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Upper Range Thermal Stress Tolerance in Channel and Hybrid Catfish Strains

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By

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Channel catfish (*Ictalurus punctatus*) have a broad distribution from Canada to Mexico, suggesting that different strains may have different thermal tolerances. In aquaculture, daily temperature maximums up to 36-40°C and fluctuations of 3-6°C occur, and may be exacerbated by future climate change. To quantify differences in thermal tolerance amongst geographically-distinct channel catfish strains and corresponding hybrid catfish (*I. punctatus* x [blue catfish] *I. furcatus*): acute critical thermal maximum (CTmax), and the effects of chronic thermal regimes on growth, survival and differential gene expression were examined. Southern channel catfish had higher CTmax than northern, and channel catfish had higher CTmax than hybrid catfish. Under chronic thermal stress, hybrid catfish had the greatest survival and most consistent growth. Further, northern channel catfish had the greatest magnitude and largest amount of upregulated gene transcripts in response to high temperatures, indicating greater thermal stress. Therefore, catfish thermal tolerance varies by geographic region and species.

DEDICATION

I would like to dedicate this research to my parents, Karen Aprill and Robert Stewart who always believed in me and have supported my passion for science. Be it taking me on nature hikes before I could walk, buying my first microscope and test tube set at the age of five or encouraging me to ask questions. To my sister, best friend, and travel companion, Winn Stewart, for delaying our trip to Machu Picchu. To my grandparents, Nita and Jerry Aprill, for their unwavering love, nurturing my curiosity, and showing me that challenges are just opportunities. In memory of Dorothy Vickers-Shelley who taught me the power of knowledge and importance of today.

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CHAPTER I

INTRODUCTION

Global climate is changing more expeditiously than previously recorded (Ficke *et al.* 2007). Natural changes in climate have been exacerbated by greenhouse gas emissions generated by fossil fuels used to create energy for humans (Mann et al 1998). Post-industrial anthropogenic impacts have been quantified by the use of paleoclimatic data obtained from sediment cores, coral, trees and glacial ice cores (Melack *et al.* 1997; Spray & McGlothlin 2002). This data shows that greenhouse gases: carbon dioxide (CO_2), ozone (O_3), nitrogen dioxide (NO_2), and methane (CH_4) are vastly increasing after remaining relatively stable for tens of thousands of years (Hansen & Lacis 1990; Ficke *et al.* 2007). Higher concentrations of these gases alter the global energy balance thus trapping infrared radiation which heats the earth's surface and lowers protection from the harmful ultraviolet rays produced by the sun known as the greenhouse effect (Solomon *et al.* 2007). Effects of the energy imbalance are expressed by increased solar radiation and atmospheric warming, eutrophication, more pronounced stratification of inland water, reduced precipitation, increased evaporation, decreased water pH, and extreme weather events (Brander 2007) .

In temperate zones, the direct impacts of climate change result in temperatures exceeding normal ranges, which also may exceed optimal ranges for cultured organisms. Climate change is particularly consequential to fish as poikilotherms, because

temperature directly affects habitat selection and physiological processes of metabolism, energy expenditure, growth, and reproductive migration (Crawshaw & Hammel 1974; Hutchinson & Maness 1979; Gunter *et al.* 2007). In aquaculture, such changes can have undesirable consequences, such as: earlier spawning (Brander 1994; Barange & Perry 2009) which can lead to logistical and environmental complications in production, decreased production, or render species no longer suitable for previously ideal culture regions. With changing climate, traditional aquaculture species might need to be moved to cooler water to maintain production or be replaced with more tolerant species (Clemmensen *et al.* 2007). Further, there are detrimental indirect impacts of climate change to the aquaculture industry, including: limitation of fishmeal and fish oil for feed production, increased prevalence of pathogens, and decreased biodiversity (Handisyde *et al.* 2006; De Silva & Soto 2009).

Rapid changes in water temperature are a concern of climate change for fish production because they lead to thermal shock and may also affect thermoregulatory behavior. In fishes, preferred and avoided temperature ranges may be modified due to damage to the central peripherial receptors or preoptic region (Crawshaw 1975; Prosser & Nelson 1981). If the thermal shock is below lethal levels, it may allow fish to alter their physiology to a point at which alternative habitats can be utilized (Bevelhimer & Bennett 2000; Browse & Xin 2001; Pörtner 2002). In catfish production ponds there is no alternative habitat for fish to utilize during times of thermal stress, therefore, the fish must acclimate or die.

Temperature dynamics in pond aquaculture systems

Pond aquaculture systems are extremely prevalent in the US catfish industry with over 95% of channel catfish grown in ponds, due to low capital cost, and relatively reliable fish production (Brune *et al.* 2004; Tucker *et al.* 2004). Although the capital costs are low, these systems will only be profitable if used in regions with temperature regimes that approach optimal temperature ranges for a species, since water temperatures in a pond setting cannot be controlled. Most ponds have a depth of less than 1.5 meters for ease of seining, minimizing electric and maintenance costs and most importantly maximizing photosynthesis and net primary productivity which in exchange oxygenates the pond and provides additional nutrition to the fish (Boyd 2004; Tucker & Hargreaves 2004). Water temperatures in these systems are susceptible to fluctuations facilitated by the shallow design under direct sunlight. Temperatures in aquaculture ponds in the Southeastern US experience fluctuations of 3-6°C from morning to afternoon (Wax *et al.* 1987; Arnold *et al.* 2013) with daily maximums up to 36-40°C for short durations of time during the summer (Liu *et al.* 2013). Global climate change may exacerbate these temperature fluctuations and increase maximum daily temperatures.

Channel catfish *Ictalurus punctatus* have a natural geographic distribution from southern Canada to northern Mexico which encompasses a thermal range from 5-35°C (Scott & Crossman 1973; Bennett *et al.* 1998; Tavares-Dias & Moraes 2007). Channel catfish are found in a vast range of environmental conditions (i.e. temperature, salinity and turbidity) (Jackson 2004). This resilience is one of the factors that make channel catfish an ideal aquaculture species. Channel catfish account for over 60% of all aquaculture production in the United States (US) with 82% of catfish production taking

place in Mississippi, making it an economically important species (Mott & Brunson 1995; Currie *et al.* 1998; Jiang *et al.* 2011). In 2012, the US catfish industry made $341 million with Mississippi, Alabama, Arkansas and Texas accounting for 95% of the total sales (NASS 2013). US catfish production is environmentally sustainable, has low impact on wild populations, minimal effluent discharge from earthen ponds and uses low levels of fishmeal (Simmons *et al.* 2006; Liu *et al.* 2008).

Strain Selection

Currently there are numerous strains of channel catfish in the US with geographically separated populations. Extensive breeding programs dating back to 1890 have led to difference in growth rate, resistance to infection and/or disease, time of spawning, dress-out percentage, feed conversion efficiency, tolerance to low oxygen, and ability to escape seining between strains (Leary 1910; Andrews & Stickney 1972; Li *et al.* 2001). Previous research on growth rates in geographically distinct populations of fish has found that warmwater species grow faster in the warm part of their range (Lagler *et al.* 1977). This is why catfish production has flourished thus far in the Southeastern US. With climate change causing increases of temperature outside of the optimal range of catfish, production in this area may decline. McCauley & Beitinger (1992) predict that the zone of primary catfish production will move 240 km north in the central part of the US with each 1°C increase of annual temperature. If current atmospheric models hold true and mean global temperature increases of 1-7°C occur (Ficke *et al.* 2007), then this could be detrimental to the Southeastern US catfish industry.

Hybridization of catfish species can improve production by selecting ideal traits from the parents to pass on to offspring. Although there are different species of catfish,

the only catfish hybridization that produces favorable commercial application is a cross between *I. punctatus* and blue catfish *I. furcatus*. Breeding programs for channel and blue catfish were established over 25 years ago (Wolters & Tiersch 2004). The best cross is with an *I. punctatus* female and an *I. furcatus* male, the reciprocal cross does not have the same heterosis (Dunham & Smitherman 1983). These crosses tend to have higher dress-out percentages, faster growth, easier harvest by seining and angling, more uniform size at harvest, greater resistance to enteric septicemia, and greater tolerance of low oxygen levels and crowding in pond systems (Giudice 1966; Yant *et al.* 1976; Dunham *et al.* 1983; Smitherman *et al.* 1983; Li *et al.* 2004; Ligeon *et al.* 2004; Dunham & Argue 2011; Kumar & Engle 2011). Blue catfish have a slightly more southern overall distribution ranging from the Mississippi River basin and coastal drainages along the northern Gulf of Mexico Coast through Mexico and into Guatemala and Belize (Graham 1999).

Heat Tolerance

Catfish have optimal temperature ranges at which best physiological performance occurs. Knowledge of the optimal temperature is essential to maximizing aquaculture yield because it indicates where maximum growth occurs and physiological functions are optimal (McCauley & Casselman 1981; Kellog & Gift 1983). This fundamental step has already been completed by previous studies, which have demonstrated superior weight gain at temperatures ranging from 26.6-32°C for channel catfish (Shrable *et al.* 1969; Kilambi *et al.* 1971; Andrews & Stickney 1972; Hariyadi *et al.* 1994; Buentello *et al.* 2000; Li *et al.* 2008; Arnold *et al.* 2013).

To determine the thermal tolerance of fish, research can examine acute thermal tolerance, chronic thermal tolerance or both. For both types of thermal tolerance, fish must display acclimation. Heat tolerance acclimation can be defined with regard to the interrelationships between magnitude of final acclimation temperature, direction of temperature change and absolute changes in thermal level. Acclimation allows an organism to tolerate new environmental conditions by going through reversible physiological changes (Rickleffs 1990). Since the 1920s research has been ongoing to determine temperature tolerance of various fish species. Acute heat tolerance has been detected using two methods. The first method is to quantify static temperatures with incipient upper lethal temperature (IULT). The second technique assesses dynamic temperatures with critical thermal maxima (CTM). With the static method IULT is determined when 50% of the fish being sampled die after being exposed to an abrupt thermal change from acclimated temperatures. The IULT method requires a large sample size and provides more physiologically relevant view rather than ecological (Currie *et al.* 1998).

Cowles & Bogert (1944) first introduced and defined the CTM method which has been modified and standardized by Lowe & Vance (1955), Hutchinson (1961) and Cox (1974) to be defined as: the collective maximum thermal points at which locomotory activity becomes disorganized and the fish loses its ability to escape conditions that will quickly lead to its death. Further, this arithmetic mean of thermal points is reached after a constant, progressive temperature increase from a previous acclimation temperature without a significant lag time (Becker & Genoway 1979). The main difference between CTM and IULT methods is that CTM requires a progressive change of temperature until

a physical disorganized response occurs, where IULT requires an abrupt change until a lethal response occurs (Becker & Genoway 1979). Hutchinson (1976) had categorized this physical disorganized response as a 'loss of muscular coordination' whereas Fry (1967) called it 'locomotory disorganization collapse.' The CTM provides a more relevant point of reference than static methods and is ecologically valuable because it identifies the first signs of stress (Paladino *et al.* 1980; Díaz & Bückle 1999). The CTM is an estimate of thermal tolerance defined as the mean temperature fish can reach at a nonlethal, yet near lethal, end point if water temperature exposure is slow and constant (Cox 1974). CTM can be calculated with a high level of certainty for acclimation levels between 10-35°C for any duration until complete acclimation with the multiple-regression model proposed by Bennett *et al.* (1998). The same equation can be reorganized to instead determine the number of days required to reach a particular acclimation level (Bennett *et al.* 1998).

In natural environments fish may encounter temperatures outside of their tolerance range, but typically only for brief periods, due to acute fluctuations. Examining the effects of such situations provided guidelines for best culture management practices (Brett 1956; Hutchinson 1976). The advantages of the CTM technique are speed, effectiveness using a small sample size, and ability to evaluate thermal stress without inducing handling stress (Currie *et al.* 1998).

Effects of Temperature on Growth

In addition to short-term, acute effects of temperature, chronic high thermal ranges are of great importance due to their effect on growth of catfish. Andrews & Stickney (1972) studied interactions of feeding rates and environmental temperature on

growth of channel catfish. In their study, fish were subjected to three different feeding rates and divided up into five temperature treatments (18, 22, 26, 30, and 34°C) for 12 weeks. The 30°C treatment had the maximum weight gain at all feeding rates. The environmental temperature increased the lipid level of the fish as temperatures continued to rise. Fish at 34°C had 43.6% lipid compared to fish at 30°C with 38.8%, yet the fish at 30°C gained more than those at 34°C. High percentages of lipids can be an issue with commercial production because fatty fish have lower dress-out percentages, lipids hold off-flavoring and overall are a less desirable product for the consumer. Therefore, in order to maximize production and product quality, they recommended rearing at 30°C. In similar studies on channel catfish, Shrable *et al.* (1969) found that temperatures from 26.6-29.4°C allow the most rapid rate of digestion. Buentello *et al.* (2000) reported maximum weight gain at 27.1°C and Kilambi *et al.* (1971) demonstrated that 32°C is the optimum condition for growth.

Objectives

To examine thermal tolerance in channel and hybrid catfish, this research was divided into three studies; acute thermal tolerance, chronic thermal tolerance and gene expression. The main objectives were:

1. to quantify acute thermal tolerance differences in two geographically distinct strains of channel catfish and the corresponding hybrid from these strains with a cross to an industry standard blue catfish strain,

2. to quantify chronic thermal tolerance differences and growth rates of two geographically distinct strains of channel catfish and the corresponding hybrid from these strains with a cross to an industry standard blue catfish strain, and
3. to explore differential gene expression under chronic optimal and upper thermal regimes of two geographically distinct strains of channel catfish and the corresponding hybrid from one these strains with a cross to an industry standard blue catfish strain.

These studies are different than those previously performed because clearly defined breeding lines were chosen and the fish were subjected to all the same conditions to allow direct comparison. These objectives will examine whether geographical strain of channel catfish or the more southern range of blue catfish may increase thermal tolerance. To get a bigger picture of thermal tolerance, both acute and chronic effects were explored. Acute tolerance studies will quantify temperature at which loss of equilibrium occurs and survival following thermal stress. For chronic tolerance, temperature effects on growth, and survival will be quantified. Further, RNA will be extracted from liver tissue of catfish in the chronic study to examine differentially expressed gene transcripts between catfish types. These studies will assist with future selection of broodstock catfish for warm water aquaculture and identify expression biomarkers related to thermal tolerance in channel and hybrid catfish.

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CHAPTER II

CRITICAL THERMAL MAXIMA OF TWO GEOGRAPHIC STRAINS OF CHANNEL AND HYBRID CATFISH

Abstract

Critical thermal maxima have been used extensively to provide physiologically and ecologically valuable reference points that identify early signs of thermal stress. In catfish pond culture, daily temperature maximums up to 36°C and fluctuations up to 6°C are observed. These extreme conditions are likely to be exacerbated by the effects of global climate change. Channel catfish (*Ictalurus punctatus*) have a broad natural distribution from southern Canada to northern Mexico. It was hypothesized that regional genetic differences would cause strains with a southern distribution to have greater thermal tolerance than strains with a northern distribution, and consequently a greater critical thermal maximum. Hybrid catfish (*I. punctatus* x [blue catfish] *I. furcatus*) strains were expected to have greater critical thermal maxima than their respective channel catfish strains due to the more southern distribution of blue catfish. To examine this, we quantified acute thermal tolerance differences of two geographically distinct strains of channel catfish and their hybrid cross with an industry standard strain of blue catfish. Catfish were subjected to water temperature increase at a rate of 2.0±0.1 °C hour^{-1} until loss of equilibrium occurred. Standard length ranged from 162-320 mm. Length had a significant effect on survival with greater survival in larger fish. Critical thermal

maximum ranged from 38.6−40.3°C. Southern channel catfish were able to tolerate higher temperatures than northern, and channel catfish tolerated higher temperatures than hybrid catfish. This study indicates that geographically distinct catfish strains differ in acute thermal tolerance, and suggests heritability for this trait as evidenced by similar responses in channel catfish and their corresponding hybrid cross with blue catfish.

Introduction

Environmental conditions such as temperature affect aquaculture productivity by altering fish growth, reproductive capacity, physiology, behavior, immune system function, and mortality (Brett 1956; Brett 1979; McCauley & Beitinger 1992; Brandt 1993; Le Morvan *et al.* 1998; Lang *et al.* 2003; Brander 2007; Ficke *et al.* 2007). Since the 1920s, research has been ongoing to determine temperature tolerance of various fish species (Hathaway 1927) to cold and heat. For heat tolerance, there are two primary methods; incipient upper lethal temperature (IULT) (Fry 1947; Allen & Strawn 1968) and critical thermal maximum (CTmax) (Becker & Genoway 1979; Díaz & Bückle 1999). CTmax provides a more ecologically relevant point of reference, identifying early signs of stress (Paladino *et al.* 1980; Díaz & Bückle 1999). Since its introduction, critical thermal methodology has been modified and standardized to be defined as: the mean maximum thermal point a fish can reach over a slow and constant exposure; at which locomotive activity becomes disorganized and the fish loses its ability to maintain dorso-ventral orientation (Cowles & Bogert 1944; Cox 1974; Becker & Genoway 1979; Currie *et al.* 1998). In natural environments fish typically encounter temperatures outside of their tolerance range for brief periods, known as acute fluctuations. Examining thermal

sensitivity to acute fluctuations using CTmax can provide guidelines for best culture management practices (Brett 1956; Hutchison 1976).

Pond aquaculture systems are extremely prevalent in the US catfish industry with over 95% of channel catfish grown in ponds (Brune *et al.* 2004; Jackson 2004). Water temperatures in ponds cannot be controlled, and due to shallow depths (<1.5 m), may have large daily fluctuations (i.e. 3-6°C) and reach high daily maximums up to 36-40°C (Wax *et al.* 1987; Arnold *et al.* 2013; Liu *et al.* 2013).

Catfish aquaculture utilizes channel catfish *Ictalurus punctatus* and the hybrid between female channel catfish and male blue catfish *I. furcatus*, with both species occupying broad geographic ranges. The natural geographic distribution of channel catfish ranges from southern Canada to northern Mexico which encompasses a natural thermal range of 5-35°C (McCauley & Beitinger 1992; Bennett *et al.* 1998; Tavares-Dias & Moraes 2007). Female channel catfish are frequently hybridized with male blue catfish to produce offspring with faster growth, better feed conversion efficiency, more uniform size at harvest, greater tolerance of low oxygen levels and crowding in pond systems, and greater resistance to enteric septicemia (Andrews & Stickney 1972; Wolters & Johnson 1994; Bosworth *et al.* 1998; Li *et al.* 2001). The natural distribution of blue catfish extends further south than channel catfish, ranging from the Mississippi River basin and Gulf of Mexico Coast through Mexico and into Guatemala and Belize (Graham 1999). Because fish are poikilotherms, an understanding of their thermal capacity is crucial for research on habitat selection, metabolism, growth rates and reproductive migration (Crawshaw & Hammel 1974).

Previous studies fail to find any acute thermal tolerance differences between channel catfish strains (Hart 1952; Allen & Strawn 1968; Cheetham *et al.* 1976; Reutter & Herdendorf 1976; Bennett *et al.* 1998). However, these studies either did not directly compare strains, used different heating rates, varied in methodology or were limited in inference by small sample sizes. Therefore, in order to address geographic influences on CTmax in catfish, research is needed that utilizes strains that have clearly defined breeding lines, are raised in an aquaculture setting, and subjected to uniform conditions (e.g. acclimation temperature), to allow for direct comparison. Although it would be highly beneficial to the aquaculture industry, little is known about CTmax values of hybrid catfish and whether thermal tolerance traits are heritable.

The objective of this study was to quantify acute thermal tolerance differences in two geographically distinct strains of channel catfish and their hybrid cross with blue catfish using rates of temperature increase that mimic pond conditions. It was hypothesized that regional genetic differences would cause strains with an originally southern natural range (Delta Select channel catfish and Delta Select x D&B blue catfish) to have greater thermal tolerance relative to strains with a natural northern range (Red River channel catfish and Red River x D&B blue catfish), and consequently a greater CTmax. The hybrid catfish strains were also expected to have a higher CTmax than their respective channel catfish strains since blue catfish have a more southern distribution than channel catfish.

Methods

Fish Source and Acclimation

Two strains of channel catfish were used: Delta Select (from the Mississippi Delta, Mississippi) and Red River (from the Red River, North Dakota). These strains were used due to their disparate geographic distributions. The Delta Select (southern channel) is a commercial strain developed at the Thad Cochran National Warmwater Aquaculture Center (NWAC) in Stoneville, Mississippi. It is a first generation composite of families collected from 80-100 random spawns from 10 commercial catfish farms in the Mississippi Delta. The Red River (northern channel) was originally collected in 1988 from the Red River, North Dakota, USA (Hudson Bay drainage basin) where it does not regularly experience the same high temperatures, and is not a typical strain utilized in the catfish industry (Li et al. 1998). In addition, two hybrid strains, which are a cross between D&B blue catfish and each of the previously mentioned channel catfish strains, were used. The D&B blue catfish strain is commonly used in aquaculture to produce hybrids for the catfish industry (Xu et al. 2012). The D&B broodstock catfish used in this experiment were from the USDA Catfish Genetics Research Unit and were originally obtained from Dycus Farms, Arkansas (Xu *et al.* 2012). Similarly, the southern channel x D&B blue (southern hybrid) is representative of commonly used strains in southeastern US commercial catfish production.

Broodfish were strip spawned at NWAC in early May 2012 following protocols described by Bosworth *et al.* (2005). The same six southern channel and five northern channel females were used for both channel and hybrid strains to minimize individual differences. Fingerlings were transported in late June to the Mississippi State University

South Farm Aquaculture Facility (South Farm) after they were eating a formulated diet, approximately 30 days after hatching.

At the South Farm, the fingerlings underwent eight months of acclimation at 30±1°C in four cylinder tanks (430 L) divided by strain. The water supplying the tanks was non-chlorinated, aerated well water maintained in a recirculating system. A sample of 10 fingerlings from each strain was measured and weighed to obtain a baseline wet weight to calculate 2% body weight feeding rate. Food was withheld 24 hours before trials to ensure a post-absorptive state and reduce stress during handling (Barton *et al.* 1988; Mørkøre *et al.* 2008).

System Design

Acute thermal trial experimental set up consisted of three primary components: a sump tank, a header tank and an insulated fiberglass tank holding the experimental aquaria (Figure 1). The sump was a 149x100x60 centimeter (cm) oval tank with two heaters inside; one 4000 Watt, 200-250 Volt heater and one 1700 Watt, 100-140 Volt heater (SmartOne, Process Technology, Mentor, OH). Two inline pumps (model 3 utility pump, Aquatic Eco-systems Inc, Apopka, FL) were used to pump heated water to the header tank and a third pump to circulate water around the heaters for consistent thermal regulation. The sump tank was connected to the header tank by inflow polyvinyl chloride (pvc) tubing with an additional overflow outlet that returned to the sump tank via pvc tubing. All junctions were fitted with uniseals to prevent leaks. The header tank was a 35.5x84x61 cm rectangular tank (PT-564, Polytank Inc, Litchfield, MN) covered in thick insulation. Inside the header tank, three airstones aerated the well water prior to the experimental aquaria. Tubing covered with insulation was used to supply the heated,

oxygenated water from header tank to the experimental aquaria. Water flow was regulated with needle valves. Within the insulated fiberglass tank (212x61x56 cm; model MT-700 min-o-cool, Frigid Units Inc, Toledo, OH) there were nine 9.5-liter aquaria (30.5x15.25x20 cm), each covered with reflective bubble insulation, and each with an external standpipe that maintained a water depth of 17 cm. The external standpipe allowed the aquaria to be submerged in a water bath to further insulate internal temperatures. Water from the insulated fiberglass tank drained into the sump tank by a pvc pipe, where it was reheated and oxygenated.

Acute thermal trials were conducted on 30 fish per strain over 14 trials, for a total of 120 fish. Only 3 strains were tested per day, with 3 replicates of each to minimize daily fluctuations, and tested strains were alternated to ensure equal trials for all 4 strains. Fish were placed in the trial set up at 30±0.03 °C for 30-40 minutes to adjust to the new setting before acute thermal trials began following the acclimation methodology of Díaz & Bückle (1999). During the summer months of 2009, data from 6 different catfish aquaculture ponds found water temperatures to increase 1-2 °C each hour (E. L. Torrans, United States Department of Agriculture Agricultural Research Service, unpublished data from 2010). Based on these data, a heating rate of 2 °C per hour was determined to give an accurate estimation of CTmax. Water temperature was increased at a rate of 2.0±0.1 °C $hour^{-1}$ until loss of equilibrium (LOE) occurred, to achieve an environmentally realistic rate of temperature increase (Pérez-Casanova *et al.* 2008). LOE was defined as the failure of a fish to retain dorso-ventral orientation for one minute (Bennett & Beitinger 1997). CTmax for each individual fish was recorded as the water temperature at which LOE was observed. Behavioral responses to increasing temperature

were monitored and recorded throughout the trial. Typical observations were erratic swimming, surfacing, aquatic surface respiration as evidenced by bubbles, rapid ventilation rate, irregular opercular movement, splashing and muscular spasms.

Dissolved oxygen levels were monitored every 30 minutes and remained close to saturation (mean 100±0.96%, 7.14±0.09 mg/L; always ≥93%) in all tanks throughout the trial. Temperature was recorded with digital hand-held thermometers to the nearest 0.1 °C every 30 minutes throughout the trial. After CTmax was reached, fish were removed, measured (standard length ±0.1 mm) and weighed (±0.5 g), and then directly returned to their original acclimation temperature (30±1 °C) to allow recovery (Table 1). Fish were placed in different recovery tanks to distinguish strains and individuals, then monitored the next 24 hours for survival (Currie et al. 1998). Mortalities were recorded and removed and mortality rates were determined.

Statistical Analysis

An analysis of covariance (ANCOVA) using the proc glm procedure in SAS (version 9.2, SAS Institute Inc., Cary, NC) was used to examine effects of geographic range (north or south), catfish type (channel or hybrid), and the interaction between all main effects on CTmax with a covariate of standard length. Weight and length are highly correlated so only length was used to measure size. A general linear model using the proc logistic procedure in SAS was used to determine significance of range, catfish type and length variables on survival following acute thermal trials. The forward selection technique was used to remove non-significant variables from the model. A logistic regression predictive model was then generated from the significant factors. A two-way analysis of variance (ANOVA) was used to determine effects of geographic range, catfish

type and the interaction of these variables on length. The Shapiro-Wilk and Kolmogorov-Smirnov tests were implemented to confirm normality. A Tukey's post-hoc test was used to compare means. Statistical significance will be determined against an α of 0.05.

Results

Significant differences in length of fish used in the experiment between geographic range ($p=0.01$) and catfish type ($p<0.01$) were observed, however, no significant differences were observed in geographic range X catfish type interaction ($p=0.24$). Northern catfish were smaller (295 mm) in length than southern (305 mm). Hybrid catfish were smaller (282.5 mm) in length than their respective channel catfish (317.5 mm). Notably, length had no significant effect on CTmax ($p=0.36$). Significant effects of geographic range ($p<0.01$) and catfish type ($p<0.01$) on CTmax were found but not the geographic range X catfish type interaction ($p=0.36$). Other non-significant interactions were length X catfish type, length X range and length X catfish type X range ($p>0.34$). Northern catfish had lower CTmax values (39.08°C) than southern catfish (39.25°C) (Figure 2A). Hybrid catfish had lower CTmax values (39.07°C) than their respective channel catfish (39.26°C) (Figure 2B).

Length had a significant effect on survival ($p=0.02$) with survival increasing as the fish increased in size (Figure 3). An apparent regional effect on survival was observed with survival being higher in southern catfish but this was not statistically significant ($p=0.08$). During acute trials fish exhibited erratic swimming and irregular opercular movement as observed in the study by Hlohowskyj & Wissing (1985). Some fish never lost equilibrium and instead stopped breathing, with death as the endpoint similar to Bettoli et al. (1985). These fish (n=11) were not attributed to a specific treatment group

and had a slightly higher thermal endpoint, therefore they were removed from statistical analyses.

Discussion

Regional differences in CTmax were observed, supporting the hypothesis that geographic distribution would affect thermal tolerance. As predicted, catfish with a southern distribution had a greater CTmax than catfish with a northern distribution. These geographic differences in thermal tolerance were also observed in the hybrid catfish, suggesting a genetic component for this trait.

CTmax values are often characterized by low variability and small changes between environmental conditions (reviewed by Beitinger *et al.* 2000), suggesting tight control of thermal tolerance. The low variability in CTmax values suggests tight control over upper lethal temperature tolerance. For example, standard deviations (SD) in CTmax values for a group of fish subjected to a similar acclimation temperature are often small, (Currie *et al.* 1998, Beitinger *et al.* 2000), such as in this study (SD: 0.2-0.4°C). However, even small changes in environmental temperature, such as 2.5-6°C as predicted by global climate models (Karl *et al.* 2009), can have large consequences for susceptible freshwater fish species (Magnuson *et al.* 1979; Regier *et al.* 1990; Morgan *et al.* 2001). Thus, in this study, although the CTmax differences between channel catfish strains and between channel and hybrid catfish were small (0.4°C), the biological importance may be large, considering that current pond temperatures reach near lethal limits (Arnold *et al.* 2013; Liu *et al.* 2013) and are likely to increase under the influence of climate change.

The only known previous study on geographic differences in acute thermal tolerance in catfish by Hart (1952) found little to no geographic variation in IULT of

channel catfish from Florida and Ohio. However, the study was limited by small sample sizes (ranging from 4 to 9 fish per site), the use of wild caught fish held for brief periods in the laboratory (i.e., 4-5 days), and greater variation in body size (51.9-436.5 g) and age. Wild caught fish may be stressed by transfer and the environment of tank settings (Clearwater & Pankhurst 1997), which can make interpretation of data more difficult. In comparison, this study used two strains maintained separately in aquaculture settings to retain their genetically distinct heritage and ensure similar rearing environments, presumably minimizing stress and allowing for clearer application to aquaculture conditions.

Few studies have examined strain or population differences in CTmax. In three strains of brook trout *Salvelinus fontinalis* originating from different geographic locations but held for multiple generations in a common hatchery environment, thermal tolerance reflected historic geographic origin (McDermid *et al.* 2012). Specifically strains with a more southern origin had higher acute thermal tolerance than those with a more northern origin. Similarly, barramundi *Lates calcarifer*, originating from different geographic locations and subsequently held in hatchery environments have been found to have different acute thermal tolerance, with tropical populations having higher tolerance than sub-tropical populations (Newton *et al.* 2010). Further, Meffe *et al.* (1995) found that in eastern mosquitofish traits for improved thermal tolerance were genetically heritable. In this study, the differences in CTmax values observed between the channel catfish strains (i.e., southern having a greater CTmax than northern) were similarly observed in the hybrid cross for both strains, suggesting heritability of acute thermal tolerance. The

genetic underpinnings and the strength for selection for this trait are unknown, but suggest that further research on genetic control and expression are needed.

Contrary to the original hypothesis, hybrid catfish had lower CTmax compared to channel catfish. Because blue catfish have a natural distribution that extends further south than channel catfish, presumably exposing them to higher temperatures, it was hypothesized that they would demonstrate a greater thermal tolerance or CTmax. However, verified knowledge about the species' thermal tolerance is limited. Possible explanations for the results include: the relative exposure of these species to thermal variability, reduced temperature tolerance in blue catfish, or epistasis (Burke & Arnold 2001). In terms of thermal variability, blue catfish occur in sub-tropical to tropical areas, where water temperatures are less variable in seasonal fluctuation compared to temperate areas where channel catfish occur (Ficke *et al.* 2007). This thermoplasticity in channel catfish would be beneficial for tolerating predicted seasonal temperature fluctuation increases (Regier *et al.* 1990). It is also possible that blue catfish have a lower CTMax than channel catfish, although this has not been tested. A third possibility is due to epistatic effects, which may occur in hybridized organisms and can lead to asymmetrical hybrid fitness (Rhode & Cruzan 2005).

Although length did not have an effect on CTmax, it did have an effect on survival following thermal trials. Similarly, Bennett *et al.* (1998) found no relationship between length and CTmax in channel catfish of the same age range. In contrast, Recsetar *et al.* (2012) found no effect of length on survival of channel catfish, possibly due to the smaller size of fish examined (62-264 mm). Barrionuevo & Fernandes (1995) found that body size will affect critical thermal minimum (CTmin) but not CTmax in *Prochilodus*

scrofa; whereas Cox (1974) found that body size has an effect on CTmax in bluegill (*Lepomis macrochirus*) and Cook *et al.* (2006) found an inverse relationship between CTmax and body size in striped bass (*Morone saxatiliis*). In terms of the effect of body size on survival or recovery, there is limited data. Similar to this study, Meffe *et al.* (1995) observed improved thermal recovery with increasing body size in eastern mosquitofish. A possible explanation could be related to energy storage or a length:weight body ratio suggested by Ospina & Mora (2004). Therefore, recovery following high temperature exposure may rely more on the immune system and body reserves, thus larger fish may have an advantage.

A heating rate of 2 °C $hour^{-1}$, comparable to Pérez-Casanova *et al.* (2008), was used in this study because it was representative of the rates observed in channel catfish aquaculture ponds. In a previous study on channel catfish, Currie *et al.* (1998) used a heating rate of 0.3 °C min^{-1} which is more standard for the literature but does not replicate environmental conditions. CTM may be affected by slower rates of temperature change, possibility allowing low levels of acclimation. Interestingly, the CTmax range for both channel catfish strains in this study overlapped with those found for channel catfish (40.3 °C) by Currie *et al.* (1998). These results are consistent with the literature suggesting that CTmax for the acclimation temperature of 29.5±0.5 °C would be around 40 °C (Cheetham *et al.* 1976; Bennett *et al.* 1998). Although Becker & Genoway (1979) recommended standardization criteria, a universally adopted heating rate for determining CTmax has not been established. For example, the rates used by Cheetham *et al.* (1976), Watenpaugh *et al.* (1985), Bennett *et al.* (1998) and Kita *et al.* (1996) were 1 °C min^{-1}, 0.3 °C min^{-1} and 0.15 °C min^{-1}, 5 °C $hour^{-1}$ respectively. Beitinger *et al.* (2000) stated that

a correct heating rate needs to be slow enough that the core temperature of the fish being studied does not lag behind the water temperature, yet not too fast that the fish do not have time to thermally reacclimate during the trial. According to Jobling (1981), using different heating rates can lead to substantial variations in values of CTmax. However the combined results of this study and the Currie *et al.* (1998) study do not support this generalization for channel and hybrid catfish.

Rapid changes in water temperatures can lead to thermal shock which may affect thermoregulatory physiology and behavior. As ectotherms, catfish rely on environmental temperatures for metabolic rate and physiological needs (Morgan *et al.* 2001). Preferred and avoided temperature ranges may be modified due to temperature-induced damage to the central peripheral receptors or preoptic region (Crawshaw & Hammel 1974; Prosser & Nelson 1981). If the thermal shock is below lethal levels, it may allow fish to alter their physiology to a point at which alternative habitats can be utilized (Bevelhimer & Bennett 2000, Browse & Xin 2001, Pörtner 2002). CTmax is biologically important, because at this temperature fish are unable to escape conditions that will quickly lead to their death (Beitinger *et al.* 2000). Climate change is a concern for biota of all ecosystems, however, freshwater fish are more vulnerable to increasing temperatures due to limited thermal refuge (Morgan *et al.* 2001). In catfish production ponds there is no alternative habitat for fish to utilize during times of thermal stress, therefore, the fish must acclimate or die.

In conclusion, this study indicates that catfish geographic strains (i.e. northern vs. southern origin) and types (i.e. channel catfish vs. hybrid catfish) differ in short-term thermal tolerance. Thermal recovery also improved with increasing body size. To obtain

a clearer, and broader representation of long-term temperature effects on survival and growth in and amongst various strains of channel and hybrid catfish, chronic elevated thermal effects should be examined (Jobling 1981). Finally, given the results of this study which imply genetic control of temperature tolerance, genetic evaluation of thermal resistance in channel and hybrid catfish strains should be further explored.

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Table 2.1 Catfish weight and length differences.

Strain	Minimum weight (g)	Maximum weight (g)	Mean weight (g)	Minimum length (mm)	Maximum length (mm)	Mean length (mm)
Southern Channel	60	241.9	162.8±7.7	200	320	275.2±4.9
Southern Hybrid	34.1	235.6	120.3±8.9	162	290	235.8±6.1
Northern Channel	59.6	258.2	137.3±10.9	198	315	251.5±6.8
Northern Hybrid	50	246.8	105.3±7.5	167	275	224.0±4.8

Catfish type body size differences depicted by minimum, maximum and mean (± standard error) weight in grams and minimum, maximum and mean (± standard error) length in millimeters. Each strain used in the experiment had a sample size of 30 individuals.

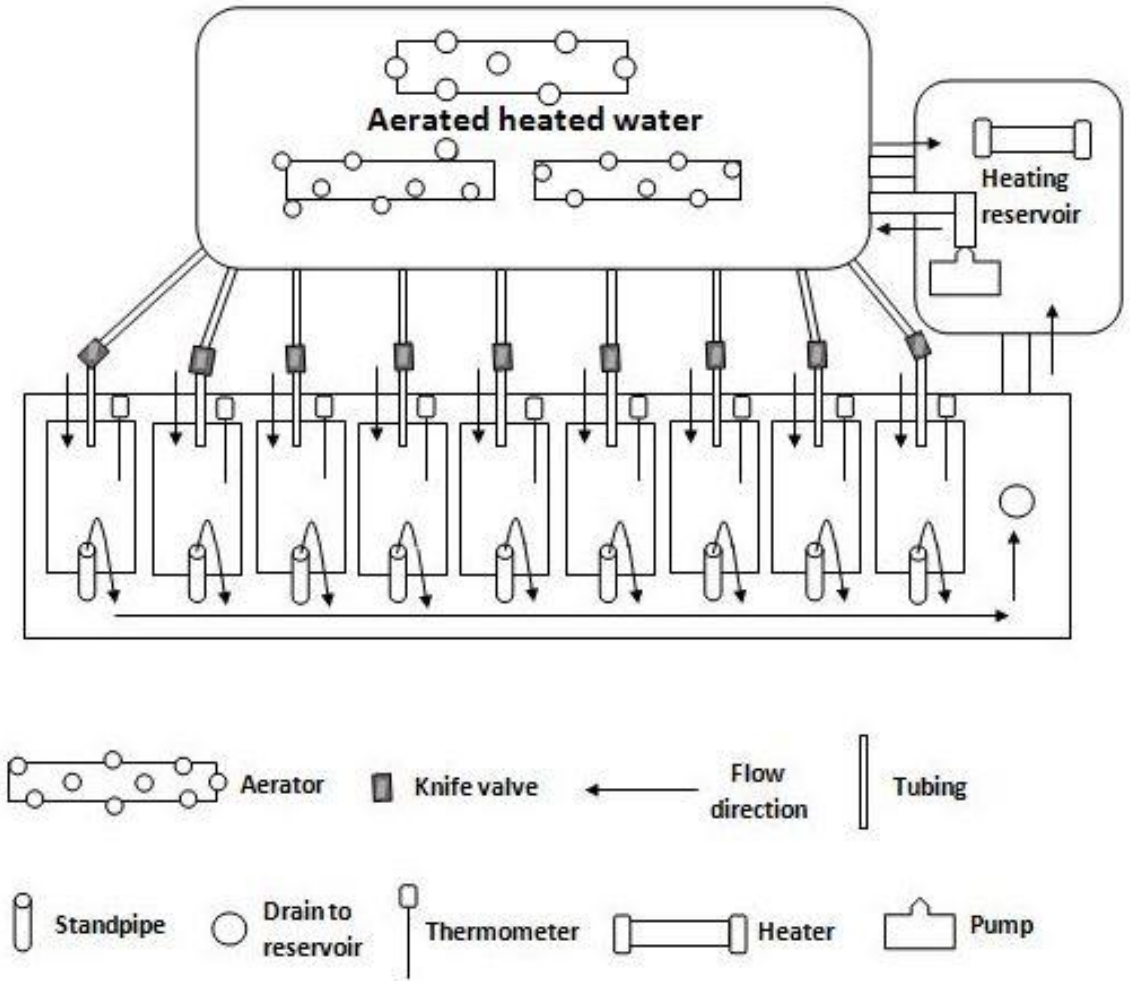

Figure 2.1 Acute thermal trial experimental set-up.

Top view diagram of the setup used to test critical thermal maxima.

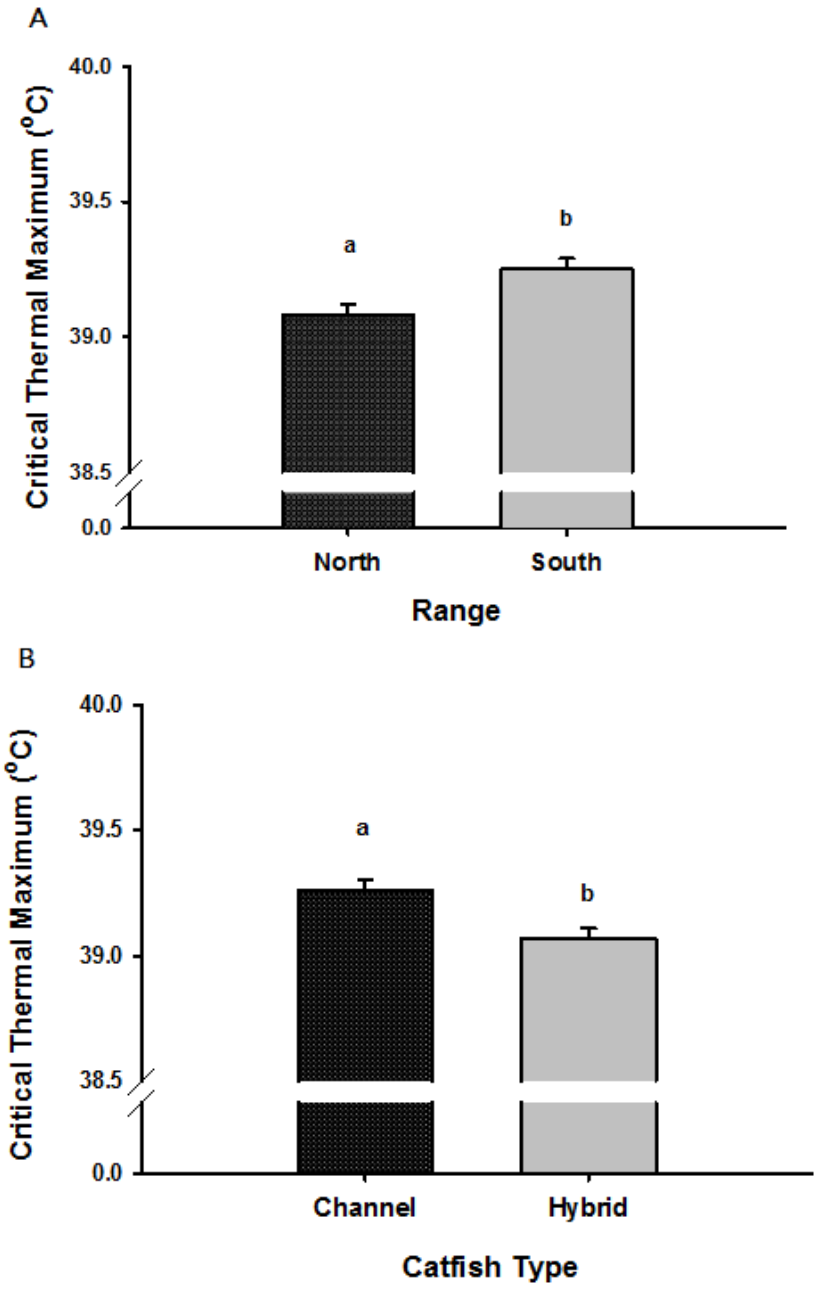

Figure 2.2 Effects of (A) range and (B) catfish type on critical thermal maximum (CTmax).

Mean (±standard error) critical thermal maximum (CTmax) by (A) geographic range (northern and southern) and (B) type (channel catfish (*Ictalurus punctatus*) and hybrid catfish (*I. punctatus* X *I. furcatus*)) of catfish. Different letters indicate significant differences between strains or types (analysis of covariance (ANCOVA), Tukey's HSD post-hoc test, $p<0.05$).

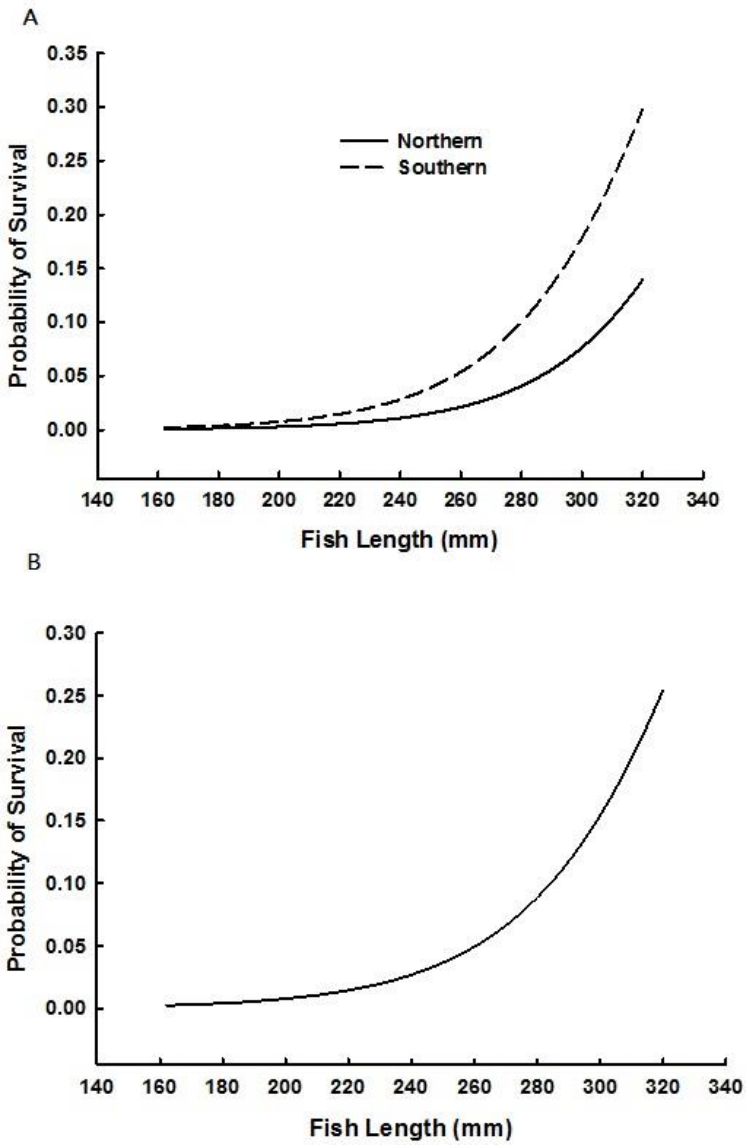

Figure 2.3 Probability of survival relative to fish length.

Probability of survival relative to fish length of (A) geographically distinct catfish strains (northern and southern) and (B) all catfish 24 hours following acute trials. A significant effect of length on survival ($p=0.02$) was observed with large fish having increased survival. A trend ($p=0.08$) of southern catfish having increased survival over northern catfish was also observed. A general linear model using the proc logistic procedure in SAS was run to determine the effect length and geographic range on survival. A logistic regression predictive model was then generated for probability of survival using the equations $pN=1/(1+\exp(\text{logit}N))$ where $\text{logit}N=11.5804-0.0335*\text{length}+0.9609$ and $pS=1/(1+\exp(\text{logit}S))$ where $\text{logit}S=11.5804-0.0335*\text{length}$. (Tukey's HSD post-hoc test, $p<0.05$).

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CHAPTER III

EFFECTS OF CHRONIC UPPER TEMPERATURE REGIMES ON GROWTH OF TWO GEOGRAPHIC STRAINS OF CHANNEL AND HYBRID CATFISH

Abstract

Climate change is a growing concern for pond culture of catfish, due to possible exacerbation of temperature fluctuations and increased maximum daily temperatures. Because channel catfish (*Ictalurus punctatus*) have a broad natural distribution from Canada to Mexico, it was hypothesized that natural differences in thermal tolerance and seasonal growth may be attributed to different geographic strains. Further, it was hypothesized that these differences would be observed in hybrid catfish (*I. punctatus* x [blue catfish] *I. furcatus*). Chronic thermal tolerance and growth rate were quantified in two geographically distinct strains of channel catfish and a corresponding hybrid catfish from one of these strains with a cross to an industry standard blue catfish strain. In a six-week growth experiment, catfish were subjected to daily cycling temperatures of either 27-31°C or 32-36°C, mimicking pond fluctuations. Hybrid catfish had the highest survival at both temperatures and both strains of channel catfish had greater growth in weight or length at 27-31°C than at 32-36°C. Therefore, these results indicate that physiological performance, in terms of growth, decreases in channel catfish at temperatures greater than 27-31°C regardless of geographic origin of strain, whereas hybrid catfish did not show a decrease in weight under the same temperature conditions.

Introduction

Channel catfish *Ictalurus punctatus* have a natural geographic distribution from southern Canada to northern Mexico which encompasses a thermal range from 5-35°C (McCauley & Beitinger 1992; Bennett *et al.* 1998; Tavares-Dias & Moraes 2007). Channel catfish are found in a wide range of environmental conditions (i.e. temperature, salinity and turbidity) (Jackson 2004). This resilience is one of the factors that make channel catfish suitable for aquaculture. In 2012, the majority of the United States (US) catfish production took place in Mississippi, with a total sales value of $341 million, making it an economically important species (USDA 2013). Male blue catfish *I. furcatus* are frequently hybridized with female channel catfish in aquaculture to produce offspring with higher dress-out percentages, faster growth, easier harvest by seining and angling, more uniform size at harvest, greater resistance to enteric septicemia, and greater tolerance of low oxygen levels and crowding in pond systems (Giudice 1966; Yant *et al.* 1976; Dunham *et al.* 1983; Smitherman *et al.* 1983; Li *et al.* 2004; Ligeon *et al.* 2004; Dunham & Argue 2011; Kumar & Engle 2011). Blue catfish also have a natural distribution that extends further south than channel catfish, ranging from the Mississippi River basin and Gulf Coast through Mexico and into Guatemala and Belize (Graham 1999).

Catfish are poikilotherms, thus their physiology is influenced by environmental temperature, with best growth performance occurring within an optimal temperature range (Hutchison & Maness 1979). Knowledge of the optimal temperature of a species is essential to maximizing aquaculture yield (McCauley & Casselman 1981; Kellog & Gift 1983). Previous studies have demonstrated superior weight gain at temperatures ranging

from 26.6-32°C for channel catfish (Shrable *et al.* 1969; Kilambi *et al.*1971; Andrews & Stickney 1972; Buentello, Gatlin & Neill 2000; Li *et al.*2008; Arnold *et al.* 2013). Díaz & Bückle (1999) found that channel catfish preferred a temperature of 29°C when allowed to move between temperatures of 10-40°C.

The majority of catfish production in the US occurs in the southeast (92%), where some of the warmest conditions are found (Mott & Brunson 1995). Temperatures in aquaculture ponds in the Southeastern US may reach daily maximums up to 34-36°C with daily fluctuations averaging 4°C in May-August (Arnold *et al*. 2013). Average temperatures in the Mississippi alluvial plain (Mississippi Delta) US have risen 3°C from 1970 to 2010 (NOAA 2013) and the US Global Change Research Program predicts that temperatures will continue to rise country wide by either 2.5°C in a lower emission scenario or by 5°C with an increase of 6°C in summer months in a higher emission scenario by 2080 (Karl *et al.* 2009). Global climate change may exacerbate pond temperature fluctuations and increase maximum daily water temperatures due to air temperature, solar radiation and humidity (Hansen *et al.* 2006; De Silva & Soto 2009).

Although fish can acclimate to temperatures higher than their optimal range, prolonged (chronic) high temperatures can cause physiological stress. Chronic thermal ranges (31-35°C) characteristic of pond conditions have been shown to decrease growth in catfish (Arnold *et al*. 2013), which can be an indicator of stress. Physiological stress can affect metabolism, fecundity and susceptibility of fish to disease or toxicants, which can result in population-level effects (Bevelhimer & Bennett 2000; De Silva & Soto 2009).

Fish species may have different chronic upper temperature tolerance or sensitivity based on their geographic distribution (Pulgar *et al.* 2005). With aquaculture species such as channel catfish, fish are unable to migrate and in some cases acclimation is hindered as well, leaving inherited genetic resilience as the main biological option to resist temperature effects (Ficke *et al.* 2007). Genetic traits may be related to a fish's ability to acclimate to temperature change, with acclimation defined as the short term adjustment of preexisting biochemical systems to temperature (Hochachka & Somero 2002). Therefore, realistic temperatures that approximate conditions fish experience, such as daily and seasonal chronic thermal fluctuations are needed.

The objectives of this study were to quantify chronic upper temperature tolerances and growth rates of two geographically distinct strains of channel catfish and one corresponding hybrid with a cross to an industry standard blue catfish strain. It was hypothesized that strains of channel catfish would have different upper temperature tolerance ranges relative to their natural geographic distribution and that this would be reflected in differences in growth and survival. Thus, strains with an originally southern natural range would have greater upper temperature tolerance relative to strains with a natural northern range and consequently greater growth and higher survival rates (Mayo 1999). Based on previous studies, it was expected that fish at temperatures above 32°C would grow at a slower rate and have decreased survival. Additionally, it was hypothesized that the hybrid catfish would outperform their relative channel strains in growth and survival since blue catfish have a more southern natural distribution and thus inhabit typically warmer climates.

Material and methods

Fish Source and Acclimation

In this study chronic upper temperature tolerance and growth rate of two geographically distinct strains of channel catfish and the corresponding hybrid catfish from these strains (with a cross to an industry standard blue catfish strain) were quantified. The strains of channel catfish used were: Delta Select (from the Mississippi Delta, Mississippi) and Red River (from the Red River, North Dakota). These two strains were selected based on their disparate geographic distributions.

The Delta Select (southern channel) strain is a commercially representative strain developed at the Thad Cochran National Warmwater Aquaculture Center (NWAC) in Stoneville, Mississippi. It is a first generation composite of families collected from 80-100 random spawns from 10 commercial catfish farms in the Mississippi Delta. Broodstock at these farms were derived from natural sources in Mississippi and Arkansas and have been domesticated for a minimum of five generations. The Red River (northern channel) strain was originally collected in 1988 from the Red River, North Dakota, USA (Hudson Bay drainage) but has since been domesticated (Li *et al.* 1998). In addition, two strains of hybrid catfish, a cross between D&B blue catfish and each of the previously mentioned pure channel catfish strains, were used. The D&B blue catfish strain is commonly used in aquaculture to produce hybrids in the catfish industry (Xu *et al.* 2012). The D&B broodstock catfish used in this experiment were from the USDA Catfish Genetics Research Unit, and were originally obtained from Dycus Farms, Arkansas (Xu *et al*. 2012), again derived from natural sources in Arkansas and domesticated for a minimum of five generations. Similarly, the Delta Select x D&B hybrid (Delta Select

hybrid) is representative of commonly used strains in southeastern US commercial catfish production. The northern channel catfish strain does not regularly experience the same high temperatures as southern channel catfish, and is not a typical strain utilized in the catfish industry. Therefore, the northern channel and northern hybrid catfish were not expected to perform as well in terms of growth rate and survival.

Six male blue catfish and six female channel catfish of each strain were strip spawned at NWAC in early May 2012 to produce the catfish used in this study (Dunham *et al.* 2000; Bosworth *et al.* 2005). The same Delta Select females were used for both channel and hybrid production to minimize individual differences. Fingerlings were transported in late June to the Mississippi State University South Farm Aquaculture Facility (South Farm) after they were eating a formulated diet, approximately 30 days after hatching.

At the South Farm, the fingerlings were separated by strain and acclimated for two weeks to 29°C in 430-l circular tanks with a flow rate of 578-L/hr. The water supplying the tanks was non-chlorinated, aerated well water maintained in a recirculating system. A random sample of 10 fingerlings from each strain per tank was measured and weighed to obtain initial total length and wet weight (Table 1).

System Design and Temperature Regulation

Chronic temperature trials were conducted for 6 weeks, during which fish were exposed to daily cycling temperatures of either optimal temperatures for best food conversion and greatest growth at 27-31°C or upper-range thermal temperatures of 32-36°C following Arnold *et al*. (2013). Cycling temperature regimes mimicked natural pond daily temperature fluctuations. Each day starting at 0900, water temperature was

increased from baseline temperatures of 27°C or 32°C to peak temperatures of 31°C or 36°C. Peak temperatures were reached by 1730, after which temperatures were slowly decreased to 27°C and 32°C for optimal and high thermal treatments respectively (Fig. 1).

At each of the two temperature regimes, there were three catfish types (southern channel, northern channel and southern hybrid) with 3 replicate 430-L tanks per temperature and treatment, and 60 fingerling (average 80.5 mm long) catfish per tank, for a total of 18 tanks. Due to space limitations, the northern hybrid catfish were limited to only one tank per temperature treatment, and were included for comparison purposes but not analyzed statistically. All tanks in each temperature treatment were connected to a large recirculating system that consisted of a sump tank (125 L), an insulated reservoir tank (1900 L), a large heat pump (HP-7, Aqualogic Inc, San Diego, CA), mechanical and biological filters and ultraviolet sterilization. Each of the recirculating systems was supplemented with new well-water which maintained high water quality and acted to slowly reduce water temperature after the daily peak period. The system was backflushed twice weekly to remove biological waste.

Fish were held under a simulated natural photoperiod (12 hours and 29 minutes L:11 hours and 31 minutes D – 12 hours and 49 minutes L:11 hours and 11 minutes D for latitude, longitude: 33 27.4’N, 88 49.3’W) which was adjusted weekly. All tanks were aerated by air stones and covered with mesh covers. All mortalities were removed immediately, recorded and taken to the Mississippi State University College of Veterinary Medicine Fish Diagnostic Laboratory for necropsy. At the end of the six week chronic trial period, each fish was anesthetized, measured and weighed after 24 hours of

fasting to determine growth according to Mississippi State University Animal Use Protocol #12-035.

Feeding Regime

Fish were fed *ad libitum* twice daily with a formulated 1.6 mm pelleted diet of 44% protein (Rangen Inc., Buhl, ID), based on feeding protocols used at Thad Cochran NWAC. Fish were fed diets with higher protein than typically used in production so not to stunt growth. Following one hour of allotted feeding time, if all food was consumed, additional pellets were provided and the fish were given an additional 15 minutes to feed. This process continued until there was a substantial amount of pellets remaining (>50 pellets). At this point all uneaten pellets were netted out of the tanks and waste was siphoned daily following feeding.

Water Quality

Total ammonia nitrogen (TAN), nitrite (NO_2^-), alkalinity and hardness of water were measured twice weekly using a commercial kit (AQ-3, LaMotte Company, Chestertown, MD). Dissolved oxygen (DO) concentrations and pH were measured three times a week using a DO meter (Y55, YSI Inc., Yellow Springs, OH) and a pH meter (pH10N, YSI Inc., Yellow Springs, OH). Temperature was monitored once daily using a digital thermometer (Traceable, VWR, Atlanta, GA) and every 15 minutes throughout the experiment using data loggers (HOBO U22 Water Temp Pro v2 data loggers, Onset Corporation, Bourne, MA). Unionized ammonia was calculated using TAN, temperature, and pH levels. Water quality conditions were maintained within guidelines presented by Tucker & Robinson (1990) for optimal growth of channel catfish: ammonia 0.1-1.0

mg/L, unionized ammonia <0.05 ppm, nitrite <0.1 mg/L, nitrate <50 mg/L, alkalinity 20-400 ppm, hardness 20-400 ppm, dissolved oxygen >5 mg/L, and pH 6.5-8.0 (Table 2).

Measuring Growth

To quantify effects of temperature on growth, fish were weighed to the nearest 0.01 g and measured to the nearest 1 mm at the beginning and end of the experiment. In a pilot study it was found that individual measurements caused post-measurement mortality. Therefore, for the growth study, group weights of ten fish were taken in five batches per tank, then individual weights and lengths were measured on the ten fish in the sixth batch for initial weigh outs. For the final weigh out, individual weights and lengths were recorded on all surviving fish.

Measuring Survival

In a pilot study, catfish were found to be susceptible to bacterial pathogens in both temperature treatments. Therefore, prior to the growth study, fish were treated for bacterial pathogens using oxolinic acid medicated feed at a rate of 24 mg/kg body weight for 6 days, a one-time 2 mg/L potassium permanganate bath for 30 minutes and several 30-minute NaCl (solar salt) baths at 3 ppt over a one week period. After this period, all fish appeared healthy and a constant salinity of 1 ppt was maintained thereafter to minimize future bacterial infections. All fish showing signs of disease were evaluated by a fish pathologist at Mississippi State University College of Veterinary Medicine.

Statistical Analysis

All statistical analyses were conducted using R software (R Core Team). Akaike's information criterion (AIC) (Burnham & Anderson 2002) was used to measure relative

goodness of fit of statistical models. We inspected the residuals and used a Shapiro-Wilk test to check for heteroscedasticity. A two-way analysis of variance (ANOVA) was conducted on initial weights and lengths using factors of temperature and strain. There were no significant differences between initial lengths of fish between strains, temperature or the interaction of strain and temperature. There was a significant relationship between initial weight and strain, although the actual difference in initial weight was only 0.9 g (Table 1). Because initial weights were significantly different between strains, weight gain was analyzed instead of final weight. A two-way analysis of covariance (ANCOVA) was run with a covariate of initial weight to account for differences in initial weight, and a Tukey's post-hoc test was used to compare means of change in weight between strains and temperature ranges.

A two-way ANOVA was run on change in length with factors of temperature and strain. Mortality data was $\log_{10}$ transformed to meet normality and then a two-way ANOVA was run with factors of temperature and strain. A Holm-Sidak test was used to compare means.

A two-way ANOVA was run on dissolved oxygen levels and percent with factors of temperature and strain. Differences among means were declared significant using an α of 0.05.

Results

Growth

Growth, in terms of weight gain or length gain was lower at the 32-36°C than 27-31°C temperature range for both northern and southern channel catfish. A significant difference (F=6.35, *P*=0.01, df=2) of weight gain was found in the interaction between

strain and temperature. Differences were found between northern channel catfish at the 32-36°C temperature range and northern channel catfish at the 27-31°C temperature range (P<0.01) as well as between northern channel catfish at the 32-36°C temperature range compared to southern channel catfish at the 27-31°C temperature range (P=0.04) (Fig. 2). Southern hybrid catfish showed no difference in growth between the temperature treatments.

In terms of length, the interaction between strain and temperature was not significant (F=1.77, P=0.22, df=2) nor the main effect of strain (F=3.25, P=0.07, df=2). The main effects of temperature (F=7.97, P<0.01, df=1) was significant. Length gain was greater at the 27-31°C than the 32-36°C temperature range (Fig. 3).

Survival

There was no significant effect of the interaction between temperature and strain on mortality. There was a significant effect of strain (F=10.98, P<0.01, df=2) and temperature (F=10.21, P<0.01, df=1) on mortality. Southern hybrid catfish had significantly greater survival than southern channel catfish (P<0.01) or northern channel catfish (P<0.01) (Fig. 4a). However there was no significant difference in mortality observed between southern and northern channel catfish. Greater mortality was observed at 27-31°C than at 32-36°C (Fig. 4b). Columnaris disease (*Flavobacterium columnare*) was the primary cause of mortality for both southern and northern channel catfish during the study, with mortality rates of 11% and 7% in southern and northern channel catfish respectively. Southern and northern hybrid catfish had mortality rates of 0.5%.

Dissolved Oxygen

Temperature had a significant effect on dissolved oxygen levels ($F=179.74$, $P<0.01$, df=1). Dissolved oxygen levels were not different between strains. All dissolved oxygen levels were maintained above growth-limiting levels of 70% (Buentello *et al.* 2000) or common management conditions of 5 mg/L (Small 2006) (Table 2).

Discussion

Growth

The findings of this study indicate that small temperature increases over current summer conditions can have detrimental impacts on growth of channel catfish. Further, this effect was observed in both geographic strains (northern or southern origin) in terms of either weight or length. In contrast, hybrid catfish did not decrease in growth with increased temperature, and had lower mortality in both temperature treatments than the channel catfish. Although it is possible that tank density differences caused by greater mortality rates of catfish at the 27-31°C temperature range may have contributed to greater growth, densities were relatively low, density differences were small (≤11%), and feed was provided in excess to all treatments. Thus, hybrid catfish may offer greater potential for the catfish industry in the future in the face of increasing climatic change.

This study demonstrated a decrease in growth of northern channel catfish reared at a temperature range of 32-36°C compared to an optimal temperature range of 27-31°C. Suja *et al.* (2009) reared stocker channel catfish averaging 111.4±1.7 g (mean±SE) at two constant temperatures of 27°C and 32°C in a recirculating system, and found that channel catfish raised at the 32°C temperature were smaller than those raised at the 27°C temperature, supporting the findings of this study. Similarly, McCauley *et al*. (1992)

found that growth rate of channel catfish increases with temperature until the thermal range of 27-30°C, after that point growth decreases rapidly. Arnold *et al*. (2013) compared growth rates of channel catfish at the 27-31°C temperature range as well as 23-27°C and 31-35°C. Channel catfish at the 27-31°C range grew larger than those at the temperature range above or below. Chronic upper temperature effects examined in this study represent realistic settings and application because in their natural environment fish experience daily and seasonal thermal fluctuations, being able to actively regulate their physiology (Ju *et al.* 2002).

Little information is known about temperature effects on hybrid or blue catfish in relation to growth. However southern populations of blue catfish have demonstrated improved growth in comparison to northern populations (Graham 1999), a difference believed to be due to southern populations becoming sexually mature sooner than northern populations (Hale & Timmons 1989), extended growing season and greater food diversity (Graham 1999). The results of this study indicate that growth in hybrid catfish is not reduced at the same high temperatures that affect growth in channel catfish. Thus, thermal tolerance of hybrid catfish may be greater than channel catfish. Hybrid catfish have demonstrated poor growth in tank systems (Small 2006), which is presumably why they did not grow as well as channel catfish at the 27-31°C temperature range. Dunham *et al.* (1990) found that hybrid catfish grew faster than channel catfish in ponds, however the opposite was true for rearing in cages. When grown in ponds from fingerling to market size, hybrid catfish significantly outperformed channel catfish at all densities (Dunham *et al.* 1987). Thus, in this experiment, hybrid catfish may have performed better relative to channel catfish if they had been grown in ponds. Notably, the conditions were

the same for hybrid catfish at both temperature treatments, therefore, the effects of temperature on hybrid catfish are comparable.

Differences in growth were seen between strains of channel catfish with geographically distinct populations, indicating that temperature effects on growth of channel catfish may be widespread geographically. It is also possible that be differences in growth may have been influenced by differences in length of domestication of strains. Channel catfish strains have been found to have varying body composition indices (Small 2006). Length and weight differences between strains was not due to lack of protein or fat content nor limitation of food because both strains were fed the same feed to satiation. Carlander (1977) observed faster growing channel catfish in the southern portion of the range, although he stated that there is little evidence of regional differences in growth. Modde & Scalet (1985) found largemouth bass (*Micropterus salmoides*) from a geographic distribution of North Dakota to Texas had increased growth rate and maximum size in the southern range. Growth, a measure of thermal sensitivity, has been influenced by geographic distribution and temperature in muskellunge (*Esox masquinongy*) as well (Wolter *et al.* 2011) with populations originating from low latitudes demonstrating maximum growth under higher thermal regimes (Wolter 2012). These differences in growth over a wide latitudinal distribution are believed to be due to temperature since it affects energy reserves via alterations in metabolism and food consumption (von Bertalanffy 1960; Lagler *et al.* 1977; Kassahn *et al.* 2007; Vergauwen *et al.* 2010). The fish used in this study were all raised at the same latitude, at the same temperature ranges and under the same conditions which implies that growth and survival may be genetically heritable traits.

Survival

Contrary to the original hypothesis, no significant difference in mortality between southern and northern strains was found. Results indicated that hybrid catfish have better survival than channel catfish at high temperatures. Although not tested statistically due to a lack of replicates, the northern hybrids also had low mortality. Similarly, hybrid catfish grown for two seasons in earthen ponds had a higher survival rate than channel catfish under the same conditions (Dunham *et al*. 1987). Given the good water quality (Table 2), daily cleaning regimen, well-fed state of fish and minimal amount of human contact with fish; mortalities were presumably related to thermal stress. Unexpectedly, fish in this study, at the temperature range of 27-31°C had higher mortality than those at 32-36°C which was witnessed by Suja *et al*. (2009) with a 4% higher survival in fish reared at 32°C compared to fish reared at 27°C.

Water temperature can increase the susceptibility of infectious disease in catfish by affecting both immunocompetence and assisting pathogens. Diseases exacerbated by high temperatures are channel catfish virus disease (CCVD), enteric septicemia of catfish (ESC) and branchiomycosis ("gill rot") followed by secondary bacterial infections such as *Aeromonas hydrophila* or *Flavobacterium columnare* (Plumb 1978; Francis-Floyd *et al.* 1987; Plumb & Shoemaker 1995; Hawke & Khoo 2004). In this study the primary pathogen found was *Flavobacterium columnare* which mostly commonly affects channel catfish at temperatures ranging from 25-32°C (Durborow *et al.* 1998). Therefore, disease could have been a contributing factor to the increased mortality rates in fish at the 27-31°C range.

Conclusion

McCauley *et al*. (1992) predict that the zone of primary catfish production will move 240 km north in the central part of the US with each 1°C increase of annual temperature. If current atmospheric models hold true and mean global temperature increases of 1-7°C occur (Ficke *et al*. 2007), then this could be detrimental to the Southeastern US catfish industry. Although this study maintained high dissolved oxygen levels, water temperature and dissolved oxygen availability are closely intertwined. With increasing temperatures dissolved oxygen levels drop and metabolism increases. Dissolved oxygen is necessary to sustain energy demands and thus if it is limited growth may be affected (Buentello *et al.* 2000). Therefore, the potential effects of climate change on the culture of channel catfish need to be considered in future management practices. Further inspection should be conducted with market sized catfish because only fingerling catfish were examined in this study. Also, northern hybrids should be examined because the necessary number of replicates were unable to be included in this study due to facility size constraints. Dunham *et al*. (1987) found there is an effect of strain or family line on growth when producing hybrid catfish. The present study relies on six male blue catfish and six female channel catfish of each strain, thus additional studies should be run on other families to compare channel and hybrid catfish performance. Use of hybrid catfish could be one potential solution to the impact of climate change on the catfish industry, although future research on genetic responses to temperature in both hybrid and channel catfish and temperature tolerance of hybrid catfish are needed. With knowledge of potentially heritable genes related to differences in physiological performance at high temperature and thermally tolerant genes, breeding programs may be developed to further

optimize growth with increasing temperatures. Hybridization of southern channel catfish strain with high performing blue catfish strains may enhance catfish production and yield.

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Table 3.1 Initial lengths and weights of catfish.

Strain	Initial Mean Weight (g)	Initial Mean Length (mm)
Northern Channel	4.95±0.09	79.50±0.72
Southern Channel	4.14±0.05	82.23±1.24
Southern Hybrid	4.05±0.05	79.92±1.04
Northern Hybrid	4.16	76.55

Mean (± standard error) initial lengths and weights of channel (*Ictalurus punctatus*) and hybrid (*I. punctatus* X *I. furcatus*) catfish by strain.

Table 3.2 Water quality parameters (mean ± standard error) during a 6-week thermal trial.

	27-31°C				32-36°C			
	Northern Channel	Southern Channel	Southern Hybrid	Northern Hybrid	Northern Channel	Southern Channel	Southern Hybrid	Northern Hybrid
pH	7.94±0.03	7.98±0.03	7.96±0.05	7.96±0.02	7.99±0.03	7.99±0.02	7.96±0.02	8.01±0.02
TAN (ppm NH_3-N)	0.26±0.03	0.23±0.03	0.23±0.03	0.20±0.00	0.28±0.03	0.26±0.04	0.22±0.02	0.20±0.00
NO2(ppm NO2-N)	0.02±0.01	0.01±0.01	0.01±0.01	0	0.02±0.01	0.01±0.01	0.01±0.01	0
Alkalinity (ppm $CaCO_3$)	84.00±1.51	83.20±1.50	85.60±0.98	84	83.43±1.62	85.00±1.92	84.67±1.61	88
Hardness (ppm $CaCO_3$)	37.50±6.37	37.14±7.24	28.00±0.00	28	36.00±5.53	28.80±0.80	37.33±8.56	28
DO (mgL-1)	6.85±0.04[a]	6.93±0.03[a]	6.94±0.03[a]	7.01±0.14[a]	6.32±0.08[b]	6.30±0.07[b]	6.31±0.07[b]	6.36±0.12[b]
DO (%)	90.50±0.43[a]	91.45±0.32[a]	91.61±0.34[a]	90.60±0.66[a]	88.90±0.47[b]	88.66±0.21[b]	88.25±0.31[b]	89.28±0.45[b]

Water quality parameters (mean ± standard error) during a 6-week thermal trial separated by treatment (strain and temperature) of channel (*Ictalurus punctatus*) and hybrid (*I. punctatus* X *I. furcatus*) catfish. Total ammonia nitrogen (TAN), nitrite (NO_2^-), alkalinity and hardness were monitored twice weekly. Dissolved oxygen (DO) and pH were monitored three times a week. Different letters indicate significant differences between treatments (two-way ANOVA, $P<0.05$).

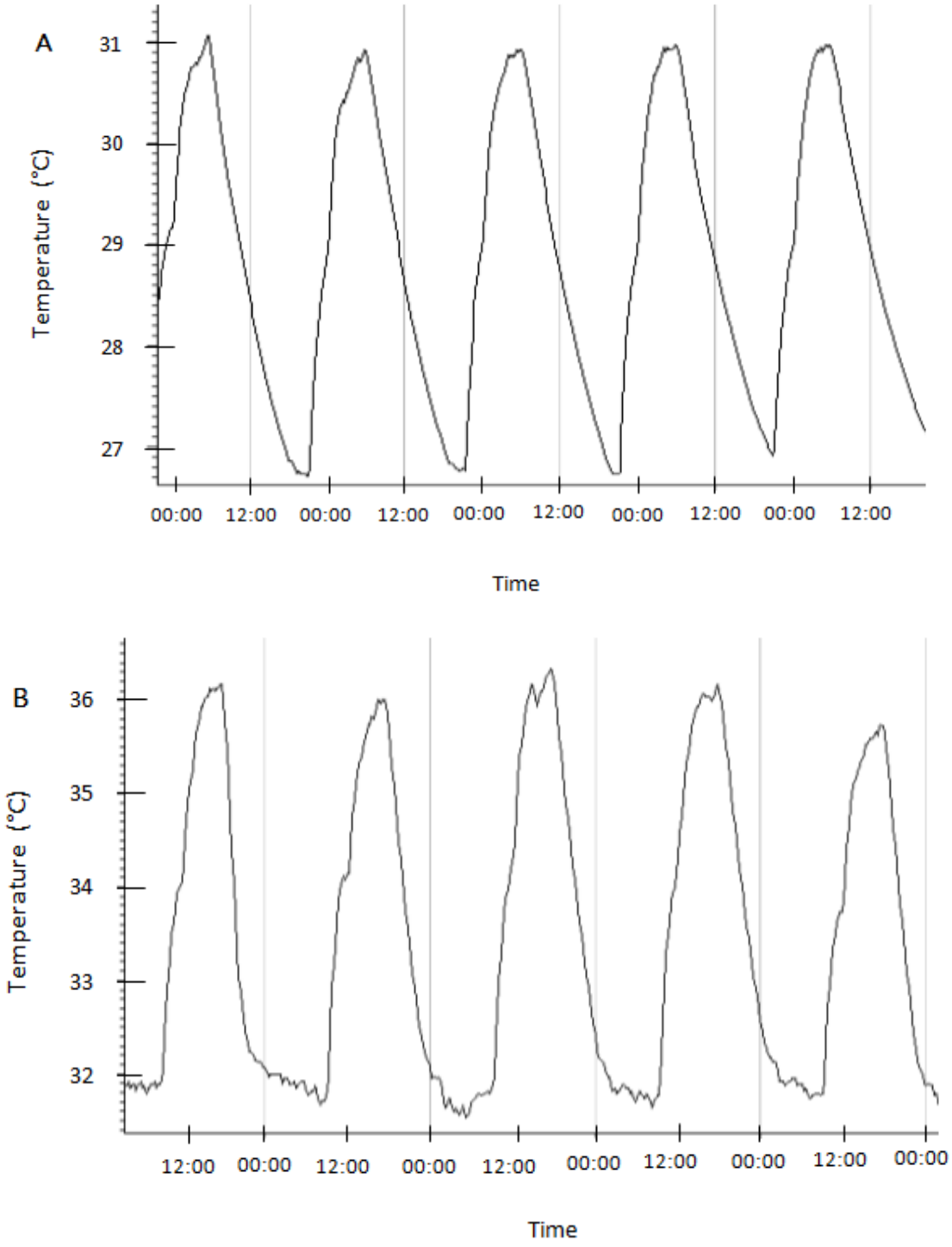

Figure 3.1 Representative daily temperature fluctuations of (A) optimal (27-31°C) and (B) upper thermal range treatments (32-36°C).

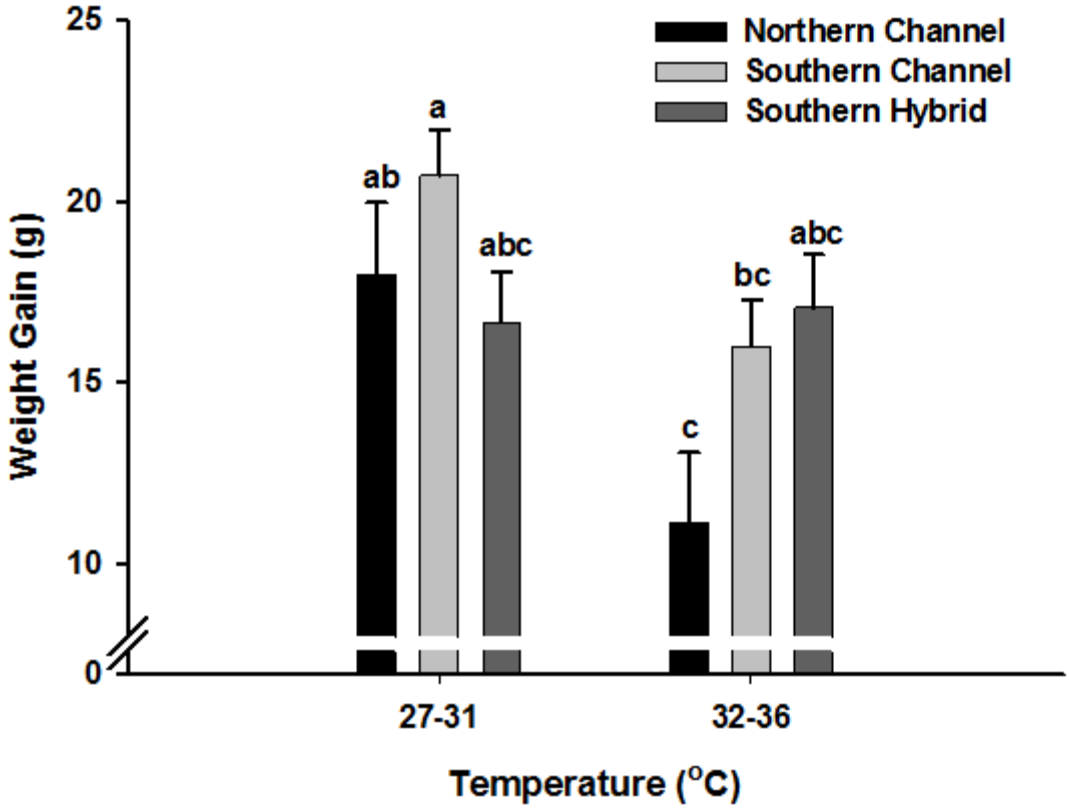

Figure 3.2 Effect of temperature on weight gain of catfish.

Mean (±standard error) weight gain of geographically distinct channel catfish (*Ictalurus punctatus*) strains and hybrid catfish (*I. punctatus* X *I. furcatus*) at optimal (27-31°C) and upper thermal ranges (32-36°C). Different letters indicate significant differences between treatments (two-way ANCOVA, covariate = initial weight, Tukey's HSD post-hoc test, $P<0.05$).

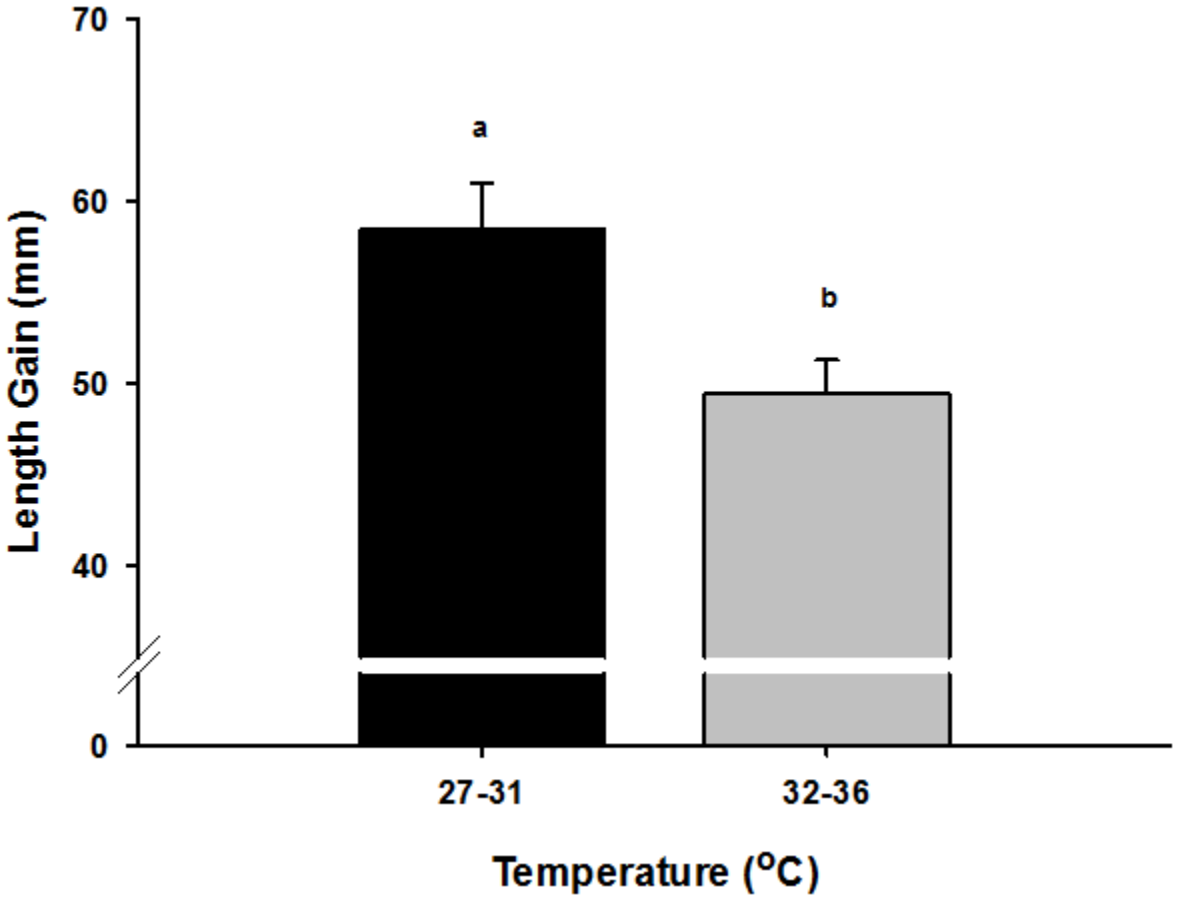

Figure 3.3 Effect of temperature of length gain of catfish.

Mean (±standard error) length gain by temperature in geographically distinct channel catfish (*Ictalurus punctatus*) strains and hybrid catfish (*I. punctatus* X *I. furcatus*). Different letters indicate significant differences between treatments (two-way ANOVA, Tukey's HSD post-hoc test, $P<0.05$).

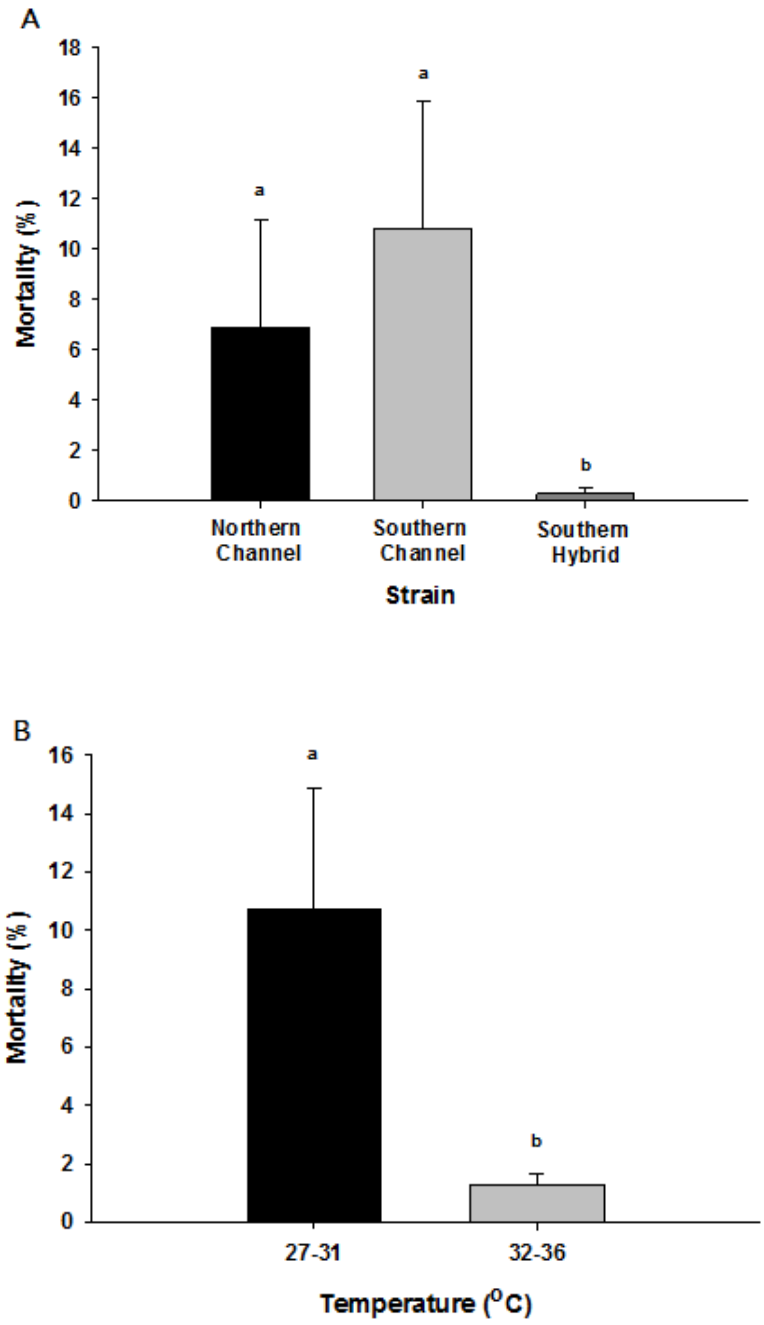

Figure 3.4 Effect of (A) strain and (B) temperature on mortality.

Mean (±standard error) percent mortality by (A) strain and (B) temperature in geographically distinct channel catfish (*Ictalurus punctatus*) and hybrid catfish (*I. punctatus* X *I. furcatus*). Temperature ($P<0.01$) and strain ($P<0.01$) had significant effects on mortality. Different letters indicate significant differences between treatments (two-way ANOVA, Tukey's HSD post-hoc test, $P<0.05$).

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CHAPTER IV

COMPARATIVE TRANSCRIPTOMICS OF CHRONIC HIGH TEMPERATURE EFFECTS ON GEOGRAPHIC STRAINS OF CHANNEL AND HYBRID CATFISHES

Abstract

Ectotherms are highly vulnerable to changes in temperature, thus global climate change is a growing concern. This study examined differentially expressed (DE) gene transcripts of and regulated pathways of two geographically distinct channel catfish (*Ictalurus punctatus*) strains and one hybrid catfish (*I. punctatus* x [blue catfish] *I. furcatus*) strain following a six-week growth experiment, where fish were subjected to daily cycling temperatures of either 27-31°C or 32-36°C, mimicking pond fluctuations. We sequenced 18 cDNA libraries of liver samples to obtain 61 million reads per library. There were 5,443 DE transcripts and 41,689 regulated pathways. Northern channel catfish had the highest amount of DE transcripts (48.6%), 5 times that of southern channel catfish, and the greatest amount of transcripts with fold changes ≥ 2. The overall amount of temperature-induced DE transcripts between southern hybrid and southern channel catfish was fairly comparable in relation to that of northern channel catfish, however, there were more transcripts up- or downregulated with ≥ 2 fold changes in channel catfish strains compared to the southern hybrid catfish. Results from this study strongly suggest genetic differences between geographic catfish types affect

physiological responses to thermal stress. Furthermore, a number of genes were linked to thermal stress tolerance, which may be beneficial for understanding geographic differences in thermal stress tolerance in ectotherms and for strain development of catfish.

Introduction

Physiological processes in ectotherms are directly influenced by environmental temperature with optimal performance occurring within a limited temperature range (Hutchison & Maness 1979). This characteristic makes ectotherms highly vulnerable to altered weather patterns such as prolonged warming periods and climate variability (Pörtner 2002; Brander 2007; Ficke *et al.* 2007). Thus, predicted increases in temperatures of 1-7°C, under climate change models (Ficke *et al.* 2007) may impose limits on the normal adaptive capacity of ectotherms (Brander 2007). Therefore, studies of thermal adaptive capacity in ectotherms are important for understanding impacts of temperature on physiological processes such as: metabolism, growth, energy expenditure, and reproduction (Crawshaw & Hammel 1974; Hutchinson & Maness 1979; Gunter *et al.* 2007).

Comparative transcriptomics, a method of gene expression profiling, has proven effective for revealing transcriptional responses contributing to adaptation to environmental changes (Cossins *et al.* 2006; Gracey 2008; Prunet *et al.* 2008). This technique is a valuable tool for examining the molecular basis of physiological plasticity, and has been widely used in fishes. Previous transcriptomic research on fishes has focused on the ability of species to survive in environments with volatile conditions:

particularly oxygen availability, salinity and temperature (Gracey *et al.* 2001; Podrabsky & Somero 2004; Buckley *et al.* 2006; Fangue *et al.* 2006; Logan & Somero 2010).

Transcriptomics can be used to detect temperature-induced stress responses (Prunet *et al.* 2008, Tomanek 2010, Vergauwen *et al.* 2010, Liu *et al.* 2013). The main transcripts observed in ectotherms under high temperature stress are related to cell cycle and apoptosis such as CDKN1B and SGK1; cytoskeleton organization and biogenesis, such as dynein and cyclin G1 genes; protein folding and translation regulation such as ribosomal proteins, ubiquitin and heat shock proteins (HSP) (Truebano *et al.* 2010; Logan & Somero 2011); and cellular repair and immune function such as DnaJB11 and elastase2 (Kassahn *et al.* 2007). Of these genes, HSPs are well described for their cytoprotective properties: improving the capability to recover and survive thermal stress by repairing denatured proteins and stabilizing DNA (Bukau & Horwich 1998; Kregel 2002; Pörtner 2002; Pockley 2003; Roberts *et al.* 2010; Dalvi *et al.* 2012). HSPs are also involved in fish immune responses, apoptosis and the inflammatory process with apoptosis occurring if the stressor is too severe (Ellis 2001; Pirkkala *et al.* 2001). Long-term HSP expression occurs at the expense of other protein synthesis (Tomanek & Somero 2000). A number of comparative gene expression studies have been conducted in aquaculture environments.

In aquaculture, warming temperatures can have undesirable consequences on culture species, such as altering: growth (Brett 1979; McCauley & Beitinger 1992; Brandt 1993), reproductive capacity (Lang *et al.* 2003), spawning timing (Brander 1994; Barange & Perry 2009), physiology (Brett 1956), behavior, immune system function and mortality (Le Morvan *et al.* 1998; Ficke *et al.* 2007). In addition, climate change can

potentially affect the productivity of aquaculture by creating logistical and environmental complications in production, limiting fishmeal and fish oil for feed production, increasing prevalence of pathogens (De Silva & Soto 2009; Handisyde *et al.* 2006) and decreasing oxygen availability in summer months (Brander 2007). Species may be rendered unsuitable for previously ideal culture regions, forcing production to move to cooler regions or necessitating replacement with more tolerant species (McCauley & Beitinger 1992; Clemmensen *et al.* 2007).

Fish species may have different chronic thermal tolerance or sensitivity based on their geographic distribution (Pulgar *et al.* 2005), which may be manifested by temperature-induced differences in growth and survival. Increased thermal tolerance is particularly important to the US catfish industry with the threat of climate change. The geographic range of channel catfish (*Ictalurus punctatus*) encompasses southern Canada to northern Mexico (McCauley & Beitinger 1992) and subsequently a vast range of environmental conditions, such as temperatures from 5-35°C (Bennett *et al.* 1998; Clark & Burns 2008; Tavares-Dias & Moraes 2007). Despite the broad thermal distribution of channel catfish, shifts in temperature can be detrimental. Energy put towards acclimation is taken away from other physiological processes. Temperatures outside of optimal range may impact growth and survival (Stewart et al in review). Since 2001, blue catfish (*I. furcatus*) have been frequently crossed with channel catfish to produce hybrid catfish for the catfish aquaculture industry (Chatakondi 2012). Blue catfish have a more tropical geographic range than channel catfish, occupying the central United States to Mexico, northern Guatemala and Belize (Graham 1999), which may indicate a greater thermal tolerance than channel catfish. Southern populations of blue catfish have demonstrated

improved growth in comparison to northern (Graham 1999); this difference is believed to be due to southern populations experiencing earlier sexual maturity (Hale & Timmons 1989), extended growing seasons and greater food diversity (Graham 1999). Hybridization produces offspring with higher dress-out percentages, faster growth, more uniform size at harvest, greater resistance to disease, and greater tolerance of low oxygen levels and crowding in pond systems (Giudice 1966; Yant *et al.* 1976; Smitherman *et al.* 1983; Dunham *et al.* 1983; Li *et al.* 2004; Ligeon *et al.* 2004; Dunham & Argue 2011; Kumar & Engle 2011). Hybrid catfish have demonstrated increased survival and more consistent growth in comparison to channel catfish between temperature ranges of 27-31°C and 32-36°C (Stewart *et al.*, in review).

Catfish aquaculture is the largest aquaculture industry in the US (Goldburg *et al.* 2001). The shallow design of catfish ponds (most are only 1.5 meters deep) makes them susceptible to changes in environmental conditions (Tidwell 2012). Water temperature fluctuations of 3-6°C from morning to afternoon have been observed (Wax *et al.* 1987; Arnold *et al.* 2013) with daily maximums up to 36-40°C for short durations during the summer (Liu *et al.* 2013). Therefore, an understanding of how high temperatures and changing climate are going to affect channel and hybrid catfish populations is needed. Breeding programs and population selection for heightened performance can lead to overall improvement of catfish strains if adaptive mechanisms for tolerating thermal stress and related genes are identified.

This study examined differential gene expression of two geographically distinct channel catfish strains and one hybrid catfish strain under simulated natural thermal regimes. Based off of previous research on acute heat stress, which found southern

channel catfish to have greater heat tolerance than northern (Stewart & Allen in press), it was hypothesized that northern channel catfish would have greater heat stress than southern channel catfish following prolonged upper range temperature exposure. It was predicted that large amounts of differentially expressed (DE) gene transcripts, representing heat stress, would be greater in northern channel catfish than southern channel catfish. Since hybrid catfish had more consistent growth and low mortality at upper range temperatures, while channel catfish had decreased growth (Stewart *et al.* in review), it was hypothesized that there would be lower amounts of DE gene transcripts in hybrid catfish compared to channel catfish of the same strain.

Methods

Strains

Two geographically distinct strains of channel catfish and the corresponding hybrid catfish from one of these strains (with a cross to an industry standard blue catfish strain) were used in this study. The strains of channel catfish used were: Delta Select (from the Mississippi Delta, Mississippi) described as southern and Red River (from the Red River, North Dakota) described as northern. These two strains were selected based on their disparate geographic distributions. Broodstock were obtained and maintained as previously described (Stewart et al in review).

System Design and Temperature Regulation

Chronic temperature trials were conducted for 6 weeks, during which fish were exposed to daily cycling temperatures of either optimal temperatures for best food conversion and greatest growth at 27-31°C or upper-range thermal temperatures of 32-

36°C following Arnold *et al.* (2013). Cycling temperature regimes mimicked natural pond daily temperature fluctuations. Each day starting at 0900, water temperature was increased from baseline temperatures of 27°C or 32°C to peak temperatures of 31°C or 36°C. Peak temperatures were reached by 1730, after which temperatures were slowly decreased to 27°C and 32°C for optimal and high thermal treatments respectively (Stewart *et al.* in review).

At each of the two temperature regimes, there were three types of catfish (southern channel, northern channel, and southern hybrid) with 3 replicate 430-l tanks per temperature and strain treatment, and 60 fingerlings (average 80.5 mm long) per tank, for a total of 18 tanks. All tanks in each temperature treatment were covered by mesh covers and connected to a large recirculating system that included mechanical and biological filtration, ultraviolet sterilization and aeration. Each of the recirculating systems was supplemented by a continuous, slow flow of new well-water, which maintained high water quality and acted to slowly reduce water temperature after the daily peak temperature period.

Fish were held under a simulated natural photoperiod (12 hours and 29 minutes L:11 hours and 31 minutes D – 12 hours and 49 minutes L:11 hours and 11 minutes D for latitude, longitude: 33 27.4'N, 88 49.3'W) which was adjusted weekly. Mortalities were removed immediately, recorded and taken to the Mississippi State University College of Veterinary Medicine Fish Diagnostic Laboratory for necropsy. At the end of the 6 week chronic trial period, each fish was anesthetized, measured and weighed after 24 hours of fasting to determine growth according to Mississippi State University Animal Use Protocol #12-035.

Feeding Regime

Fish were fed *ad libitum* twice daily with a formulated 1.6 mm pelleted diet of 44% protein (Rangen Inc., Buhl, ID), based on feeding protocols used at Thad Cochran National Warmwater Aquaculture Center (NWAC) as described by Stewart *et al.* (in review).

Water Quality

Water quality was monitored as described by Stewart *et al.* (in review), with acceptable water conditions maintained: ammonia 0.1-1.0 mg/L, unionized ammonia <0.05 ppm, nitrite <0.1 mg/L, nitrate <50 mg/L, alkalinity 20-400 ppm, hardness 20-400 ppm, dissolved oxygen >5 mg/L and pH 6.5-8.0 (Jensen 1988, Tucker et al 1990).

Sampling and RNA isolation

At the end of the 6 week chronic thermal trial, liver tissue was collected from fish euthanized with tricaine methanesulfonate (MS 222) (Argent Chemical Laboratories Inc., Redmond, WA) at 500 mg/L. Six fish from each tank were randomly chosen to extract samples. Two tissue samples (~50 mg each) were excised in a sterile setting using a sterile #24 stainless steel blade and each immediately placed in a polypropylene sterile 2 ml round bottom cryogenic vial (VWR, Atlanta, GA) containing 500 uL of TRI-reagent (Zymo Research, Irvine, CA), and flash frozen in liquid nitrogen. All samples were stored at -80°C until analysis.

For RNA extraction, one vial of liver tissue from each fish was allowed to thaw and was then homogenized in TRI-reagent using a TissueLyser (Qiagen, Germantown, MD). Liver was chosen for RNA extraction because of its metabolic and physiological

importance with stress response and high rates of protein turnover (Podrabsky & Somero 2004; Logan & Somero 2011; Liu *et al.* 2013). The Direct-zol RNA mini prep kit (Zymo Research) was used following homogenization according to manufacturer's recommendations. To prevent degradation, only four to six samples were run at a time and kept on ice until transfer to a zymo-spin IIC column and collection tube (Zymo research). Samples were eluted twice for more complete RNA extraction. Spectrophotometry (NanoDrop, Thermo Scientific, Wilmington, DE) was run on extracted RNA samples to determine purity and concentration. Bioanalysis was performed with a 2200 TapeStation System (Agilent, Santa Clara, CA) at the Warmwater Aquaculture Research Unit in Stoneville, Mississippi to determine RNA quality. RNA samples that had the best RNA integrity numbers (RIN, ranging 8.2-9.9) were pooled from 3 fish per tank for a total of 18 samples to reduce the effects of individual genetic variation.

Illumina Sequencing, Mapping and Differential Expression

RNAseq libraries were prepared with the Tru-seq RNA Sample Preparation Kit V2 Illumina (catalog #RS-122-2001) at Global Biologics (Columbia, Missouri). DNA libraries were sequenced (50 bp, single-end reads) on an Illlumina HiSeq 2000 using Illumina v3 chemistry and OLB1.9.4 software for base calling by the Institute for Genomics, Biocomputing & Biotechnology at Mississippi State University. Illumina was chosen due to being relatively cost-effective and technically efficient (Liu *et al.* 2011). Reads, a DNA sequence generated from a sequencer, were mapped to the Liu *et al.* (2013) transcriptome using Bowtie2 version 2.1.0 default parameters, which return the best mapping result for each read (Langmead & Salzberg 2012; Liu *et al.* 2013). The

number of reads that map to each transcript were counted and used in the DE expression. DE was calculated using the DESeq package for the R programming language (Anders & Huber 2010), by comparing the high and low temperature treatment of each fish strain. DE genes were characterized by a p-adjusted value equal to or less than 0.05 after adjustment for multiple testing with the Benjamini-Hochberg procedure (Benjamini & Hochberg 1995) using a false discovery rate of 10%.

Annotations

The National Center for Biotechnology Information (NCBI) nonredundant protein (nr) and UniProtKB uniprot_sprot (UniProt) databases were downloaded 6/10/2013 and 8/25/2013 respectively (NCBI Resource Coordinators, The UniProt Consortium). The highest scoring BLASTX alignment (Blastall 2.2.20) for each Liu *et al.* (2013) transcript was reported for each database (Altschul et al 1990). Open Reading Frames (ORFs) for each Liu *et al.* (2013) transcript were predicted with with OrfPredictor using BLASTX alignments to nr database using an E-value cutoff of 1e-10 (Min *et al.* 2005 a). ORF coverage was determined using the Targetid/annotator (Min *et al.* 2005 b). Functional annotation of the translated predicted ORFs was conducted with WebMGA using HMMER3 (hmmscan 3.0) against the Pfam (PFAM 24.0) database using the parameter e-value cutoff for prediction 0.001 (Eddy 1998, Finn *et al.* 2008, Wu *et al.* 2011) and Batch CD-search of the NCBI Conserved Domains CD database (CDD – 44354 PSSMs) using the parameters 'Search mode = Automatic, Expected Value threshold = 0.01, Maximum number of hits = 500' (Marchler-Bauer et al 2011). Pathway annotation of the translated predicted ORFs was conducted with WebMGA using BLASTP (blastall 2.2.15) alignments to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (KEGG

2/12/2012) using the parameter e-value cutoff for prediction 0.001 (Ogata *et al.* 1999). Signal peptide and transmembrane proteins were predicted in the translated predicted Lui *et al.* (2013) ORFs using SignalP (signal 4.0) (Petersen *et al.* 2011). DE transcripts were annotated using GOanna on the Agbase (Agbase Version 2.0) web server (McCarthy 2006; McCarthy 2011). A one to one reciprocal blast (BLASTX-TBLASTN, minimal bit score of 60) was conducted with the Liu *et al.* (2013) transcriptome and the zebrafish protein sequences in Genbank (downloaded 8/14/13) (Benson 2013). BLASTX results were analyzed two ways. The first way, they were narrowed down to significant DE ($P<0.05$) and divided into five primary biological function groups: protein folding, protein biosynthesis, proteolysis, metabolism and stress response (Table 3) from which counts of number of transcripts upregulated or downregulated could be summed. Some transcripts were involved in multiple processes and thus could not be easily categorized. Results were also evaluated by narrowing down transcripts to significant DE ($P<0.05$), dividing them into two categories: upregulation and downregulation for each type of catfish and then filtering by $\geq$ 2-fold change, a accepted standard of transcript profiling studies (Gracey *et al.* 2001; Podrabsky & Somero 2004; van der Meer *et al.* 2005; Buckley *et al.* 2006). Channel catfish have been found to have enough phylogenetic similarity to zebrafish *Danio rerio* to be comparable (Steinke *et al.* 2006), and it is believed that genetic predictions can be made by using zebrafish as a model (Jiang *et al.* 2011) which is why we performed a one to one reciprocal BLAST to compare pathways. The pathways associated with the zebrafish proteins were obtained from the wiki pathways, reactome and KEGG orthology (KO) databases (Croft 2011; Kelder 2012).

Results

Illumina Sequencing, Mapping and Differential Expression

The sequencing resulted in an average of 61 million reads per library with 100 million and 48 million representing the maximum and minimum values (Table 1). Between 36 and 76 million reads from each library were mapped to the Liu *et al.* (2013) transcriptome (Table 1). There were 136,463 transcripts found unique to the database. A total of 5,443 significant DE transcripts were found amongst the southern and northern channel catfish and the southern hybrid catfish, of which 608 (11.2%) were found DE in all. The northern channel catfish had the highest amount of DE transcripts at 2,644 (48.6%) compared to the 544 (9.9%) in southern channel catfish and 600 (11%) in southern hybrid (Figure 1).

Annotations

Of the Liu *et al.* (2013) transcripts, 43% and 34% had a BLASTX hit with a bit score above 60 against the NCBI nr and UniProt databases (Table 2). OrfPredictor translated 59,101 transcripts into the peptide sequence 27,266 were full length (Table 2). Of the predicted protein sequences 18,276 and 11,846 were assigned annotation based on the Pfam profiling and Batch CD-search, respectively (Table 2). KEGG pathway analysis resulted in the association of 7,927 different pathways to 41,689 transcripts (Table 2). Signal peptides were identified in 3,872 predicted protein sequences with the SignalP software. Of the predicted protein sequences with signal peptides 3,608 were not predicted to have a transmembrane domain (Table 2). GOanna produced 4,893 GO term annotations for 3,446 DE transcripts (Table 2). The zebrafish one to one reciprocal blast

resulted in 14,224 homologs being identified in which 627 were associated zebrafish pathways reported in Wikipathways, Reactome, and KO.

There was far more transcriptional upregulation than downregulation for all catfish types (Tables 3 and 4). For proteolysis, protease was only slightly more downregulated than upregulated. Northern channel catfish had triple the amount of downregulated protease related transcripts compared to southern channel catfish and twice the amount of hybrid catfish. Glycosidase was upregulated in northern and southern channel catfish but downregulated in southern hybrid catfish. Northern channel catfish had about twice the amount of transcripts being upregulated as southern channel catfish (Table 3).

For stress response, the adaptive immune system was upregulated primarily with northern channel catfish having twice the amount of transcripts as southern channel and three times the amount of southern hybrid catfish. The innate immune system was upregulated primarily with northern channel catfish having four times the amount of transcripts as southern channel (equal amount of transcripts up- and downregulated) and twice the amount of southern hybrid catfish. Apoptosis was primarily upregulated with the amount of upregulated transcripts being over twice as high in northern channel catfish compared to southern channel and slightly under twice as many in southern hybrid catfish. Angiogenesis was primarily upregulated in all catfish types with northern channel catfish having four times the amount of transcripts as southern channel and 1.5 times the amount of southern hybrid catfish. Cell development and growth was mainly upregulated with northern channel catfish having about 1.5 times the amount of transcripts as southern hybrid catfish and 3 times the amount of southern channel catfish. Ubiquitin was

mostly upregulated with the largest amounts of transcripts upregulated in southern and northern catfish. Ubiquitin was mainly upregulated with northern channel catfish having 1.5 times the amount of transcripts of southern channel and 2.5 times the amount of southern hybrid catfish. Significant upregulation of HSPs 1, 4, 8, 40, 47, 60, 70, 71 and 90 were observed with the greatest amount of transcripts upregulated in HSP 70 (northern channel 59, southern channel 34, southern hybrid 37) and HSP 90 (northern channel 56, southern channel 51, southern hybrid 51), followed by HSP 40 and the least amount of transcripts upregulated in HSP 8 (northern channel 2, southern channel 1, southern hybrid 0) (Table 3). Northern channel catfish generally had higher upregulation of all HSP transcripts than southern channel catfish and southern hybrid catfish (Table 3). Northern channel catfish had the largest amount of upregulated heat response transcripts, over three times the amount of southern channel or hybrid catfish. HSP 70 had the greatest fold change in all catfish types, with the largest being in northern channel catfish (8.9-9.4), followed by southern hybrid (8.0-8.8) and southern channel catfish (7.2-7.4) (Table 4).

For metabolism; amino acid metabolism was primarily downregulated with about equal amounts in northern and southern channel catfish, twice the amount found in southern hybrid catfish. Carbohydrate metabolism was equally upregulated and downregulated in northern channel catfish, mostly downregulated in southern channel catfish and in southern hybrid catfish there was twice the number of transcripts being upregulated as downregulated. Overall, lipid metabolism was primarily downregulated, with the greatest amounts in northern channel catfish, followed by southern hybrid and then southern channel catfish; although upregulation was present as well. Southern

channel catfish had no nucleotide metabolic activity, while northern channel catfish had five times the amount of transcripts being downregulated compared to southern hybrid catfish. Protein metabolic activity was again low in southern channel catfish, with northern channel catfish having about twice the amount of downregulated transcripts as southern hybrid catfish. Cytochrome P450 transcripts were equally upregulated and downregulated in northern channel catfish, while mostly downregulated in southern channel catfish and mostly upregulated in southern hybrid catfish (Table 3).

For protein folding; tubulin was relatively consistent amongst catfish types, with only upregulation of transcripts. Protein folding, however, was extremely varied between catfish types. Northern channel catfish had over twice the amount of transcripts upregulated as southern hybrid and 12 times the amount of southern channel catfish (Table 3). Dynein transcripts were among the top upregulated fold changes in all catfish types with 5.2-5.6 in northern channel, 4.5-6.7 in southern channel and 5.7-6.8 in southern hybrid catfish (Table 4). DnaJ homolog subfamily A was present in the greatest upregulation fold changes of all catfish types. Again, northern channel had the greatest fold change (6.2-6.7), followed by southern hybrid (5.2-6.3) and southern channel catfish (4.5-5.3) (Table 4).

For protein biosynthesis; northern channel catfish expressed over twice the amount of upregulated transcripts for glycosyltransferase than southern hybrid catfish and more than three times the amount of southern channel catfish. Northern channel catfish also had a large amount of transcripts downregulated which neither of the other two types of catfish had. Southern hybrid catfish had over double the amount of downregulated fatty acid biosynthesis transcripts compared to northern channel catfish and southern

channel catfish had no DE transcripts with this function. Dihydropyrimidinase was also downregulated in all catfish types with southern channel catfish having twice the amount of transcripts downregulated as southern hybrid and northern channel catfish having three times the amount of southern hybrid catfish. Southern hybrid catfish was the only catfish type to have any transcripts regarding the mevalonate pathway which was entirely downregulated (Table 3).

Northern channel catfish had the greatest amount of classified transcripts with significant fold changes (256), followed by southern channel catfish (182) and southern hybrid catfish (150). Of the northern channel catfish transcripts, 47% were shared with the other two catfish types; compared to 64% shared in southern channel and 70% shared in southern hybrid catfish. About half (48%) of the remaining northern channel catfish transcripts were unique to that catfish type and 44% were shared with southern channel catfish alone. For southern channel catfish, over 58% of the remaining transcripts were shared with northern channel catfish and 25% were unique to southern channel catfish. Southern hybrid catfish had equal amounts of transcripts shared between southern and northern channel catfish. Of the remaining transcripts, over 55% were unique to southern hybrid catfish.

Discussion

Northern channel catfish clearly showed greater temperature-induced stress than southern channel catfish or southern hybrid catfish, as revealed by transcriptional expression. In response to chronic high temperature regimes, northern channel catfish had greater numbers of DE transcripts and greater magnitudes of change in upregulated DE transcripts. Comparing channel catfish with hybrid catfish, there were more transcripts

that experienced large fold changes (≥ 2) in both the southern (219) and northern (311) channel catfish strains compared to the southern hybrid (163) catfish. However, the overall number of DE transcripts between southern hybrid (600) and southern channel catfish (544) was fairly comparable in relation to that of northern channel catfish (2644). Thus, from an overall measure of transcriptional regulation change, these results do not indicate differences in stress between southern channel and hybrid catfish. In contrast, whole-body measures of growth indicate that southern channel catfish may have greater impacts of temperature on physiological performance than hybrid catfish (Stewart *et al.* in review).

To create a better understanding of function, transcripts were divided into five primary categories for analysis: proteolysis, stress response, metabolism, protein folding and protein biosynthesis, following similar techniques used by Liu *et al.* (2013) and Logan & Somero (2010).

Proteolysis between the studies had no direct overlap of DE transcripts. The closest relationship was that ubiquitin-conjugating enzyme E2 G2 and ubiquitin-conjugating enzyme E2 L3 were present under acute thermal stress and ubiquitin-conjugating enzyme E2 C was present under chronic thermal stress. Northern channel catfish had the greatest amount and magnitude of upregulated DE transcripts for ubiquitin, followed by southern hybrid catfish. Ubiquitin was one of the transcripts that overlapped pathways, however transcripts were only counted once to avoid over representation.

Stress response can be indicated by upregulation of HSP 70, ubiquitin, and apoptosis. In this study, although the number of upregulated transcripts were relatively

constant between southern channel and hybrid catfish for HSP 70, ubiquitin and apoptosis; northern channel catfish had about double the amount. The magnitude of fold change indicates that HSP 70 had the greatest upregulation, followed by ubiquitin, and apoptosis did not have fold changes >2. Logan & Somero (2011) argue that there is a correlation between severity of stress and expression of different categories of genes. In *Gillichthys mirabilis*, under an environmentally realistic heating rate of (4°C/h), they observed upregulation of HSP 70 with mild temperature stress, upregulation of ubiquitin at high temperature stress, and upregulation of apoptosis only with extreme temperature stress. Thus, based on Logan & Somero's (2011) and Stewart & Allen (in press) observations, these fish were under high but not extreme stress.

As found by Kassahn *et al.* (2007), one of the main transcripts seen in relation to high temperature stress was cytoskeleton organization. Dynein was among the greatest magnitude fold change in all catfish types with the greatest being found in southern hybrid catfish. Transcripts relating to angiogenesis and neurogenesis also had large amounts of upregulation with northern channel having 4-5 times the amount of southern channel catfish. Whereas Kassahn *et al.* (2007) found DnaJB11 to be associated with stress in reef fish; catfish demonstrated the greatest magnitude fold change of DnaJA(1,4) with thermal stress, greatest in northern channel catfish. Ribosomal transcripts also had large magnitude fold changes with upregulation, greatest being in northern channel catfish. Ribosomal protein genes have been found previously with heat stress (Buckley *et al.* 2006; Buckley & Somero 2009; Truebano *et al.* 2010) and it is believed that the upregulation of ribosomal protein genes indicates the presence of DNA repair and transcription (Lindström 2009).

Cellular effects of stress were indicated by the upregulation of HSPs across all catfish types. Northern channel catfish had both the greatest amount of HSP 70 transcripts upregulated and the largest fold changes in HSP 70, providing further evidence that this catfish type was under the greatest thermal stress. Similarly, Podrabsky & Somero (2004) observed upregulation of HSP 70 and HSP 90 at chronic high temperatures. Southern hybrid catfish had the second greatest amount of HSP 70 transcripts upregulated and second largest magnitude of fold changes in HSP 70 and HSP 90, which was unexpected, considering that these same temperature regimes did not result in a decrease in growth or an increase in mortality (Stewart *et al.* in review). HSP 70 is generally the most responsive heat-shock protein (Kurtz *et al.* 1986; Locke 1997) and an important product of protein synthesis in cells under thermal stress in channel catfish (Luft *et al.* 1996). It promotes the proper re-folding and re-assembly of denatured proteins that can be caused by environmental stress and is believed to play a fundamental role in cellular functions as well as responses to stress because it is evolutionarily highly conserved (Luft *et al.* 1996). Since HSP 70 can also be expressed under other types of stress (i.e., oxidative stress; Roberts *et al.* 2010), it is important that there was triple the amount of upregulated heat response transcripts found in northern channel catfish, indicating that northern strains of catfish have lower thermal tolerance than southern (Morimoto 1998). HSPs not only play a role in refolding and maintaining proteins, they also have immunogenic properties (Schmitt *et al.* 2007).

Also related to stress response is immunity. Gene transcripts associated with the innate immune system have been found to be highly upregulated during chronic thermal stress in annual killifish (*Austrofundulus limnaeus*) (Podradsky & Somero 2004).

Although the innate immune response could be self-harming in the long term causing tissue damage (Roitt *et al.* 1993), high levels of upregulated transcripts encoding for the complement system continue to be expressed with chronic high cycling temperatures (Podradsky & Somero 2004). In northern channel catfish and southern hybrid catfish the innate immune system was primarily upregulated with northern channel catfish having twice the expression of southern hybrids and four times the expression of southern channel catfish. The adaptive immune system, which provides long lasting protection from potentially harmful antigens (Ostberg *et al.* 2007), was also primarily upregulated with northern channel catfish having over twice the expression of southern channel or hybrid catfish.

In terms of metabolism-related transcripts, inositol-3-phosphate synthase 1-A was DE under the chronic thermal conditions of this study and the acute conditions of Liu *et al.* (2013). Liu *et al.* (2013) observed cytochrome c oxidase subunit II and cytochrome c oxidase subunit VIIa 2 transcripts with DE under acute thermal stress, however they were not seen under chronic thermal stress, instead cytochrome b-c1 complex subunit 6 was observed. The majority of transcripts listed in the Liu *et al.* (2013) study were not observed under chronic thermal stress. Differences in molecular effects on metabolism were expected to be greatly varied under chronic versus acute stress because alternative processes are required to maintain homeostasis. During acute thermal stress, genes involved in lipid metabolism are the first to be upregulated to allow for ATP production, supplying energy to escape rapidly changing temperatures (Sargent *et al.* 1989; Hazel 1995; Hochachka & Somero 2002). With chronic stress, genes involved with protein metabolism were upregulated more (Tseng & Hwang 2008). Northern channel catfish had

the greatest amount and magnitude of upregulated DE transcripts related to protease, followed by southern hybrid catfish. This may indicate that the northern channel catfish were in greater need for an immediate fuel source, as found in amino acids.

Protein folding related gene transcripts found by Liu et al (2013) under acute thermal exposure, and also found in this study under chronic thermal exposure were: DnaJ homolog subfamily A member 1, DnaJ homolog subfamily A member 4, endoplasmin, stress-induced-phosphoprotein 1, cysteine and histidine-rich domain-containing protein 1, peptidyl-prolyl cis-trans isomerase FKBP4, and protein disulfide-isomerase family A member 6. The primary difference in presence of DE transcripts between acute and chronic thermal stress was lack of calreticulin, calnexin, calumenin-B precursor and T-complex protein 1 subunit delta in this chronic trial. In this study, northern channel catfish had the greatest amount and magnitude of upregulated DE transcripts related to protein folding, followed by southern hybrid catfish. For categorization purposes, HSPs were included in the stress response, even though they are known to have important roles in protein folding.

Protein biosynthesis-related transcripts found by Liu *et al.* (2013) under acute thermal stress had no clear relationship to those found in this study under chronic thermal stress. The most closely related transcripts found between these studies were 40S ribosomal protein S27 compared to 40S ribosomal protein S15, 40S ribosomal protein S15a and 40S ribosomal protein S23 found under acute thermal stress. Different proteins are synthesized under acute thermal stress compared to chronic thermal stress because with acute stress energy is partitioned to immediate recovery. Thus the principle proteins being synthesized are heat shock proteins. Under chronic thermal stress there is some

acclimation which allows for energy to be allocated to longer-term survival strategies (Vergauwen *et al.* 2010).

In addition to evaluating gene transcript expression changes by categories, overall differences in expression in catfish types revealed differences in responses to high temperatures. Southern hybrid catfish had a greater number of upregulated DE transcripts than expected, although still comparable to southern channel catfish, for several possible reasons. First, instead of hybrid catfish exhibiting hybrid vigor or thermal tolerance expected from a blue catfish parent; thermal tolerance may be an epistatic trait and thus hybridization is breaking up the associated gene complexes (Burke & Arnold 2001). Second, blue catfish may not have as high of a thermal tolerance as predicted, which may have decreased the thermal tolerance in hybrid catfish. Limited research has been conducted on blue catfish so their relative thermal tolerance is unknown. In support, Stewart & Allen (in press) found that hybrid catfish had lower thermal tolerance in comparison to channel catfish. Third, blue catfish occur in sub-tropical to tropical areas, where water temperatures are less variable in seasonal fluctuation compared to temperate areas where channel catfish are found (Ficke *et al.* 2007), thus channel catfish may have greater thermoplasticity. Fourth, the blue catfish broodstock originated from the same general region as the channel catfish broodstock so heat related traits hypothesized to be passed on might not have been. Results may have been different if blue catfish broodstock were obtained from a more southern distribution, within their natural range.

Differences observed between catfish from different geographic ranges were as expected, with northern channel catfish having both the greatest number of DE transcripts as well as the greater magnitudes of change in upregulated DE transcripts. A potential

explanation, is that northern channel catfish may have already been at the edge of their limits of thermoplasticity or perhaps they have lost more of their upper thermal tolerance over generations due to selection processes, making it more difficult to acclimate to high thermal temperatures than southern channel catfish.

Previous transcriptome studies have observed an effect of thermal extremes decreasing growth rates and expression of growth-related gene transcripts of ectotherms (Logan & Somero 2010; Pankhurst & King 2010; Vergauwen *et al.* 2010; Quinn *et al.* 2011). Under thermal stress, there is a decrease in expression of cell growth and proliferation related genes (Gracey *et al.* 2001; Gracey *et al.* 2008). Following their observation that gene ontology associated with metabolism remained upregulated following prolonged heat stress in coral reef fish (*Pomacentrus moluccensis*), Kassahn *et al.* (2007) suggest that thermal stress causes sustained reallocation of energy reserves. Vergauwen *et al.* (2010) observed a depletion in energy reserves and a decrease of condition factor in zebrafish (*Danio rerio*) near their upper thermal tolerance. Similarly, growth rates of channel catfish were observed to decrease under chronic thermal stress, with northern channel catfish strains having lesser weight gain in comparison to southern channel catfish strains (Stewart *et al.* in review). Chronic thermal trials in tanks also found hybrid southern catfish had the highest survival and most consistent growth between temperature ranges (Stewart *et al.* in review). Under acute thermal stress, southern channel and southern hybrid catfish had no difference in survival while southern strains of channel catfish had higher survival than northern strains. Similar trends in acute thermal tolerance were observed in channel catfish strains and the hybrid cross for each (i.e. southern channel and southern hybrid following the same pattern) suggesting

heritability (Stewart *et al.* in review), which the results of this study further support. Some of the shared magnitude fold change DE transcripts between southern channel and hybrid catfish were: collectin-12, complement receptor type 1, heat shock 70 kDa protein 4 and microfibril-associated glycoprotein 4.

While many transcriptomic studies have been conducted examining acute thermal stress in fishes (Buckley *et al.* 2006; Kassahn *et al.* 2007; Logan & Somero 2010; Dalvi *et al.* 2012; Liu *et al.* 2013), only a few have been conducted on chronic thermal stress, and very few on environmentally realistic temperature regimes. Many of the molecular processes that occur under chronic thermal stress are first switched on under acute thermal stress (Horowitz 2002). Thus, comparisons between the Liu *et al.* (2013) acute upper temperature study on hybrid catfish were made, however many differences were found that are likely due to the duration of the thermal stress. Logan & Somero (2010) found that multiple classes of stress-related proteins, such as HSP, were upregulated during acute heat stress but were no longer present under long term acclimation; indicating that current studies on acute thermal trials may not detect many of the physiological processes occurring under long-term thermal stress. In *Gadus morhua*, increases in temperature (1°C/5 days) that mimicked seasonal water temperature changes upregulated immune-related genes (Pérez-Casanova *et al.* 2008). Similarly, Kassahn *et al.* (2007) found that prolonged heat exposure challenged the immune system of coral reef fish. Both of these studies concur with the temperature-induced upregulation of immune-related genes in this study, described above, and emphasize the importance of examining environmentally realistic temperature regimes.

Results from this study strongly suggest genetic differences between catfish types affect physiological responses to thermal stress. The amount of DE transcripts was consistently similar between southern channel and southern hybrid catfish compared to northern channel catfish (Table 3). Further, the number of overall DE transcripts with large fold changes was closer between northern and southern channel catfish than southern hybrid catfish. Therefore, future breeding programs should take this information into consideration to provide stronger and more selective strains for the catfish industry. Genes identified in this study, may be beneficial for the development of strains of catfish with greater thermal tolerance. Studies on channel and hybrid catfish gene expression will continue to make great strides in comprehending cellular effects of thermal stress by identifying genetic pathways controlling traits and genetic variation (Liu *et al.* 2008). Using catfish as a model, the same concept can be applied to other species for increased aquaculture productivity or conservation.

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Table 4.1 Number of reads and mapping results.

Treatment	Reads	Percent Mapped
Red River 27-31°C	48,220,749	75.159
Red River 27-31°C	52,770,631	75.262
Red River 27-31°C	54,419,520	74.773
Red River 32-36°C	62,857,772	75.356
Red River 32-36°C	65,720,616	75.021
Red River 32-36°C	50,925,243	75.699
Delta Select 27-31°C	64,224,524	74.842
Delta Select 27-31°C	63,123,693	75.138
Delta Select 27-31°C	59,619,074	74.447
Delta Select 32-36°C	100,849,111	75.505
Delta Select 32-36°C	65,438,804	75.005
Delta Select 32-36°C	60,697,855	75.844
Delta Select hybrid 27-31°C	50,148,185	75.139
Delta Select hybrid 27-31°C	61,918,937	74.408
Delta Select hybrid 27-31°C	48,732,371	74.851
Delta Select hybrid 32-36°C	62,630,052	75.293
Delta Select hybrid 32-36°C	52,993,798	75.381
Delta Select hybrid 32-36°C	65,770,666	75.207

Each sample was made by pooling the three RNA samples with the top RNA integrity numbers (RIN value) from that tank. Treatment of each sample, number of reads generated for each RNAseq library and the percent successfully mapped to the Liu et al (2013) transcriptome with Bowtie2

Table 4.2 Number of transcripts with annotation.

Analysis	Number of Liu Transcripts	Percent Liu Transcripts	Number of DE Transcripts	Percent DE Transcripts
Blastx				
NCBI nr	62,655	43.29	2,813	51.68
UniProt	48,509	33.51	2,470	45.38
ORFpredictor				
Predicted Proteins	59,101	40.83	2,705	49.70
Full Length	27,266	18.84	1,305	23.98
Functional Analysis				
Pfam	18,276	12.63	1,010	18.56
Batch CD-search	11,846	8.18	546	10.03
Goanna	NA	NA	3,446	63.31
Signal Peptide	3,872	2.68	237	4.35
Signal Peptide – no TM	3,608	2.49	220	4.04
Pathway analysis				
KEGG	41,689	28.80	2,203	40.47
Reciprocal Blast *Danio rerio*				
Homologs	14,224	9.83	825	15.16
Pathways	627	0.43	30	0.55
No Annotations				
	80,710	55.76	1,640	30.13

Table 4.3 Differentially-expressed transcripts of catfish under sustained 27-31°C and 32-36°C cycling temperature regimes.

Function	Northern (Red River) Channel		Southern (Delta Select) Channel		Southern (Delta Select) Hybrid	
	Number of Transcripts Upregulated	Number of Transcripts Downregulated	Number of Transcripts Upregulated	Number of Transcripts Downregulated	Number of Transcripts Upregulated	Number of Transcripts Downregulated
Proteolysis	105	67	50	29	54	28
Glycosidase	8		5			4
Metal ion binding	3	2	1		1	
Methyltransferase	1	2		2		
Protease	13	22	5	7	8	11
RNA binding	2		2		2	
Stress Response	1081	565	493	123	527	209
Adaptive immune system	18	4	8	1	6	1
Angiogenesis	18	3	4	2	12	1
Apoptosis^{+}	53	14	20	1	28	4
ATP synthesis	13	3	7	1	5	
Cell adhesion	6	11	1	1	3	1
Cell cycle arrest	3	2	2		2	
Cell development and growth	12	5	4	2	9	4
DNA repair	1	1			1	1
Endocytosis	6	10		3	2	1
Heat response	16		5		5	
Heat shock protein 1	4				4	
Heat shock protein 4	5		5		4	
Heat shock protein 8	2		1			
Heat shock protein 47	8		8		8	
Heat shock protein 60^{+}	8		8		8	

Table 4.3 (Continued)

Function	Northern (Red River) Channel		Southern (Delta Select) Channel		Southern (Delta Select) Hybrid	
	Number of Transcripts Upregulated	Number of Transcripts Downregulated	Number of Transcripts Upregulated	Number of Transcripts Downregulated	Number of Transcripts Upregulated	Number of Transcripts Downregulated
Heat shock protein 70+	59		34		37	
Heat shock protein 71	13		10		11	
Heat shock protein 90	56		51		51	
Heme biosynthesis	2	1				1
Innate immune system	20	10	5	5	10	1
Neurogenesis	11	6	2	5	4	
Signal transduction	42	18	15	3	16	
Transcription+	76	39	21	2	16	14
Ubiquitination+	57	23	35	5	23	5
Metabolism	183	127	34	44	78	87
Amino acid biosynthesis	7	4	1	1	2	5
Amino acid metabolism	2	14		7		13
Aminotransferase		3		3		3
ATP binding	46		2		15	
Carbohydrate biosynthesis	5	1	4		1	1
Carbohydrate metabolism	12	12	1	6	12	5
Carbonic Anhydrase	7		1		3	
Cytochrome P450+	11	8	1	1	3	6
DNA binding	4	7	1		2	1
Lipid biosynthesis	8	9	3	3	4	9
Lipid metabolism	18	25	6	10	10	17
Nucleotide biosynthesis	5	1			2	
Nucleotide metabolism	4	11				2
Nucleotidyltransferase	1				7	

Table 4.3 (Continued)

Function	Northern (Red River) Channel		Southern (Delta Select) Channel		Southern (Delta Select) Hybrid	
	Number of Transcripts Upregulated	Number of Transcripts Downregulated	Number of Transcripts Upregulated	Number of Transcripts Downregulated	Number of Transcripts Upregulated	Number of Transcripts Downregulated
Protein biosynthesis	4	8		6		6
Protein metabolism	6	8	1		2	5
Transferase	3	8		2		4
Protein Folding	73	3	52	0	59	0
Cellular respiration	3		5			
Heat shock protein 40+	21	4	19		16	2
Iron-sulfur cluster assembly	3		2		1	
NAD-specific glutamate dehydrogenase	1				1	
Protein folding	48	1	4		18	
Tubulin folding pathway	6		5		5	
Protein Biosynthesis	66	58	18	19	20	41
Aminotransferase+	5	1		3		3
C/M/P thioester hydrolase		4				
Dihydropyrimidinase		6		4		2
Eukaryotic translation	14	5	3	6	2	6
Fatty acid biosynthesis		4				10
Glycosyltransferase+	10	8	3		4	
GTP cyclohydrolase		1		1		2
Mevalonate pathway						5
Phosphatase	2	2	2		1	
Sulfotransferase	1	2				1

Differentially-expressed transcripts ($p<0.05$) of two strains of channel catfish (northern and southern) and one hybrid strain (southern), following 6 weeks of cycling temperature regimes (27-31°C and 32-36°C). Numbers indicate the number of differentially-expressed transcripts either up- or downregulated between these thermal regimes for each type of catfish. The + symbol indicates a transcript involved in multiple processes.

Table 4.4 Magnitude of change in differentially expressed transcripts of catfish under sustained 27-31°C and 32-36°C cycling temperature regimes.

Upregulated Genes and Pathways	Northern Channel		Southern Channel		Southern Hybrid	
	Fold Change	Number of Transcript	Fold Change	Number of Transcript	Fold Change	Number of Transcript
2-acylglycerol O-acyltransferase 2-A					2.4	1
40S ribosomal protein S27	3.5-3.8	2	2.0-2.4	2	2.7	1
78 kDa glucose-regulated protein	3.7-4.2	5	2.2-2.8	5		
Activator of 90 kDa heat shock protein ATPase homolog 1	3.8-4.0	3	2.0-2.7	3	2.3-3.0	3
Adenine phosphoribosyltransferase					2.6	1
Adrenomedullin 2					2.3	1
Alkaline phosphatase					2.3	1
Alpha-actinin-2			2.2	3		
Ankyrin repeat and SOCS box protein 5	4.1	1	3.3	1		
Aquaporin-12	2.1-2.3	3	2.1	1		
ATP synthase coupling factor 6			2.0-2.2	2		
BAG family molecular chaperone regulator (2, 3)	4.2-4.5	4	3.0-5.0	4	3.4-3.8	4
Bestrophin-2	2.3-3.2	2	2.2-3.3	3	2.3-3.0	2
Beta-2-microglobulin	2.6-2.6	2				
Calmodulin	2.2-2.3	2				
Calponin-3	2.1	1			2.0	1
C-C chemokine receptor type 11	2.9	1	3.3	1		
CDP-diacylglycerol--serine O-phosphatidyltransferase	2.5	2	2.5-2.7	1	2.6-2.7	2
Ceramide synthase 2					3.3	1
Chaperonin Cpn60	3.4	1				
Chromobox protein homolog 7	2.0-3.6	2	3.1	1	2.3-2.8	2

Table 4.4 (Continued)

Upregulated Genes and Pathways	Northern Channel		Southern Channel		Southern Hybrid	
	Fold Change	Number of Transcript	Fold Change	Number of Transcript	Fold Change	Number of Transcript
Class I histocompatibility antigen, F10 alpha chain			2.8	1		
Clusterin	2.4-2.5	2	2.2	1	2.2	1
Coiled-coil domain-containing protein (65, 148)	2.5-2.8	2	3.1	1	2.1	1
Collectin-12			2.1-2.7	2	3.1	1
Complement factor H			2.9	1		
Complement receptor type 1			2.6	1	3.0	1
Cyclin-dependent kinases regulatory subunit 2	3.2-3.4	3	2.2-2.3	3	2.7-3.0	3
Cysteine and histidine-rich domain-containing protein 1	6.9-7.7	4	4.5-5.9	4	3.6-4.9	3
Cysteine-rich with EGF-like domain protein (1, 2)	2.5-2.8	3	2.2-2.3	2	3.0	1
Cytochrome b-c1 complex subunit 6	2.9-3.1	3	2.6-2.9	2	2.3-2.9	3
DEP domain-containing protein 1B			2.6	1		
DNA damage-inducible transcript 4-like protein	3.5	1				
DNA replication ATP-dependent helicase/nuclease DNA2					2.0-4.1	4
DnaJ homolog subfamily A member (1, 4)	6.2-6.7	5	4.5-5.3	5	5.2-6.3	5
DnaJ homolog subfamily B member (1, 4, 6, 9)	2.0-4.5	10	2.0-2.7	3	2.2-3.4	4
DnaJ homolog subfamily C member 3			2.5-3.2	5		
Dual specificity phosphatase DUPD1	2.6	1	3.1	1		
Dual specificity protein phosphatase 14	6.4	1	4.1	1	6.0	1
Dynein assembly factor 1	4.3	1			5.2	1
Dynein intermediate chain (1, 2)	5.2-5.6	3	4.5-6.7	3	5.7-6.8	2
Dynein regulatory complex protein 1	2.4-3.0	9	2.8	1		

Table 4.4 (Continued)

Upregulated Genes and Pathways	Northern Channel		Southern Channel		Southern Hybrid	
	Fold Change	Number of Transcript	Fold Change	Number of Transcript	Fold Change	Number of Transcript
E3 ubiquitin-protein ligase MYLIP-B	2.0-2.2	2				
Endoplasmin	2.4-2.7	4	2.3-2.5	3		
Eosinophil peroxidase			3.0	1		
Erythroid differentiation-related factor 1	2.3	1				
Excitatory amino acid transporter 1	3.1	1			2.7	1
Exportin-1	2.0-2.1	2				
Follistatin-related protein 3					2.1	1
Glutamate carboxypeptidase 2	2.1	1				
Growth hormone-inducible transmembrane protein	2.1-2.3	4				
GTPase IMAP family member 8			2.3	1		
Heat shock 60 kDa protein	3.4-3.6	7	2.2-2.7	8	2.4-2.7	8
Heat shock 28 kDa protein	2.0	1				
Heat shock 70 kDa protein (1, 1A/1B)	8.9-9.4	6	7.1-7.4	6	8.0-8.8	6
Heat shock 70 kDa protein 4			2.3-2.9	6	2.3-3.4	6
Heat shock 70 binding protein	2.6-2.7	2				
Heat shock cognate 71 kDa protein	2.5-2.5	6	2.2	1		
Heat shock protein 4	3.8	1	2.8	1		
Heat shock protein 90-alpha (1, 2)	4.1-5.9	11	2.5-3.6	12	3.6-4.5	11
Heat shock protein 90-beta	2.5-5.7	4	2.2-3.3	3	2.4-4.5	3
Hepcidin	2.0-2.3	2				
Homeobox protein SIX4	2.0	1				
Hyaluronan and proteoglycan link protein 2	2.3	1				
Hypoxia up-regulated protein 1	3.5-3.9	6	2.7-3.0	6	2.0	1
Inositol-3-phosphate synthase 1-A	3.8-4.1	2	2.5	2		

Table 4.4 (Continued)

Upregulated Genes and Pathways	Northern Channel		Southern Channel		Southern Hybrid	
	Fold Change	Number of Transcript	Fold Change	Number of Transcript	Fold Change	Number of Transcript
Insulin-like growth factor-binding protein 1	2.1	1				
Interferon-induced protein 44					3.2	1
Intraflagellar transport protein 43 homolog A	2.3	1			2.0	1
Kelch-like protein 38					2.1	1
Kynurenine--oxoglutarate transaminase 3	2.7	1				
Leukotriene B4 receptor 1			2.1	1		
Lon protease homolog	2.2	1				
Major facilitator superfamily domain-containing protein 2A-B	2.7	2				
Membrane cofactor protein	2.3	1	2.2-3.0	3	2.8-2.9	2
Mesencephalic astrocyte-derived neurotrophic factor	2.3-2.5	3	2.0	2		
Microfibril-associated glycoprotein 4			2.1-2.6	3	2.0-2.4	4
M-protein	2.2	1				
Myeloid leukemia factor 1	5.7	1	5.0	1	4.5	1
Myomesin-2	2.2-2.4	2			7.4	1
NACHT, LRR and PYD domains-containing protein 12	2.3	1				
Neoverrucotoxin subunit alpha					2.7	1
Nicotinamide nucleotide transhydrogenase	2.0	1				
Nuclear receptor coactivator 7	2.7-3.1	4				
Nucleoside diphosphate kinase 3	2.2	1				
Olfactomedin-like 3			2.0	1		
Peptidyl-prolyl cis-trans isomerase D	2.4-4.8	2	3.3	1	2.1-3.1	2
Peptidyl-prolyl cis-trans isomerase FKBP4	3.7-3.8	2	2.8-3.0	2	3.1	2

Table 4.4 (Continued)

Upregulated Genes and Pathways	Northern Channel		Southern Channel		Southern Hybrid	
	Fold Change	Number of Transcript	Fold Change	Number of Transcript	Fold Change	Number of Transcript
Period circadian protein homolog 2	2.1	1				
PEX5-related protein	2.1-2.4	4	2.3-2.8	4		
Phospholipase (D1, D2)	2.7-5.3	2	3.3	1	2.2-3.0	3
Pleckstrin homology-like domain family B member 2	2.10	1				
Podocan-like protein 1	3.7	1	3.3	1		
Polyubiquitin			2.4	1		
Potassium-transporting ATPase alpha chain 1			2.3	1		
Probable ATP-dependent RNA helicase (DDX11, DDX47)	2.1-2.7	3	3.0-3.4	2	5.9	1
Procollagen galactosyltransferase 1	3.5-6.0	2	2.8	1	2.2-2.8	3
Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3	2.4-2.6	3	2.2-2.2	3		
Prolyl 4-hydroxylase subunit alpha-1	3.0-3.2	3	2.8-3.1	3	2.1	1
Prostaglandin E synthase 3	2.0	1	2.2	1		
Protein canopy homolog 2	2.1	1				
Protein CYR61	2.1	1				
Protein-cysteine N-palmitoyltransferase porcupine					2.1	1
Protein disulfide-isomerase A6	2.5	1	2.0-2.1	5		
Protein FAM135B					3.9	1
Protein Hikeshi	2.4-2.6	2				
Protein kinase C alpha type					2.9	1
Protein lifeguard 2	3.5	1	3.1	1	2.3	1
Protein naked cuticle homolog 2	2.4-2.5	2				

Table 4.4 (Continued)

Upregulated Genes and Pathways	Northern Channel		Southern Channel		Southern Hybrid	
	Fold Change	Number of Transcript	Fold Change	Number of Transcript	Fold Change	Number of Transcript
Protein NLRC3	2.4-2.5	3	2.1	1		
Protein phosphatase 1K	2.1	1				
Ras and Rab interactor 2					2.5	1
Receptor tyrosine-protein kinase erbB-4					2.8	1
Receptor-type tyrosine-protein phosphatase R					2.6	1
Retrotransposable element Tf2 155 kDa protein type 1-like	2.3-2.4	3			2.1-2.4	3
Rho GDP-dissociation inhibitor 1	2.2	1				
Sarcalumenin	2.4	1				
Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	2.2	1				
Scavenger receptor class A member 3	2.6-3.0	2	3.2-3.3	2	2.4	2
Serine/threonine-protein kinase Sgk1	2.6-2.9	3	2.1-2.2	3		
Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit C	2.1	1				
Serpin H1	2.5-3.0	7	2.3-2.8	6	2.1-2.8	7
SH3 and cysteine-rich domain-containing protein 3	2.0	1				
Sodium/myo-inositol cotransporter			2.6	1		
Solute carrier family 25 member 38-A	2.2-2.4	2				
Solute carrier family 35 member B1	2.2-2.3	2				
Sphingomyelin phosphodiesterase 3					2.1-2.8	3
Stress-70 protein	2.0	1				
Stress-induced-phosphoprotein 1	3.4	1	2.7	1	2.5	1
Suppressor of G2 allele of SKP1 homolog	2.2	1				

Table 4.4 (Continued)

Upregulated Genes and Pathways	Northern Channel		Southern Channel		Southern Hybrid	
	Fold Change	Number of Transcript	Fold Change	Number of Transcript	Fold Change	Number of Transcript
Synaptotagmin-like protein 5	2.4	1				
TC1-like transposase	2.6	1			2.5	1
Thioredoxin domain-containing protein 11	2.9	1				
Thiosulfate sulfurtransferase/rhodanese-like domain-containing protein 1	2.4	1				
TIMELESS-interacting protein	3.3	1				
Transforming acidic coiled-coil-containing protein 3	2.1-2.3	2				
Transmembrane protein 33	2.7-2.8	3	2.2	1		
Transposable element Tcb1 transposase	2.6-4.6	2	2.4-3.8	2	2.6-3.2	3
Transposase	4.1	1	3.4	1		
Transposon TX1 uncharacterized 149 kDa protein	2.1	1				
Tubulin-folding cofactor B	2.1	1				
Tyrosine-protein kinase STYK1	2.3	1	2.4	1	2.3	1
Tyrosine-protein kinase transforming protein SEA			2.6	1	2.2	1
Ubiquitin carboxyl-terminal hydrolase isozyme L1	7.3	1	6.4	1	7.1	1
Ubiquitin-conjugating enzyme E2 C			2.0	1		
Unconventional myosin-IXa					2.2	1
Verrucotoxin subunit beta	2.3-3.4	2				
VIP36-like protein	3.6-3.7	2	2.6	2	2.5-2.6	2
Zinc finger BED domain-containing protein 1-like	2.7	1				

Table 4.4 (Continued)

Upregulated Genes and Pathways	Northern Channel		Southern Channel		Southern Hybrid	
	Fold Change	Number of Transcript	Fold Change	Number of Transcript	Fold Change	Number of Transcript
Zinc finger protein 16					3.0	1
Zinc finger protein ZPR1	2.2-2.3	2	2.0	1		
Zona pellucida-like domain-containing protein 1	3.5-3.9	3	3.6-3.7	4	3.2-5.4	4
Total Number of Transcripts with > 2 Fold Change		256		182		150
Downregulated Genes and Pathways	**Northern Channel**		**Southern Channel**		**Southern Hybrid**	
	Fold Change	Number of Transcript	Fold Change	Number of Transcript	Fold Change	Number of Transcript
25-hydroxyvitamin D-1 alpha hydroxylase	4.1-4.2	2			3.1-3.2	2
Acyl-CoA dehydrogenase family member 9			2.0-2.2	2		
Adenosine deaminase domain-containing protein 2	2.1	1				
Alpha-protein kinase 2	2.1	1				
Beta-1 adrenergic receptor			2.4	1		
Bloodthirsty-related gene family, member 9	3.3	1				
Calcium-binding and coiled-coil domain-containing protein 1	2.1	1			2.1	1
CCAAT/enhancer-binding protein epsilon					2.0	1
Chymotrypsin-C			2.1	1		
Chymotrypsin-like elastase family member 2A			2.0	1		
Complement C3			9.8	1		
Cysteine sulfinic acid decarboxylase			2.1	1		
Cytochrome P450 family 26 subfamily b polypeptide 1			2.6-2.8	2		

Table 4.4 (Continued)

Downregulated Genes and Pathways	Northern Channel		Southern Channel		Southern Hybrid	
	Fold Change	Number of Transcript	Fold Change	Number of Transcript	Fold Change	Number of Transcript
Cytokine-inducible SH2-containing protein	4.7	2				
Dedicator of cytokinesis protein 4	2.2	1				
Dehydrogenase/reductase (SDR family) member 3a	3.5	1				
Dihydropyridine-sensitive L-type skeletal muscle calcium channel subunit alpha-1			2.4	1		
Espin-like protein	2.1	1				
Fatty acid desaturase 2					2.0-2.2	2
Glypican-5			4.3	1		
High mobility group protein B3	3.2	1	3.0	1		
Homeobox expressed in ES cells 1	2.7	1				
Hypothetical protein LOC100001066					2.3	1
Importin-13	2.1-2.5	3	2.3-4.4	2		
Inositol oxygenase			4.5	1		
Insulin-like growth factor II	2.2-2.5	3				
Keratin, type I cytoskeletal 13	2.3	1				
Large neutral amino acids transporter small subunit 4			2.94	1		
LIM domain kinase 1	2.1	1				
Low-density lipoprotein receptor (1, 2)	2.5-3.0	7				
Lysoplasmalogenase	2.1	1				
Methylmalonyl-CoA epimerase			2.5	1		
Mitochondrial glutamate carrier 1			2.7	1		
NADH-ubiquinone oxidoreductase chain 6					2.5	1
NK-lysin type 2	2.1	1				

Table 4.4 (Continued)

Downregulated Genes and Pathways	Northern Channel		Southern Channel		Southern Hybrid	
	Fold Change	Number of Transcript	Fold Change	Number of Transcript	Fold Change	Number of Transcript
NOD3 protein-like			2.3	1		
Nuclear receptor subfamily 1 group D member 1	2.1	1				
Oatx protein	2.1	1				
Ornithine carbamoyltransferase					2.1	1
PDZ and LIM domain 3b	4.2	1	3.2-3.8	2		
Pentraxin fusion protein	3.1	1				
Phosphatidate phosphatase LPIN1	2.2-2.6	2				
Phosphomannomutase 2	3.4	1				
Phosphorylase b kinase regulatory subunit alpha			2.2	1		
Predicted protein [Nematostella vectensis]	2.7-3.2	3				
Proline-rich protein 18			2.4	1		
Protein CYR61	2.9-4.3	2				
Protein FAM186A	2.1	1				
Protein NLRC3-like			2.1	1		
Receptor-type tyrosine-protein phosphatase-like N	2.1	1				
Rho-related GTP-binding protein RhoE	2.6-2.8	3				
Ribonuclease-like 3			2.0	1		
RING finger protein 207	3.9-4.5	2				
rRNA-processing protein FCF1 homolog			2.1	1		
Short-chain dehydrogenase/reductase 3			2.8	1		
Sodium-dependent phosphate transport protein 2B			2.1-2.3	2		
Solute carrier family 12 member 2					3.1	1

Table 4.4 (Continued)

Downregulated Genes and Pathways	Northern Channel		Southern Channel		Southern Hybrid	
	Fold Change	Number of Transcript	Fold Change	Number of Transcript	Fold Change	Number of Transcript
Solute carrier family 22 member 6			2.1	1		
Transcriptional regulator Myc	3.2	2				
Transmembrane protease serine 13			6.1	1		
Transmembrane protein 260			2.1	1		
Transposase	2.6	1	2.1	1		
Trichohyalin	2.7	1				
Trypsin					2.5-2.9	3
Twinfilin-2			2.3	1		
U7 snRNA-associated Sm-like protein LSm10			2.1	1		
Viral macrophage inflammatory protein 2			3.3	1		
Xin actin-binding repeat-containing protein 2	3.0	1				
Zinc-binding protein A33-like	2.5	1	2.3	1		
Total Number of Transcripts with > 2 Fold Change		55		37		13

Magnitude of change (> 2) in differentially-expressed ($p<0.05$) transcripts following 6 weeks of cycling temperature regimes (27-31°C and 32-36°C) in two strains of channel catfish (southern and northern) and one hybrid strain (southern). When more than one transcript was differentially-expressed for the same gene, the range of the changes are given. Transcript descriptions were derived from NCBI and UniProt.

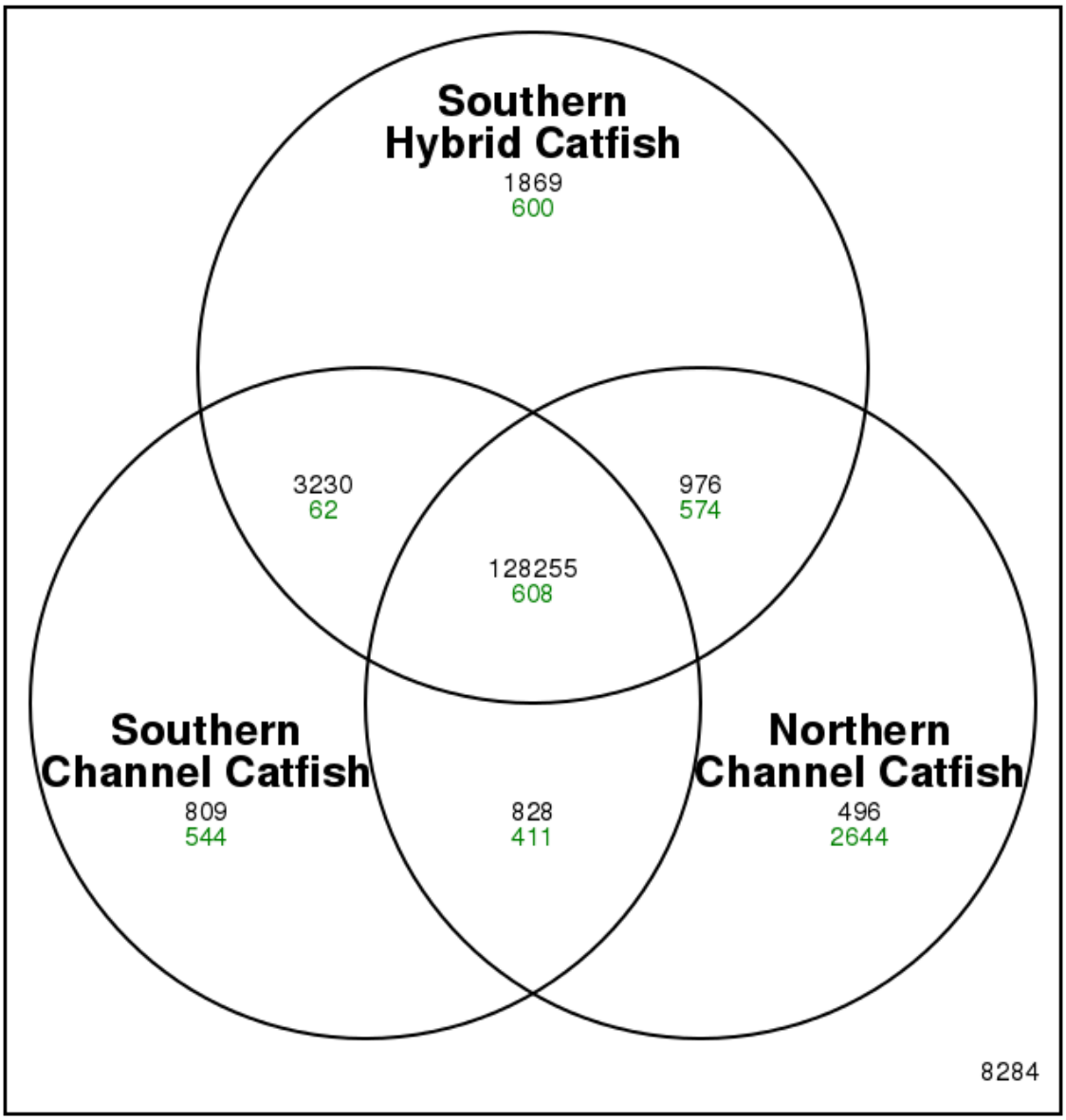

Figure 4.1 Venn diagram of differentially expressed transcripts in liver of catfish under sustained 27-31°C and 32-36°C cycling temperature regimes.

Venn diagram of differentially expressed transcripts in liver of two strains of channel catfish (Southern and Northern) and one hybrid strain (Southern). For each pair of numbers, the top number indicates the number of transcripts mapped to the Liu et al (2013) database. The bottom number represents the number of differentially expressed transcripts. The number of transcripts in the Liu et al (2013) database not observed in this experiment is listed in the bottom right hand corner.

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CHAPTER V

SUMMARY AND CONCLUSION

Channel catfish (*Ictalurus punctatus*) have demonstrated the ability to acclimate to a wide array of environmental conditions and temperatures (Allen & Strawn 1971, Kent *et al.* 1988, Seddon & Prosser 1997, Urrutia & Tomasso 2007). Acclimatory processes are directed by gene expression, varying in accordance with the severity and duration of the stressor (Logan & Somero 2011). Cellular stress responses are activated first with acute thermal stress causing rapid changes to gene expression to repair damage (Kültz 2005). If the acute thermal stress is prolonged as a chronic stress, then cells, tissues and organs begin to remodel (Gracey 2008). The processes of: acute stress, chronic stress and alternations in gene expression must be understood to comprehend the full thermal effects on ectotherms.

Although channel catfish have a large geographic range (Scott & Crossman 1973), these studies show that there are differences in thermal tolerance between fish from greatly separated latitudes. Acute thermal tolerance results indicated that southern channel catfish have higher thermal tolerance than northern, based on CTM results. In addition, gene expression analyses reveal that northern strains of channel catfish had greater differential gene expression (DE) at high temperatures in comparison to optimal growth temperatures, indicating higher stress levels. Northern strains of channel catfish had five times greater overall differential gene expression than southern and 1.5 times the

amount of upregulated DE gene transcripts with magnitude fold changes in response to high temperatures.

Hybridization of channel catfish with blue catfish (*I. punctatus* x *I. furcatus*) has been used in the catfish aquaculture industry since 2001 (Chatakondi 2012) due to their improved feed conversion (Li *et al.* 2004), faster growth (Giudice 1966) and disease resistance (Bosworth *et al.* 1998) which make them more economical to produce (Ligeon *et al.* 2004); thus it is important to examine how hybrid catfish tolerate thermal stress in comparison to channel catfish. Acute thermal trials found that channel catfish had a higher critical thermal maxima (CTmax) than hybrid catfish and there was no difference in survival following acute thermal stress. However in chronic thermal trials hybrid catfish had greater survival and more consistent growth between temperature ranges than either channel catfish strain. During chronic high temperature trials, temperatures reached a maximum of 36°C with a heating rate of 0.5 °C per hour, whereas during acute trials maximum temperatures reached 40.3°C with the heating rate of 2 °C per hour, which could explain the difference seen in survival. Without a follow up study, it cannot be determined whether the rate of heating or the maximum temperature had a stronger influence on survival. Since hybrid catfish have the blue catfish parent with a distribution range spanning from the southern United States to Mexico, Guatemala and Belize (Graham 1999), which is more of a tropical climate than that of the channel catfish, this could indicate that hybrid catfish would be better adapted to high temperatures. Hybrid catfish had the least amount of DE up- or downregulated transcripts with significant fold changes compared to the two channel catfish strains in genetic analyses, indicating lower stress response and possibly higher thermal tolerance.

In addition to geographic location and hybridization, this study found two other primary factors that affect survival of fishes. The first factor that affected survival was length; during acute thermal trials, probability of survival increased with fish of greater length, however length had no affect on CTmax. Thus length was not a factor for the temperature at which loss of equilibrium occurred but was a factor for recovery immediately following loss of equilibrium. As suggested by Ospina & Mora (2004) this could be due to energy storage or a length:weight body ratio. Larger fish have greater body reserves, which can be metabolized to provide energy necessary for recovery. The new energy can be used to: synthesize proteins such as heat shock proteins, support the immune system, replace cells lysed under stress; giving an advantage to fish allow to do so following high temperature exposure. A possible explanation for why CTmax was not affected by length could be that thermal stress was too rapid, affecting the central nervous system and not allowing enough time for the fish to acclimate (White 1983, Hernández Rodríguez *et al.* 1996). Following the acute thermal stress, fish were kept within optimal temperatures, enabling the body to recover. The second factor was water temperature increasing the susceptibility of disease. Under chronic thermal stress, the greatest mortalities occurred in the 27-31°C temperature regime despite it being the optimal temperature range for growth of juvenile channel catfish (Arnold *et al.* 2013). *Flavobacterium columnare*, which commonly affects channel catfish at temperatures ranging from 25-32°C (Durborow *et al.* 1998), was the primary pathogen found in deceased fish of the chronic thermal trial.

In order for global fisheries production to be sustainable, reliance upon aquaculture must increase. This is because an estimated 70% of the world capture

fisheries are fully exploited or overexploited (Brander 2007). Ensuring stable aquaculture production depends on environmental conditions, such as climate. Therefore, climate change can alter growth, reproductive capacity, physiology, behavior, immune system function, natural distribution patterns or structure of the ecosystem and mortality (Brander 2007, Brandt 1993, Ficke *et al.* 2007, McCauley & Beitinger 1992, Brett 1979, Lang *et al.* 2003, Le Morvan *et al.* 1998). This research sought to explore the impacts of high temperature and potentially climate change on channel and hybrid catfish. Temperatures above optimal for catfish are occurring in ponds and will continue to do so. In terms of application to the catfish industry, understanding how these high temperatures will affect fingerlings is important for greatest production (Barange & Perry 2009). These studies demonstrate lower impact of high temperatures on hybrid catfish, suggesting use of such fish in regions experiencing high temperatures. Only thermal stress impacts on fingerling catfish were explored in these studies, however, optimum growth temperature and temperature tolerance has been observed to decrease as fishes grow, so implications can still be made about food-sized catfish (Buentello *et al.* 2000, Cook *et al.* 2006, Fowler *et al.* 2009). In addition, Lang *et al.* (2003) observed an impact of high temperatures (i.e., 36°C) on broodstock, suggesting susceptibility at multiple life stages.

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