The effects of fasting on aspects of thermal tolerance in juvenile shortnose sturgeon (*Acipenser brevirostrum*)

by

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ABSTRACT

Sturgeon populations continue to decline because of damming of rivers (water diversion) and climate change. Both factors can affect the thermal profiles of the river, which can modify a species' food resources. Studies on the stress response in sturgeon examine the impacts of a single stressor. The current study investigated the effects of food deprivation on the critical thermal tolerance (CTmax) and the physiology of shortnose sturgeon; the goal was to understand how fasting interacts with the physiological performance of sturgeon when challenged with a temperature stress. The CTmax and physiological responses of juvenile shortnose sturgeon fasted for one, three and seven days were investigated. CTmax did not change with increasing fasting levels. Plasma glucose, lactate and osmolality increased following thermal stress, but did not differ between different fasting periods. Overall, sturgeon can tolerate thermal stress well; however, the mechanism involved in thermal tolerance is unlikely related to nutritional status.

ACKNOWLEDGEMENTS

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STATEMENT OF RESEARCH CONTRIBUTION

In May of 2018 I began reading literature and learning the laboratory techniques that would be used in the completion of my honours thesis. I began sampling fish in June, and blood samples were collected with the assistance of my supervisor, Jim Kieffer throughout the duration of June. Following the completion of sampling, I completed all assays in September and October and statistical analyses were completed in conjunction with Jim in January 2019.

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CTmax: Critical thermal maximum

LOE: Loss of equilibrium

Introduction

Sturgeon species have inhabited regions of North America for approximately 66-100 million years (Billard & Lecointre 2001). Nearly all sturgeon are anadromous (Bemis & Kynard 1997), which allows them to move freely between salt and freshwater habitats. Anthropogenic activities such as overfishing, damming and climate change have directly resulted in the decline of many sturgeon populations (COSEWIC 2005; Lee et al. 2016). Human activities also affect the distribution, quantity, and quality of sturgeon prey items (COSEWIC 2005). The presence of dams often modify water flow patterns within rivers, as well as cause changes in water quality and habitat conditions (e.g. food sources) of fish (COSEWIC 2005); changes in food resources subsequently can increase the competition between individuals for potentially limited resources, which could make any impact of food deprivation more prominent (Lessard & Hayes 2003; Cai et al. 2016). Additionally, fluctuations in temperature can also affect the spatial and temporal patchiness of aquatic food sources (Zeng et al. 2017).

Feeding style and habitat requirements can vary significantly between adult and juvenile sturgeon. Dadswell et al. (1984) showed that adult shortnose sturgeon are selective feeders, where juveniles appear to be non-selective and have been found to have up to 90% of non-digestible food items, such as rocks and gravel in their gut. Carrying an abundance of non-digestible food items with little or no nutritional value could potentially lead to incidental fasting. The effects of fasting on fish energy reserves are wide-spread (Navarro et al. 1992; Navarro and Gutierrez 1995; Kieffer and Tufts 1998; Hoseini et al. 2013; Shrivastava et al. 2017). Fasting in fish has been shown to reduce muscle energy reserves (Kieffer & Tufts 1998), metabolic rate (Aslop and Wood 1997; Gingerich et al. 2010), ammonia excretion rates

(Shrivastava et al. 2017), and body mass (McCue 2010) and elevate levels of glucose (Scarabello et al. 1991).

While there are ample studies of the effects of fasting and starvation in bony fish (Black et al. 1966; Gingerich & Philipp 2010; Verhille et al. 2015; Cai et al. 2017), less research has been conducted on primitive and endangered fish, such as sturgeon (but see Gillis and Ballantyne 1996; Verhille et al. 2015). Specifically, there is a lack of information pertaining to the basic hematological and biochemical responses to starvation in sturgeon (Kiessling et al. 1993; Falahatkar 2012). Of the 27 extant species, the shortnose sturgeon, *Acipenser brevirostrum* inhabits 25 segments of water on the east coast of North America, extending from the Saint John River (New Brunswick, Canada) to the St. John's River (Florida, United States) (Dadswell 1979; National Marine Fisheries Service 1998). All populations of shortnose sturgeon are faced with increases in water as a result of climate change; the fact that shortnose sturgeon are an anadromous species (Bemis & Kynard 1997) may make them particularly susceptible to the effects of temperature changes during migration, both for feeding and reproduction.

The thermal tolerance of various sturgeon species is fairly well understood (Ziegweid et al. 2008; Verhille et al. 2015; Spear and Kieffer 2016; Zeng et al. 2017), however, the combined effects of temperature on the relationship with other stressors is not as well described. While sturgeon can tolerate a variety of environmental conditions through plasticity in phenotypic traits (Zeng et al. 2017), such as cardiovascular and neuronal processes (Oufiero & Whitlow 2016), a change in some of these traits may play a role in a fishes ability to withstand the impacts of environmental stressors, including those of temperature changes (i.e. during migration, moving between fresh and brackish water).

Since fasting and thermal tolerance influence a fish's ability to withstand environmental stressors, understanding the relationship between the two variables is significant (Chatzifotis et

al. 2018). Recently, Verhille et al. (2015) demonstrated a weak, but significant effect of food restriction (fish fed 0.25, 05, 1.0 and 2.0% body mass per day) on the thermal tolerance patterns of green sturgeon; in contrast, Gilbert and Miles (2016) showed no relationship between fasting and thermal tolerance in the lizard, *Urosaurus ornatus*. These conflicting findings provide the rationale for the current study. Specifically, the objective of this study is to investigate whether various levels of fasting (1, 3, or 7 days) influenced the thermal tolerance, as measured using a standard critical thermal maximum (CTmax) test, of shortnose sturgeon. An overarching goal of the current study is to gain information regarding the relationship between periods of fasting and aspects of thermal tolerance, including the critical thermal maximum and secondary stress indicators (e.g., hematocrit, glucose, osmolality, chloride, protein). In addition, this study will further contribute to our understanding of the various factors (e.g. body size, acclimation temperature, heating rate, repeat thermal stress) that can influence the thermal tolerance in shortnose sturgeon (Zhang & Kieffer 2014; Zhang et al. 2017; Bard & Kieffer 2019). The CTmax for a fish fasted for one day, weighing ~130g should be between 30℃ and 32℃ (Zhang & Kieffer, 2014; Spear & Kieffer, 2016). A lower level of energy reserves with extended fasting (i.e. 3 or 7 days) should reduce the thermal tolerance of sturgeon; thus, there should be a lower CTmax when fish are fasted for longer periods. If a greater CTmax results in a greater stress response, then the levels of stress indicators (e.g., plasma lactate and glucose) should be higher in fish with a higher CTmax. Conversely, a decrease in secondary stress indicators should be expected in fish with a lower CTmax.

Materials and Methods

Fish Culture and Husbandry

Shortnose sturgeon were obtained from Acadian Sturgeon and Caviar (Carter's Point, New Brunswick Canada. The sturgeon were held in three, 1m diameter (160L) cylindrical flow-through holding tanks at 15℃, until the desired sizes for experimentation were achieved (see below). While in these tanks, sturgeon were fed EWOS VITA Complete Fish Feed for Salmonids (43% protein, 14% fat) twice daily to satiation. Once the fish grew to approximately 130g, fish were assigned to one of three fasting groups: 1, 3 or 7 days. Two fish from each fasting group were placed in one of two identical, aerated 130L cylindrical flow-through tanks at 15℃ and held there for the appropriate fasting period. Fish mass (to the nearest gram) was obtained prior to placing fish in the holding tank. The pair of fish in the fasting tanks had different body markings and features (e.g., fin shape, colour of body, length) for easy identification. One thermal tolerance experiment (see below for details of set-up and experiment) was conducted in the morning and one in the afternoon; experimental groups were alternated between fasting tanks to account for possible tank effects. The University of New Brunswick Animal Care Committee approved the following experimental protocol, meeting Canadian Council of Animal Care guidelines.

Experimental Design

The CTmax procedure followed the general protocol outlined in Zhang and Kieffer (2014) and Spear and Kieffer (2016). The thermal tolerance (CTmax) experiments were performed in a rectangular, insulated tank (test arena), filled with approximately 30L of 15℃ fresh, de-chlorinated water. An elevated heating tank (45 by 56cm) was located beside the test

arena and equipped with a 1000W heater (Pentaire Aquatic Ecosystems, Florida, USA) and air diffusers (Pentair Aquatic Ecosystems, Florida, USA). The heater was programmed to increase the temperature of the water by ~8℃ per hour (Mean (±SE) =8.3 ±0.05). This heating rate was consistent with those used in previous studies (Spear and Kieffer 2016; Zhang et al. 2017). Water flowed from the heating reservoir to the test arena, and back to the heating reservoir via a submersible pump (Logilo Systems, Denmark) to maintain the temperature of the setup. A black perforated plexiglass shield isolated the submersible pump from the test arena. Electronic temperature probes (Logilo Systems, Denmark) were inserted on both ends of the test arena to record the temperature within the test tank.

CTmax Test Design

Following the completed period of fasting (i.e. 1, 3 or 7 days), one sturgeon (N=8 for each fasting period) was placed in the test arena at a time at 15℃, for one hour to recover from handling stress (Zhang & Kieffer, 2014; Spear & Kieffer 2016; Bard & Kieffer 2019). The fish was then exposed to a thermal stress (or not, in the case of control fish), by increasing the temperature at a constant rate by 8℃ per hour. Temperature was recorded prior to heating, and every 10 minutes throughout the thermal test. Loss of equilibrium as an endpoint is indicated when the fish rolled dorso-ventrally and is unable to right itself within 10 seconds (Ziegweid et al. 2008; Spear & Kieffer 2016; Bard & Kieffer 2019). Individuals were manually righted three times to ensure the fish had reached its CTmax (Spear & Kieffer 2016; Bard & Kieffer 2019). Water temperature was measured in the test arena throughout the trials, until the third incidence of LOE, at which point the CTmax was recorded. At the point of CTmax, fish were anaesthetized with a buffered TMS solution (250mg L^{-1} tricaine methanesulfonate buffered in $NaHCO_3$). Once fully anaesthetized (lack of ventilation and no response to touch), the sturgeon was removed

from the anaesthetic bath, weighed, and measured for length. It was blotted dry, weighed (nearest gram), length was taken (nearest cm), and a blood sample was obtained. The percent (%) mass loss of the fish was calculated using the following formula: % mass loss= 100-[(100 x final mass)/initial mass]. To acquire a blood sample, a lithium-heparinized needle was inserted in the caudal vasculature. For the control (N=6 fish per fasting group), fish were introduced to the test arena for three hours (i.e. the time to complete a typical CTmax test; Zhang and Kieffer 2014) and were sampled as noted for the other experimental groups. Fish from this group served as a no thermal stress control. Following blood sampling, the fish gastrointestinal tract was removed, and contents of the stomach and intestine were analyzed to determine if any food was present across the fasting periods. After the completion of each individual trial, the test arena was drained, cleaned, and refilled with de-chlorinated water at 15℃.

Table 1. Mean (±S.E.) total length, and initial and final mass of shortnose sturgeon fasted for 1, 3 and 7 day periods.

Fasting duration and condition	Variable	Mean	Standard Error	Sample Size (N)
1 day fast (thermal stress)	Initial mass (g)	140.5	3.5	8
	Final mass (g)	135.9	3.2	
	Length (cm)	31.9	0.2	
1 day fast (no thermal stress)	Initial mass (g)	131.2	4.6	6
	Final mass (g)	126.2	4.4	
	Length (cm)	30.9	0.2	
3 day fast (thermal stress)	Initial mass (g)	127.9	6.3	8
	Final mass (g)	122.0	6.3	
	Length (cm)	31.0	0.5	
3 day fast (no thermal stress)	Initial mass (g)	134.8	7.4	6
	Final mass (g)	127.0	5.9	
	Length (cm)	30.7	0.9	
7 day fast (thermal stress)	Initial mass (g)	132.4	11.6	8
	Final mass (g)	126.9	10.9	
	Length (cm)	32.1	0.7	
7 day fast (no thermal stress)	Initial mass (g)	131.3	5.1	6
	Final mass (g)	120.0	5.0	
	Length (cm)	31.2	0.3	

Blood Analysis

The blood sample was placed in a 1.5 mL centrifuge tube. Approximately 100uL of the whole blood was used for duplicate hematocrit determination (Zhang and Kieffer 2014). The remainder of the blood was then centrifuged for two minutes at 6700g, and the resulting plasma was pipetted into labeled tubes and frozen at -20℃ for further testing. Plasma glucose levels were measured using a glucose meter (OneTouch Ultra 2; Code 25 test strips; www.onetouch.ca; Penny and Kieffer 2014). Plasma protein concentrations were measured by a standard colorimetric assay at 540 nm wavelength (Total Protein Reagent, Buiret Method & Protein Standard Set P5495; Sigma; www.sigmaaldrich.com; Penny and Kieffer 2014). Plasma lactate concentrations were measured using a standard spectrophotometric assay at 540nm wavelength (Lactate Reagent 735-10, Lactate Standard Solution 826-10 & Lactate Standards Set 735-11; Trinity Biotech; www.trinitybiotech.com; Zhang and Kieffer 2014; Bard and Kieffer 2019). Plasma osmolality was measured using a micro-osmometer (3300 micro-osmometer, Advanced instruments) and appropriate standards.

Statistical Analysis

Statistics were analyzed using SIGMASTAT 3.5 software (www.sigmaplot.com). Normality for all variables was evaluated using the Shapiro-Wilks test. Critical thermal maximum values were compared between groups (1, 3 and 7 days of fasting) using a one-way ANOVA and a linear regression. The mean blood parameters were compared between conditions (thermal stress versus no thermal stress) and fasting level (1, 3 or 7 days of fasting) and their interaction using two-way ANOVAs. When the ANOVA results were significant, a Holm Sidak (α= 0.05) multiple comparison test was used to compare mean blood parameter values between the various groups. Tank effect within each fasting period was assessed using a t-test.

Results

CTmax

There was no effect of holding tank on mean CTmax values within each fasting period (t-test; P>0.1). Mean CTmax values were variable and did not differ significantly between fasted groups of sturgeon (one-way ANOVA between groups. P>0.05, linear regression; P=0.550). The CTmax values of sturgeon fasted for one, three and seven days were 30.6, 30.1 and 30.9℃, respectively (Figure 1).

Percent Mass Loss

Percent mass loss in control, non-thermally stressed fish varied between groups and ranged from 3.8% to 8.7% (Figure 2). The greatest mass loss in thermally stressed fish occurred at 7 days (one-way ANOVA, P>0.05). In contrast, percent mass losses in thermally stressed fish were consistent and ranged from 3.3% to 4.7% (Figure 2). There was a significant difference in percent mass loss between control and thermally stressed fish at seven days.

Glucose and Protein

Mean glucose concentrations (mmol/L) did not differ significantly among thermally stressed groups across different fasting levels (one-way ANOVA; P=0.163) (Figure 3a). Fasting time had a significant effect on the control glucose levels, with levels in the day 3 and day 7 fasted groups being lower than fish fasted for one day (Figure 3a). Compared with thermally stressed fish, glucose levels in control fish were significantly lower across all fasting periods than noted in stressed fish (2-way ANOVA; P>0.05).

Mean protein concentration was consistent across all groups and between thermally stressed and control fish (Figure 3b). Mean protein concentration for all fish combined was approximately 27 ug/ml).

Secondary Stress Response

Lactate levels increased significantly following thermal stress in all fish, however mean lactate concentration (mM) did not differ significantly between thermally stressed fasted fish and averaged about 4.2 mM (Figure 4a). Lactate levels were very low (<0.5mM) in control fish; there were significant differences in lactate between thermally stressed and control fish across all fasted groups (2-way ANOVA, $P<0.05$; Figure 4a).

Thermal stress increased plasma osmolality relative to non-stressed fish (2-way ANOVA, $P<0.05$) Mean osmolality significantly increased following a thermal stress after one day of fasting (Figure 4b). Plasma osmolality was not significantly different across fasting times in thermally stressed fish (Figure 4b). In contrast, plasma osmolality was found to be lower at 3 and 7 days of fasting relative to 1 day of fasting (Figure 4b).

Overall, mean hematocrit (HCt) was higher in thermally stressed fish relative to non-stressed fish (2-way ANOVA, $P<0.05$). Mean hematocrit did not differ significantly between thermally stressed groups, or between control groups (Figure 4c)

Gut Content

While analyzing the gut contents, it was observed that fish that were fasted for longer periods of time had no food in their intestine; the majority had a green coloured liquid. Fish fasted for one day had no pellets in the gut but showed evidence of food in the intestine. Intestinal contents of fish fasted for one day was not identified as pellet, but rather as a yellow slime, of thicker consistency. As fasting continued, there was less liquid found in the gut. Fish

fasted for three days demonstrated a yellow coloured slime in their gut. The intestinal contents of non-thermally stressed fish mirrored that of thermally stressed fish.

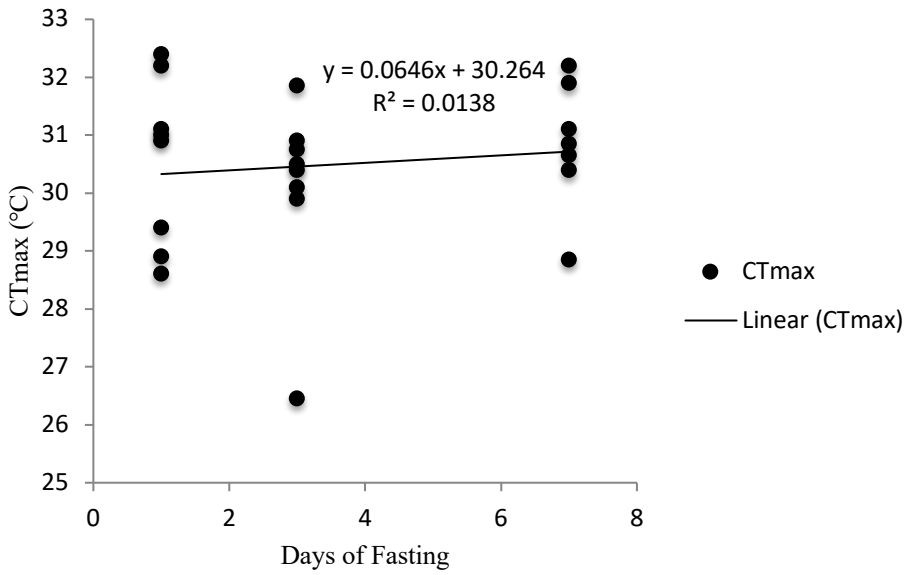


Figure 1. Mean critical thermal maximum (CTmax) values of individual shortnose sturgeon (Acipenser brevirostrum) fasted for one, three or seven days, acclimated to 15°C.

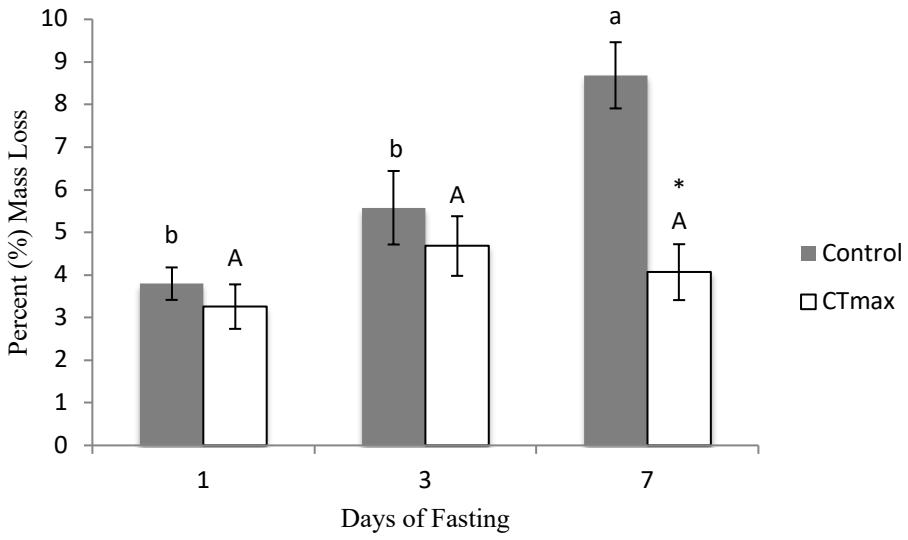
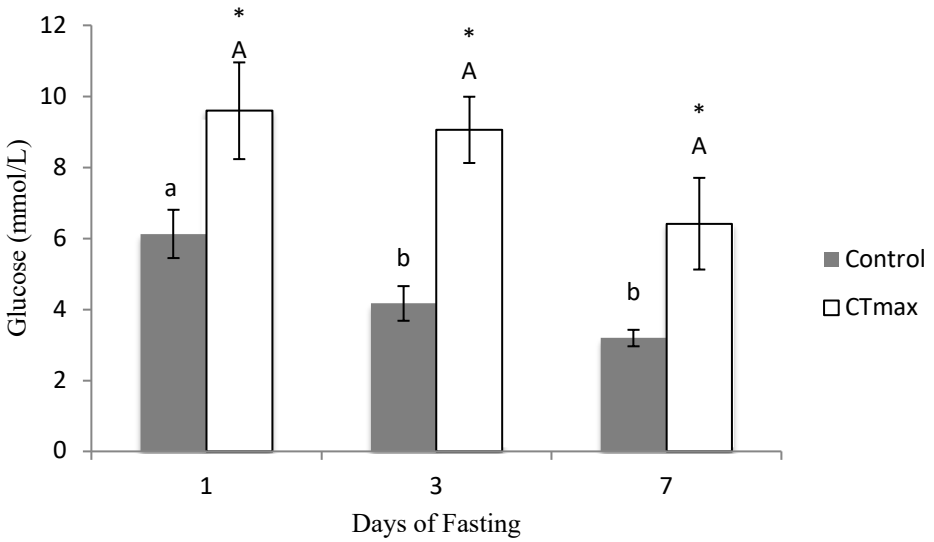


Figure 2. Mean (±SE) percent (%) mass loss of shortnose sturgeon (Acipenser brevirostrum) fasted for one, three or seven days, in the control group (n=6) and thermally stressed groups (n=8). Different lowercase letters represent significant differences between non-thermally stressed groups, and different uppercase letters represent significant differences between thermally stressed groups. Asterisks (*) represent a significant difference between the control group and the thermally stressed group.

a



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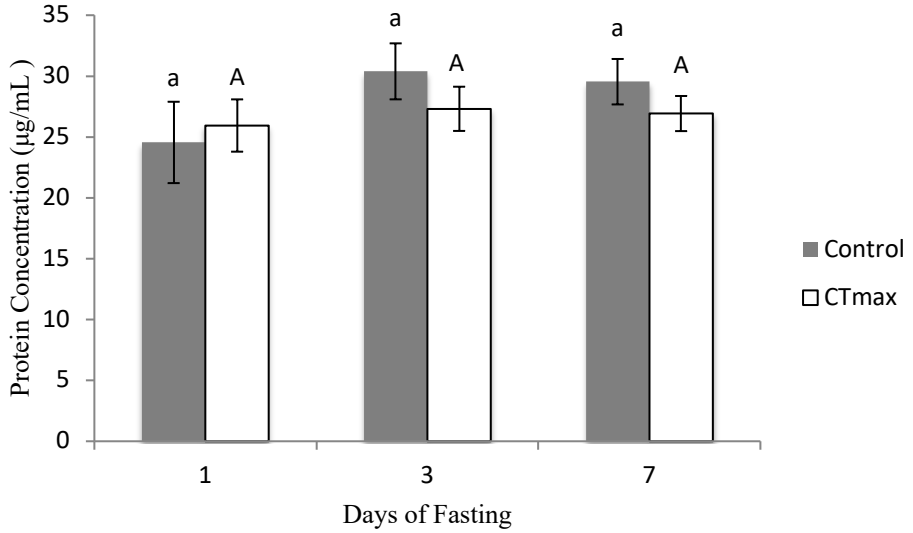

Figure 3. Mean (±SE) (a) glucose (mmol/L) and (b) protein concentration (ug/ml) of shortnose sturgeon (Acipenser brevirostrum) fasted for one, three or seven days, acclimated to 15°C in the control group (n=6) and thermally stressed group (n=8). Different lowercase letters represent significant differences between control, non-thermally stressed groups, and different uppercase letters represent significant differences between the control group and the thermally stressed group, within a fasting group.

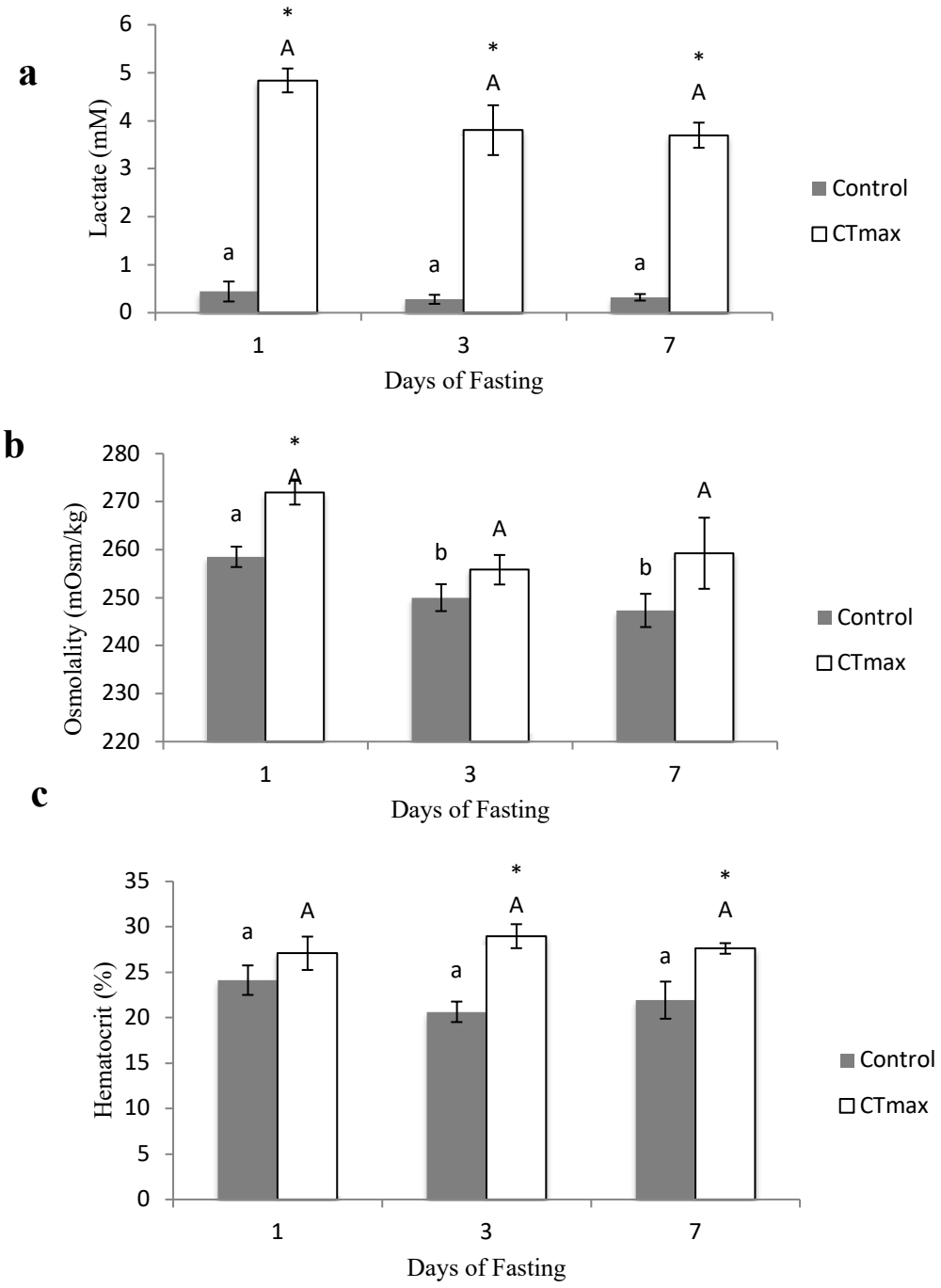

Figure 4. Mean (±SE) (a) lactate (mM), (b) osmolality (mOsm/kg) and (c) percent hematocrit (%) of shortnose sturgeon (Acipenser brevirostrum) fasted for one, three or seven days, acclimated to 15°C in the control group (n=6) and thermally stressed group (n=8). Different lowercase letters represent significant differences between non-thermally stressed groups, and different uppercase letters represent significant differences between thermally stressed groups. Asterisks (*) represent a significant difference between the control group and the thermally stressed group.

Discussion

It is known that several factors affect the CTmax of shortnose sturgeon, including: acclimation temperature (Ziegeweid et al. 2007; Zhang & Kieffer 2014), and body size (Ziegweid et al. 2007; Zhang & Kieffer 2014). The results from the current study and those for green (*Acipenser medirostris)* and white sturgeon (*Acipenser transmontanus*) on the effects of food deprivation and fasting on thermal tolerance (Verhille et al. 2015; Lee et al. 2016) is less clear. The present study demonstrates that CTmax values for shortnose sturgeon fasted for 1, 3 and 7 days are comparable to previous studies involving only one day of fasting (Zhang & Kieffer 2014; Spear & Kieffer, 2016; Bard & Kieffer 2019), and suggest that fasting up to 7 days does not impact thermal tolerance of shortnose sturgeon. Studies on green and white sturgeon fed limited rations (i.e., food limitations) reveal mixed findings on the impact of nutritional deprivation on thermal tolerance. (Verhille et al. 2015; Lee et al. 2016). Specifically, Verhille et al (2016) showed that CTmax decreased with increasing feed ration in juvenile green sturgeon; however, the difference in CTmax between fish fed 0.25% body mass per day and fish fed 2% body mass per day for two weeks was 0.4℃. Gilbert et al. (2016) found no relationship between fasting time and thermal tolerance of lizards (*Urosaurus ornatus*), deprived of food for up to 13 days. Lee et al. (2016) found a significant effect of thermal tolerance on nutritional deprivation/limitation for green and white sturgeon fed at 50% of their optimal feeding rate, but not at other rations. The available data for ectothermic species therefore suggests that nutritional status may be less important than other factors, such as acclimation temperature, as a limiting factor to explain patterns of thermal tolerance in fish. It is possible that the duration of the fasting/food deprivation in the current and published studies isn't severe (i.e., long enough) to affect thermal tolerance.

To further understand the effect of fasting on thermal tolerance in sturgeon, a pilot study in which juvenile (~125g) Atlantic sturgeon (*Acipenser* oxyrinchus)were fasted for up to 26 days at temperatures ranging from 14.1-16.3℃ prior to being exposed to a CTmax test. A multiple linear regression revealed that a combined effect of fasting and temperature influenced the CTmax (P=0.013); however, the multiple linear regression indicated that days of fasting alone did not account for the ability to predict CTmax (P=0.801). Since acclimation temperature affects CTmax in sturgeon (see Zhang and Kieffer 2014), the small 2.2℃ difference in acclimation temperature from the beginning to the end of the fasting period likely explained the slightly higher CTmax values found at the end of the experimental period. Thus, additional experiments with more defined and controlled temperature conditions are required to better understand the effects of fasting on thermal tolerance in sturgeon.

Due to the uncertainty of the direct mechanism that causes a fish to roll over at its critical thermal maximum point, further research is required. Some suggestions that have been made are failure of system-level neural processes (i.e. regulation of ventilation or brain circulation) (Lagerspetz 1974), failure to maintain muscle membrane potential (Hosler et al. 2000), and failure of cardiac function (Portner & Farrell, 2008). Although it has yet to be determined, nutritional deprivation does not appear to influence the mechanism involved in establishing thermal tolerance.

Percent Mass Loss

Percent mass loss following stressors is not always provided following exposure to stressors in fish. This study noted an average percent mass loss of approximately 4% for thermally stressed fish and approximately 6% for non-thermally stressed fish following fasting, which is consistent with Gilbert and Myles' (2016) study that demonstrated a percent mass loss

of between 7 and 9 percent in lizards fasted for thirteen days. These results demonstrate a substantial loss in mass following periods of complete food deprivation. Losses could represent food passing through the digestive system over time, shifts of water due to use of lipids during fasting (Smith 1981; Idler & Bitners 1958), or the loss of muscle tissue that could accompany fasting (Smith 1981). Non-thermally stressed control fish fasted for seven days demonstrated a percent mass loss of more than twice the amount than thermally stressed fish fasted for seven days. This drastic difference in percent mass loss between the two groups of fish at 7 days of fasting is not fully understood, but mechanisms surrounding water retention in the muscle during fasting and expulsion of water during thermal tolerance tests may help explain this.

Hematological Response

Blood parameters were used to determine the magnitude of the secondary stress responses in fasted fish that were either exposed or not exposed to thermal stress. There was a secondary stress response associated with temperature stress but not fasting, as indicated by an increase in blood parameters following thermal stress, regardless of the fasting period. These results are consistent with the lack of a relationship between fasting and CTmax. Thermal stress lead to drastically elevated levels of lactate, in comparison to non-thermally stressed fish. This increase is likely explained by the utilization of anaerobic pathways (Spear & Kieffer 2016; Bard & Kieffer 2019). Anaerobic pathways are activated due to the overly high demands of aerobic metabolism (Zhang & Kieffer 2014), increased activity and decreased oxygen with increasing water temperatures, and no drastic changed in oxygen carrying capacity in the blood (Spear & Kieffer 2016). This increase in lactate levels is consistent with similar studies on sturgeon that have tested the effect of thermal stress on hematological responses (Spear & Kieffer 2016; Bard & Kieffer 2019).

Thermal stress increases plasma glucose levels, which has been shown in previous studies on shortnose sturgeon (Zhang & Kieffer 2014; Spear & Kieffer 2016; Bard & Kieffer 2019). The magnitude of difference between thermally stressed and non-thermally stressed groups was between 3.2 and 4.9 mmol/L, indicating a significant increase in glucose following thermal stress. Levels of plasma glucose decreased after three days of fasting, and remained stable for non-thermally stressed fish, however, levels did not significantly change for thermally stressed fish, regardless of the fasting length. The drop, followed by stabilization in glucose levels is likely due to the activation of energy reserves, which are reserved to fuel a number of tissues (Gillis & Ballantyne 1996). This result has been previously demonstrated by Gillis and Ballantyne (1996), who deprived lake sturgeon (*Acipenser fulvescens*) of food for 60 days and demonstrated that glucose levels were significantly lower after 10 days of fasting but showed no difference between fasted and fed fish for the remainder of the fasting period.

Plasma osmolality was not significantly different among thermally stressed and non-thermally stressed groups, with the exception of fish fasted for one day. A 24-hour fasting period resulted in higher levels of osmolality for thermally stressed fish, and a significant difference in osmolality level between thermally stressed and non-thermally stressed fish. The decrease shown in three and seven day fasted control fish, followed by stability in levels of osmolality may be as a result of stabilization of energy reserves, which is an important strategy during starvation (Gillis & Ballantyne 1996). Though energy reserves may drop initially, the utilization of stored energy once entering starvation is a necessary survival strategy for fish (Gillis & Ballantyne 1996).

As shown in various studies, plasma protein concentration is not affected by thermal stress (Zhang & Kieffer 2014; Spear & Kieffer 2016; Bard & Kieffer 2019). Fasting has been shown to have no effect on plasma protein as well, which was demonstrated in lake sturgeon by

Gillis and Ballantyne (1996). These findings are consistent with the current study, as levels of plasma protein remained constant regardless of length of fasting or the presence of thermal stress.

Hematocrit, an indicator of oxygen carrying capacity, has been previously shown to increase following thermal stress in shortnose sturgeon (Zhang & Kieffer 2014); however, this was not the case when fasting was introduced. Fasting, combined with thermal stress resulted in a constant oxygen carrying capacity throughout groups, indicating that the lack of change may be as a result of the activation and utilization of anaerobic pathways (Spear & Kieffer, 2016).

In conclusion, fasting, regardless of the duration used in the current study does not significantly impact the thermal tolerance of juvenile shortnose sturgeon. Shortnose sturgeon fasted for one, three or seven days responded similarly to other studies on shortnose sturgeon in terms of thermal tolerance and secondary stress indicators. Fasting may be more appropriate than feeding fish in limited rations because of the uncertainty present in the amount of food consumed by each fish when feeding. In terms of blood parameters, juvenile shortnose sturgeon were not significantly impacted by fasting, overall. Thermal tolerance had a substantial impact on levels of lactate, as expected, and all other secondary stress indicators fell within a typical range following a thermal tolerance test, regardless of the length of the fasting duration. It is not currently known what the driving force behind CTmax is, but it can be confirmed that it likely is not related to nutritional status; in the future, further testing needs to be performed to determine the driving force behind CTmax. It can be noted that fasting in cold temperatures is shown to have a greater effect on plasma insulin levels, insulin-link growth factor-I and thyroxine in coho salmon (*Oncorhynchus kisutch*) (Larsen et al. 2001). These effects of fasting and cold temperatures on plasma levels may also be present in shortnose sturgeon, and cold temperatures may make the effects of fasting may be more prominent. If the effect of fasting is more

prominent in cold water for shortnose sturgeon, this introduces great implications for sturgeon that may experience periods of nutritional deprivation during the winter months. Further research is required to determine if the effects of fasting on secondary stress response in cold temperatures is applicable to the blood parameters of shortnose sturgeon.

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Appendix

Fish #	Heating Rate	CTmax (°C)	Length (cm)	Pre-exp mass (g)	Post-exp mass (g)	% change in mass	Mean HCT (%	Mean glucose (mmol/L)	Avg. Protein (ug/mL)	Lactate (mM)	Osmolality (mOsm/kg)
1 Day Ctmax:											
30	8.34	31.1	32	130	128	1.54	26.75	7.85	27	5.42	272
31	8.26	32.2	32	124	120	3.23	27.95	15.8	18.9	5.7	262
32	8.07	31	32	145	138	4.83	27.00	5.4	37	5.40	265
33	8.4	32.4	31	133	130	2.26	18.00	7.7	24	3.90	267
34	8.14	30.9	32	150	148	1.33	33.35	13.6	25	3.90	282
35	8.6	28.6	31	145	140	3.45	24.30	11.2	19.6	5.20	280
36	8.45	28.9	32	148	142	4.05	34.40	10.3	24	4.80	272
37	8.3	29.4	33	149	141	5.37	25.00	4.9	32	4.40	275
	8.32	**30.5625**	**31.875**	**140.5**	**135.875**	**3.25653**	**27.093**	**9.59375**	**25.9375**	**4.84**	**271.875**
3 Day Ctmax:											
1	8.262	30.4	33	132	126	4.55%	33	8.7	19.86	2.93262	254
2	8.34	30.5	31	152	143	5.92%	34.5	5.5	31.66	5.93961	256.5
7	8.25	30.1	28.5	107	99	7.48%	23.4	11.25	26.66	5.48451	240
8	8.55	26.45	31.5	138	130	5.80%	29.7	8.05	27.16	1.40304	258
9	8.022	31.85	32	125	123	1.63%	25.5	10.75	27.06	3.40992	253
10	8.286	30.9	30	149	146	2.05%	29.4	8.75	21.26	4.11144	257
13	8.13	29.9	31	110	105	4.55%	29.8	13.35	35.66	2.93706	272
14	8.184	30.75	31	110	104	5.45%	26.5	6.1	29.16	4.21578	256
	8.253	**30.10625**	**31**	**127.875**	**122**	**4.67875**	**28.975**	**9.05625**	**27.31**	**3.8042475**	**255.8125**
7 Day Ctmax:											
3	8.364	30.4	30.5	133	129	3.01%	26.7	7.5	23.56	3.68631	239
4	8.07	32.2	32.5	180	173	3.89%	26.1	12.3	24.66	4.55211	241
5	8.79	30.65	35	166	154	7.23%	30.9	2.35	32.26	3.29115	306.5
6	8.724	28.85	34.5	155	149	3.87%	28.1	4.25	32.76	3.10467	259.5
11	7.434	31.1	31	112	109	2.68%	28.5	7.2	24.46	4.29792	250
12	8.64	31.9	33	128	124	3.13%	25.9	2.6	23.06	4.10367	257
15	8.418	30.85	30.5	94	92	2.13%	26.7	4.5	24.46	4.20912	259
16	8.196	30.95	30	91	85	6.59%	28.1	10.6	30.16	2.35098	262
	8.3295	**30.8625**	**32.125**	**132.375**	**126.875**	**4.065625**	**27.625**	**6.4125**	**26.9225**	**3.69949125**	**259.25**
1 Day Control:											
Control (1)			31	131	125	4.58%	25.69	7.5	36.66666667	0.180697674	263
Control (1)			31	125	120	4.00%	30	4.3	23.33333333	0.172093023	251
Control (1)			31	114	110	3.51%	26.67	3.95	26.25	0.189302326	260
Control (1)			31.5	145	140	3.45%	20.27	6.15	19.16666667	1.462790698	257
Control (1)			31	142	135	4.93%	22.22	8.05	12.91666667	0.421627907	265
Control 13			30	130	127	2.31%	20	7.25	28.9591836	0.22422	255
			30.9166667	**131.1666667**	**126.1666667**	**3.7957**	**24.1344**	**6.125**	**24.54875282**	**0.441788605**	**258.5**
3 Day Control:											
Control 1			30	107	105	1.87%	19.3	3.75	21.61225	0.17205	244
Control 2			32	160	148	7.50%	23.3	5.15	28.65306	0.14874	255
Control 3			27	125	119	4.80%	23.2	3.55	33.14285	0.12654	257
Control 4			32.5	144	134	6.90%	22.8	6	37.93878	0.73926	255
Control 12			32.5	140	130	7.14%	17.4	3.85	33.14266	0.23865	249
Control 11			30	133	126	5.26%	17.9	2.7	27.83673	0.24642	240
			30.6666667	**134.8333333**	**127**	**5.58%**	**20.65**	**4.17**	**30.38772167**	**0.27861**	**250**
7 Day Control:											
Control 5			31	141	129	8.51%	23.4	3.4	38.2449	0.4773	251
Control 6			31	132	121	8.33%	18.3	3.65	30.4898	0.14874	248
Control 7			30	108	96	11.10%	29.8	3.25	26.5102	0.27639	260
Control 8			32	142	127	10.56%	25	3.8	25.69388	0.23088	245
Control 9			32	135	127	5.93%	17.2	2.75	27.93878	0.21645	246
Control 10			31	130	120	7.69%	17.9	2.3	28.34694	0.57498	234
			31.1666667	**131.3333333**	**120**	**8.69%**	**21.93333333**	**3.19**	**29.53741667**	**0.32079**	**247.3333333**