to migrate long distances in thermally stressful water, they would become more susceptible to predation, disease, or death. These effects would be exacerbated if fish were forced to remain in sub-optimal habitats for extended periods.

CONCLUSIONS

The results of this study provide information that should help structure future YOY shortnose sturgeon investigations to evaluate the hypotheses advanced in this discussion. First, the thermal tolerances in this study place physiological limits on suitable habitats in southeastern rivers. Suitable habitats can be sampled to collect wild YOY shortnose sturgeon. Field collections of YOY shortnose sturgeons would facilitate identifications of critical habitats of YOY shortnose sturgeon in southeastern rivers. Furthermore, results of this study may be used to mitigate future modifications that could alter thermal regimes of southeastern rivers. In addition, results can be used to develop laboratory experiments that further evaluate the effects of temperature on growth and development.

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CHAPTER 4

CONCLUSIONS

In this thesis, two years of experimental data were used to examine salinity and temperature tolerances of young-of-the-year (YOY) shortnose sturgeon, *Acipenser brevirostrum*. All experiments were conducted with artificially-propagated fish that were raised in a laboratory. Several salinity and temperature LC_{50} experiments and one factorial experiment were conducted in 2005. Results of 2005 experiments were used to design the factorial and thermal maxima experiments that were conducted in 2006.

Salinity has significant lethal and sub-lethal effects on juvenile sturgeons. In the first study presented in this thesis, survival of YOY shortnose sturgeon decreased with increased salinity, but lethal salinity thresholds increased with increased fish size. Salinity LC_{50} values ranged from 14.8-20.9 ppt for YOY shortnose sturgeon that ranged from 56-128 mm TL. Salinity tolerance data from this thesis and other studies indicate that juvenile white sturgeon, *Acipenser transmontanus*, Adriatic sturgeon, *Acipenser naccarii*, and shortnose sturgeon have similar salinity tolerances at similar sizes (McEnroe and Cech 1985, Jenkins et al. 1993, Cataldi et al. 1999). Furthermore, the results of this thesis and previous studies demonstrate that plasma osmolalities of juvenile sturgeons are highly similar among sturgeon species and increase with increases in salinity and body size (LeBreton and Beamish 1998, McKenzie et al. 1999, McKenzie et al. 2001, Martinez-Alvarez et al. 2002, Rodriguez et al. 2002, Jarvis and Ballantyne 2003). Therefore, salinity tolerance in juvenile sturgeons is likely based on a combination of

organ development and ion flux rates. Ion flux rates are a function of gill surface area in relation to body size (Muir 1969, McEnroe and Cech 1985, Cataldi et al. 1999).

The results of the studies presented in this thesis demonstrate that temperature has significant lethal and sub-lethal effects on YOY shortnose sturgeon. Survival of YOY shortnose sturgeon decreased with increased temperatures, but lethal temperature thresholds increased with increased size and acclimation temperature. Temperature LC_{50} estimates ranged from 28.2 °C to 30.7 °C for YOY that ranged from 68-128 mm TL. Upper limits of safe temperature were similar to LC_{50} estimates and ranged from 28.7 °C to 31.1 °C. However, hemolysis and reduced hematocrit levels demonstrated that fish that survived exposure to a temperature of 31 °C were significantly stressed. The preferred and optimum growth temperature of fish acclimated to 19.5 °C was about 26 °C. In contrast, the preferred and optimum growth temperature of fish acclimated to 24.1 °C was about 28 °C. The results of this thesis suggest that summer water temperatures of rivers in the southeastern United States may exceed safe thermal limits of YOY shortnose sturgeon and thus limit juvenile recruitment in some years.

Results of factorial experiments demonstrate that salinity (ppt), temperature (°C), and fish weight (g) significantly interact to affect the survival of YOY shortnose sturgeon. The survival probability model that was generated from the results of the factorial experiments has several potential applications. First, the model can be used to identify seasonal and flow-related changes in physiologically suitable habitat of YOY shortnose sturgeon for rivers in the southeastern United States. Second, the model can be used to design field experiments with the goal of collecting YOY shortnose sturgeon and identifying critical habitats for rivers in the southeastern United States. If critical habitats are identified, the model can be used to estimate effects of

proposed river modifications on shortnose sturgeon recruitment. This may lead to further regulation of anthropogenic disturbances to rivers in the southeastern United States.

Although the results presented in the two papers of this thesis are important, tolerance thresholds presented here should not be considered absolute thresholds. Laboratory experiments such as these do not consider effects of several factors that can influence survival of YOY shortnose sturgeon. Such factors include parental fitness, the timing, availability, and abundance of prey resources, effects of flow regulation on downstream movement patterns, distance from spawning grounds to estuaries, predation, parasitism, disease, competition, dissolved oxygen concentrations, and variability of growth rates among wild populations. Finally, results should only be applied to southern populations of shortnose sturgeon, because northern and southern populations are genetically and ecologically different (Kynard 1997).

In summary, salinity, temperature, and body weight affect YOY shortnose sturgeon survival, both individually and interactively. Increases in salinity and temperature decrease the probability of survival for YOY shortnose sturgeon, and increases in body weight increase the probability of survival for YOY shortnose sturgeon. Hematocrit and plasma osmolality values demonstrate that YOY shortnose sturgeon undergo substantial physiological changes to survive changing salinity and temperature conditions. Further studies are needed to evaluate how salinities and temperatures affect the distribution, abundance, and condition of wild YOY shortnose sturgeon and their prey.

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