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The effects of repeat acute thermal stress on the critical thermal maximum (CT_{max}) and physiology of juvenile shortnose sturgeon *Acipenser brevirostrum*

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Abstract

The shortnose sturgeon *Acipenser brevirostrum* (LeSueur, 1818) is a species of special concern in Canada, but little is known about their thermal biology. Information on the upper thermal tolerance of shortnose sturgeon becomes valuable for predicting future survival particularly with climate change and improving species management. Using a modified critical thermal maximum (CT_{max}) methodology, the objective is to determine whether previous thermal stress affects the thermal tolerance of juvenile shortnose sturgeon when exposed to a second thermal stress event. Prior exposure to thermal stress (CT_{max} 1) did not affect the thermal tolerance (CT_{max} 2) of juvenile shortnose sturgeon when a 24h recovery period was allotted between tests. However, a significant increase in thermal tolerance occurred when the recovery time between the two thermal challenges was 1hr. Plasma glucose, lactate and osmolality were all significantly affected by thermal stress, but values returned to control levels within 24hrs. Hematocrit and plasma chloride concentrations were not significantly affected by thermal stress. All fish survived the CT_{max} testing. The data indicate that the thermal tolerance of juvenile shortnose sturgeon is modified when multiple thermal stresses occur closer together (1hr) but not if separated by a longer time period (24hrs).

Key words: thermal tolerance, repeat, CT_{max}, fish, shortnose sturgeon, *Acipenser brevirostrum*, critical thermal maximum

Introduction

The shortnose sturgeon *Acipenser brevirostrum* Lesueur 1818 is within the order Acipenseriformes, which contains 27 extant species of sturgeon and paddlefish (Birstein et al. 1997). All sturgeon species date back to the lower Jurassic, approximately 200 million years ago, with little changing morphologically in all species over this geological time scale (Havelka et al. 2011). For most sturgeon species, including shortnose sturgeon, populations continue to decline primarily because of damming of rivers, water pollution, and overfishing (i.e., for the roe and flesh) (Kynard et al. 2016). Because of various anthropogenic factors, all species of sturgeon are listed as endangered or a species of special concern (IUCN 2004).

The biogeographic range of shortnose sturgeon encompasses only the northern hemisphere, from the southern reaches of the St-John's River, Florida (USA) to the northern areas of the Saint John River, New Brunswick (Canada) (Kynard et al. 2016). Shortnose sturgeon are amphidromous (Kynard et al. 2016), and thus can be found in a variety of habitats such as estuaries, rivers, lakes and oceans (see Dadswell et al. 1984 and Kynard et al. 2016 for additional life-history and biological data for shortnose sturgeon). Consequently, sturgeon can experience abrupt changes in temperature when transitioning between saltwater, freshwater and brackish water (Kynard et al. 2016). Variation in water temperature can also result from dredging practices, damming of rivers (e.g., changing water flows), factory wastewater and water run-off, which can lead to exposure of repeated thermal stress (Rajaguru 2002; Kynard et al. 2016). These thermal changes, coupled with the predicted increases of 2.0-4.8°C by the end of the 21st century due to climate change (Ficke et al. 2007; Habary et al. 2015) can challenge the ability of fishes, including sturgeon species, to tolerate thermal stress (Ficke et al. 2007; Hupfield et al.

2014; Vinagre et al. 2015). The distribution of fish populations is often limited by their upper thermal tolerance limit (Beitinger et al. 2000); thus, having a general understanding of the factors that affect thermal tolerance of a species provides researchers with valuable information about fish distribution in populations currently facing fluctuating thermal environments (Beitinger et al. 2000; Deslauriers et al. 2016).

From previous studies on shortnose sturgeon, it has been demonstrated that acclimation temperature (Ziegeweid et al. 2008; Zhang and Kieffer 2014) and body size (Zhang and Kieffer 2014) significantly affect thermal tolerance (as assessed using a critical thermal maximum, CT_{max}, test), but heating rate has little impact (Zhang et al. 2017). Through the measurement of various physiological endpoints, such as plasma lactate and ion concentrations, the physiological response following a CT_{max} thermal stress test has also been documented for shortnose sturgeon (Zhang and Kieffer 2014; Spear and Kieffer 2016; Zhang et al. 2017). Other studies have examined the thermal tolerance of sturgeon species (see Spear and Kieffer 2016, for a summary; Deslauriers et al. 2016); however, no other studies have focused on how repeated temperature stressors affect thermal tolerance within any sturgeon species.

The majority of thermal tolerance studies focused on other fish species (Lutterschimdt and Hutchison 1997; Beitinger et al. 2000; Deslauriers et al. 2016) will subject individuals to a singular thermal stressor (i.e., a single CT_{max} test), without any assessment of post-test physiology (Zhang and Kieffer 2014; Spear and Kieffer 2016). Furthermore, thermal tolerance studies are largely concentrated on the effects of acclimation temperature in freshwater and/or game fish, such as rainbow trout (*Oncorhynchus mykiss* Walbaum 1792), channel catfish (*Ictalurus punctatus,* Rafinesque 1818) and largemouth bass (*Micropterus salmoides,* Lacépède,

1802) (Cheetman et al. 1976; Field et al. 1987; Bennett et al. 1997; Currie et al. 1998). In general, researchers have often overlooked the study of multiple thermal stressors in fishes. Presently, there are only a few fish studies that have explored multiple CT_{max} tests to investigate aspects of thermal tolerance in fish (e.g., Bilyk et al. 2012; Healy and Schulte 2012; Gallant et al. 2017; Morgan et al. 2018). Less is known about the physiological/stress responses to multiple thermal stressors, and recovery profiles are not well defined in fishes. Information about the physiological changes from repeated thermal stress can be useful in the management of fish species, development and application of bioenergetics models and conservation efforts of endangered species, such as sturgeon (Deslauriers et al. 2016). As sturgeon have been the focus of intensive aquaculture in North America and abroad, an understanding of the thermal biology of sturgeon, in general, is required.

The overarching objective of this study was to determine if a repeated thermal stress affects the upper thermal tolerance (CT_{max}) of juvenile shortnose sturgeon, and to identify any differences in the physiological response between first and second thermal stress events through examination of aspects of the secondary stress response. The current study tested the following three predictions: (1) Repeated thermal stress would increase the temperature tolerance of shortnose sturgeon (2) The differences in thermal responses would be reflective in changes in secondary stress response indicators. In particular, secondary stress indicators (e.g., hematocrit, lactate, glucose, chloride) would be elevated following thermal stress events, and these levels would be greater following the second thermal stress. (3) The period of recovery (e.g., 1hr or 24hr) between thermal stressors will affect the upper thermal tolerance in sturgeon. If a reduced

recovery period (i.e., 1 hr) between thermal stresses increases the thermal tolerance of sturgeon, it may suggest that heat/temperature hardening may be occurring.

Methods

Fish Culture and husbandry

We obtained all fish from Acadian Sturgeon and Caviar (Carter's Point, Kingston, New Brunswick, Canada). Once the fish arrived at the University of New Brunswick (Saint John, New Brunswick, Canada), they were placed into a holding tank (~1300L) and fed once daily until satiated (Corey Aquafeed: 1.5mm optimum, 52% protein, Fredericton, N.B. Canada), but fasted 24 hours prior to all experimentation. The tank contained an inflow of de-chlorinated Saint John city water at 12°C. We used a 12h light and 12h dark photoperiod throughout the study. The University of New Brunswick Animal Care Committee approved the following experimental protocol, meeting Canadian Council of Animal Care guidelines.

Experimental Setup

Thermal tolerance was determined using the modified critical thermal maximum (CT_{max}) methodology as described in Zhang et al. (2017). We performed thermal tolerance experiments in a rectangular insulated tank (test arena) (41.9 x 24.8 x 29.8 cm), filled with ~30L of 12°C fresh de-chlorinated water (Zhang and Kieffer 2014). An elevated heating tank (45 x 56 cm) was located beside the test arena and equipped with 1000W heater (Pentair Aquatic Ecosystems, Florida, USA) and air diffusers. The heater was calibrated to heat inflowing water at a mean ($\pm$S.E.) constant rate ~9°C $hour^{-1}$ (see below) throughout the study. This heating rate was chosen as it was within the range of heating rates (6-15 ^{o}C $hour^{-1}$) used in our previous studies (Zhang

and Kieffer 2014; Spear and Kieffer 2016; Zhang et al. 2017). Since heating rate does not affect CT_{max} values in shortnose sturgeon (Zhang et al. 2017), the use of a heating rate of 9 ^{o}C hour^{-1} allows for a comparison of CT_{max} values between studies conducted in our laboratory. The water from the elevated heating tank flowed to the test tank by gravity and was then pumped back to the heating tank via a submersible pump (Loligo Systems, Denmark) to maintain temperature within the setup. The submersible pump was isolated from the test chamber by a perforated black Plexiglas shield. We placed an electronic thermometer (Loligo Systems, Denmark) at each end of the test tank to record the temperature within the test tank.

Experiment 1: CT_{max} design

All the fish used for CT_{max} experiments had an average weight of ~132g (Table I). We selected individuals from the holding tank and assigned each fish to one of four experimental groups (Figure 1). For the control, *Group 1* (*Control*) (*N*=8), fish were introduced to the test arena for 3 hours (i.e., the time to complete a typical CT_{max} test) and then sampled as noted for the other experimental groups. Fish from this group served as a no thermal stress control. *Group 2* (CT_{max}1) (*N*=8) consisted of the individual fish being introduced into the test arena for an acclimation period of 1 hour (Zhang and Kieffer 2014) at 12°C, then exposed to CT_{max} protocol (see below). *Group 3* (CT_{max} 1-24h) (*N*=8), same procedure as Group 2, except the fish was placed in a holding tank (45x56 cm) following CT_{max}, for 24 hours (see below for rationale for separating tests by 24hrs), and then sampled for blood. *Group 4* (CT_{max} 2) (*N*=8), same procedure as Group 3, except after the 24-hour recovery period, the fish is re-introduced into test area for 1

hour, then re-exposed to a second CT_{max} protocol and sampled for blood. *Group 5* (CT_{max} 2- 24h) (*N*=8), same procedure as Group 4 except placed back into a holding tank for a 24-hour recovery period following second thermal stress and then sampled for blood. We did not feed fish between the first and second CT_{max} tests. We used a 24hr period between $CT_{max}1$ and $CT_{max}2$ for the following reasons: *(i)* survival rates could be easily followed, *(ii)* this time period is consistent with other studies (e.g., Healy and Schulte 2012; Gallant et al. 2017), and *(iii)* to reduce the possibility of time of day effects on CT_{max} in fish (Healy and Schulte 2012).

Experiment 1: Experimental Protocol

Following the 1hr acclimation period, the fish that were exposed to thermal stress (i.e., Groups 2-5), were subjected to a linear increase of temperature of ~9°C $hour^{-1}$ (±S.E.) (calculated from each CT_{max} test; 9.28±0.05) at a constant rate until loss of equilibrium (LOE) occurred. Loss of equilibrium as an endpoint is indicated when the fish rolled dorso-ventrally and is unable to right itself within 10 s (Ziegeweid et al. 2008; Zhang and Kieffer 2014; Spear and Kieffer 2016). We manually righted individuals three times to ensure that each fish reached its CT_{max} (Spear and Kieffer 2016). We measured the water temperature in the test arena throughout the trials, until the third incidence of LOE, at which point we recorded the CT_{max} for the fish. At this point, the individuals were removed from the test arena, placed into a holding tank for further experimentation depending on the group it was assigned to, or anaesthetized (with a buffered TMS solution; 1g/L with 2g/L buffered sodium bicarbonate, Spear and Kieffer 2016) and measured for length and weight (Table I), and a blood sample taken. With a lithium heparinized needle and syringe, approximately 1ml of blood was removed from the caudal vasculature (Zhang and Kieffer 2014; Spear and Kieffer 2016), and analysed for various

physiological endpoints (see below). The test arena was drained, wiped down and rinsed, and filled with new de-chlorinated water at 12^{o}C following each trial.

Experiment 1: Blood Analysis

We used approximately100 µl of the whole blood for duplicate hematocrit determination (Zhang et al. 2017). We centrifuged the remaining blood sample for 2 minutes at 6700g, and pipetted the resulting plasma into two labelled tubes and frozen for further testing. The frozen samples were thawed and plasma lactate concentrations were measured using standard spectrophotometric assay at 540nm (Lactate Reagent 735-10, Lactate Standard Solution 826-10 and Lactate Standard Set 735-11; Trinity Biotech). We measured plasma glucose using an OneTouch Ultra glucose meter (OneTouch Ultra 2; Code 25 test strips; www. onetouch.ca; Penny and Kieffer 2014; Downie et al. 2018), plasma chloride using a Labconco digital chloridometer (Labconco, U.S.A), and osmolality using the Advanced Micro Osmometer (Advanced Instruments Inc., U.S.A).

Experiment 1: Statistical Analysis

Statistics were analysed using SIGMASTAT 3.5 software (www.sigmaplot.com). Linear regression (α=0.05) was used to determine the relationship between the first critical thermal maximum (CT_{max} 1) and the second critical thermal maximum (CT_{max} 2) (CT_{max} values from Groups 4 and 5). Paired t-test (α=0.05) was performed to verify if a difference existed between

the mean values of $CT_{max}1$ and $CT_{max}2$. One-way ANOVA tests ($\alpha = 0.05$) were used to examine the effects of acute thermal stress on the various blood parameters. When the ANOVA results were significant, a Holm Sidak ($\alpha = 0.05$) multiple comparison test was used to compare mean blood parameter values between the various groups.

Experiment 2: Potential for Heat Hardening in sturgeon

To explore the possibility of heat hardening in juvenile shortnose sturgeon, individuals (N=8) were exposed to the repeat CT_{max} protocol (described above), but with a reduced recovery period of 1hr. We placed individuals in an aerated container filled with water at the acclimation temperature following the first CT_{max} test. The test arena was quickly drained, and refilled with water at the acclimation temperature. This water replacement took about 20 minutes. Following this, the fish were placed back in the test arena and left to acclimate for the next 40 minutes (i.e., for a total of 1hr at the acclimation temperature), and then subjected to the second CT_{max} test. We did not sample blood from these fish.

Results

Experiment 1:

Weight and Length

Mean (± S.E.) weight and length did not vary among any of the groups (Table I). On average, juvenile sturgeon weighed 132.45 g, and were 31.91 cm in total length.

Modified Critical thermal maximum (CT_{max})

There were no significant differences between the values of the first CT_{max} ($CT_{max}1$) and the second CT_{max} ($CT_{max}2$) when separated by 24hr (Paired t-test; t= -0.594; df= 15; P>0.05), and

the mean (± S.E.) $CT_{max}1$ and $CT_{max}2$ values were 26.59± 0.457 (*N*=16) and 26.78± 0.440 (N=16), respectively (Figure 2). There was a significant, positive relationship between $CT_{max}1$ and CT_{max} 2, which was described by the equation: $CT_{max}2 = (0.725 * CT_{max}1) + 7.495$ (R^2=0.567, *P*<0.001) (Figure 2). When the apparent outlier (denoted as * on the graph) was removed from Figure 2, the relationship between $CT_{max}1$ and $CT_{max}2$ was stronger, and represented by the equation: $CT_{max}2= (0.937 * CT_{max}1) + 2.109$ (R^2= 0.847, *P*<0.001). All fish survived the CT_{max} testing, and there was a recovery rate between CT_{max} tests (i.e., $CT_{max}2$/ $CT_{max}1$) of 1.009.

Hematological Response

We used multiple stress indicators to determine the effects of thermal stress on the physiology of juvenile shortnose sturgeon. Acute thermal stress did not affect mean hematocrit, or plasma Cl^- concentration among the control or the thermally stressed groups (Figures 3a and b). We found significant differences in mean plasma osmolality immediately after $CT_{max}1$ and $CT_{max}2$; these levels recovered within the 24-hour recovery period (Figure 3c). Mean plasma lactate increased immediately after both $CT_{max}1$ and $CT_{max}2$ but values recovered to control levels following a 24 hr period (Figure 4a). There was a significant difference in mean plasma lactate between the first thermal stress event (Group $CT_{max}1$) and the second thermal stress event (Group $CT_{max}2$) (Figure 4a). Mean glucose levels were affected by thermal stress immediately after CT_{max} (Group $CT_{max}1$ and $CT_{max}2$) but levels returned to control levels following a recovery period (Group CT_{max} 1- 24h and CT_{max} 2-24h) (Figure 4b).

Experiment 2: $CT_{max}1$ and $CT_{max}2$ separated by 1hr

Sturgeon used in this experiment ranged in size from 87-130g (mean 107.5±5.2). There were significant differences between the values of the first CT_{max} ($CT_{max}1$) and the second CT_{max} ($CT_{max}2$) (paired t-test; t= -3.74; df= 7; P=0.007; Figure 5), and the mean (± S.E.) $CT_{max}1$ and $CT_{max}2$ values were 27.41 ± 0.576 (N=8) and 28.48± 0.718 (N=8), respectively. There was a significant, positive relationship between $CT_{max}1$ and $CT_{max}2$, which can be described by the equation: $CT_{max}2 = (1.154 * CT_{max}1) - 3.16$ (R^2=0.835, P<0.001) (Figure 5).

Discussion

This study examined the repeatability of CT_{max} performances following a recovery period in shortnose sturgeon. The use of repeated CT_{max} tests provides the researcher with three important measures of thermal tolerance: (i) the CT_{max} values, (ii) relationships between the two CT_{max} values, and (iii) recovery ratios ($CT_{max}2/CT_{max}1$). The CT_{max} values are known for many species of vertebrates and invertebrates (Lutterschmidt and Hutchison 1997; Beitinger et al. 2000), and thus allow for a direct comparison of values between studies. Although upper thermal tolerance (CT_{max}) values in shortnose sturgeon, and other fish species, are affected by many factors, including acclimation temperature and body size (Zhang and Kieffer 2014), values for CT_{max} from the present study are in agreement with previous sturgeon studies, including shortnose sturgeon (see Spear and Kieffer 2016 and Deslauriers et al. 2016, for discussion).

The current study showed that there were no significant differences between the mean CT_{max} values of the first and second test in juvenile shortnose sturgeon following a 24hr recovery period. Similar studies have been conducted on killifish *Fundulus heteroclitus* (Linnaeus 1766),

where individuals were subjected to CT_{max} experiments 1, 2, 3, 5, 10, 13 and 21 days after the initial CT_{max} experiment (Healy and Schulte 2012). It was concluded that the repeated exposure to the same thermal stress protocol did not significantly affect the CT_{max} of *F.heteroclitus* (Healy and Schulte 2012). It was also shown that prior heat shock did not affect the CT_{max} during a subsequent thermal stress event in Atlantic salmon *Salmo salar* (Linnaeus 1758) (Gallant et al. 2017). Taken together, the findings from these studies suggest that these fish species maintain their thermal tolerance during multiple thermal stress events. Conversely, Bilyk et al. (2012) found evidence of heat hardening (i.e., a short-lived increase in heat tolerance; Bilyk et al. 2012) in notothenioid fish when subjected to a CT_{max} experiment, followed by a 4 to 24h recovery period and, then another CT_{max} experiment (Bilyk et al. 2012). The difference in CT_{max} values between tests ranged between 0.6± 0.29°C and 1.8±0.45°C (Bilyk et al. 2012). Although the species within Bilyk et al's (2012) study were all Antarctic species instances of heat hardening were also recorded among temperate species such as the fathead minnow *Pimephales promelas* (Rafinesque 1820) and the red shiner *Cyprinella lutrensis* (S.F. Baird and Girard, 1853) (Maness and Hutchinson 1980). Nevertheless, heat hardening in temperate species is found to peak at 2 hours after the thermal stress event and can return to normal as soon as 4 hours (Maness and Hutchinson 1980; Bilyk et al. 2012). A 24-hour recovery period was provided to the sturgeon in the present study (i.e., experiment 1); therefore, any signs of heat hardening would not likely have been detected during the second thermal stress event. However, the findings from our second experiment, where the recovery period between subsequent CT_{max} tests was 1hr, revealed a slightly higher CT_{max} value during the second CT_{max} test. Even so, heat hardening would only temporarily aid a fish under thermal stress in the wild, as it would likely not withstand long-term

exposure at those elevated temperatures (Maness and Hutchison 1980; Bilyk et al. 2012). This would likely only give the fish a limited time to find thermal refuge or die (Maness and Hutchinson 1980; Bilyk et al. 2012). The increase in CT_{max} within these studies are minimal, and the fluctuations seen by heat-waves and factory run off can be as much as 10°C, in which heat hardening would likely prove to be of little to no advantage (Rajaguru 2002). However, from an ecological perspective, these findings for sturgeon may provide a context in regards to temperature changes over the short term in the wild, such as tidal cycles, and how individuals respond to these changes.

Similar to previous studies on shortnose sturgeon, plasma chloride levels, and hematocrit levels were unaffected by thermal stress (Zhang and Kieffer 2014; Spear and Kieffer 2016; Zhang et al. 2017). Plasma lactate levels increased following CT_{max} experiments, to levels which have been previously noted (Zhang and Kieffer 2014; Spear and Kieffer 2016; Zhang et al., 2017). The increase in lactate during the thermal tolerance tests (i.e., $CT_{max}1$ and $CT_{max}2$ in experiment 1) was likely due to the activation of anaerobic pathways of sturgeon related to increased activity and a decrease in oxygen levels in the experimental chamber with warmer water (Zhang and Kieffer 2014). The significant difference in plasma lactate between $CT_{max}1$ and $CT_{max}2$ group may suggest that the fish may have obtained an energetic advantage from prior exposure to thermal stress reducing the use of anaerobic metabolism during the second test. It is also possible that that the lower lactate levels observed during the $CT_{max}2$ is related to the additional fasting period prior to the second CT_{max} test. Specifically, the fish that had undergone a second CT_{max} test were fasted for an additional 24hrs, which may have influenced the availability of metabolic resources used during stressful situations. While the effects of fasting on CT_{max} values in sturgeon have not been previously addressed, Lee et al. (2016) noted the

potential impacts of nutritional status/food quantity on thermal tolerance of green sturgeon (*Acipenser medirostris*, Ayres 1854). In contrast to lactate levels during the $CT_{max}2$ test, however, there were no differences in glucose levels following the second CT_{max} test. Thus, it is plausible that the added short-term fasting period had limited effects on storage of necessary metabolites used to fuel the energetic requirements of thermal stress, at least in juvenile shortnose sturgeon. Some caution, however, is required with these conclusions since the plasma metabolites were measured in different fish between the two groups. Future research should validate this conclusion using the same individual during testing and blood sampling.

There were no mortalities recorded for the duration of the study and there were no significant differences in any of the blood parameters among the control and recovery groups ($CT_{max}1$- 24h and $CT_{max}2$-24h) suggesting that the fish could fully recover from the thermal stress within a 24hr period (Gallant et al. 2017). There was also a recovery ratio (i.e., $CT_{max}2$/ $CT_{max}1$) of 1.009 between the values of $CT_{max}1$ and $CT_{max}2$ in experiment 1, suggesting that prior thermal stress did not hinder the individual's ability to tolerate a secondary thermal stress.

In conclusion, it appears that the thermal tolerance of juvenile shortnose sturgeon are not affected by prior thermal stress. Neither plasma chloride nor hematocrit changed with thermal stress. Plasma osmolality, glucose and plasma lactate increased with thermal stress but recovered within 24 hours. From the blood parameters data and survivability of this study, it also appears that juvenile shortnose sturgeon can make a full recovery from thermal stress. Further studies should focus on mechanism that might explain the decrease in lactate between the first and second thermal stress event immediately after CT_{max}. Additionally, determine if instances of heat

hardening could occur in shortnose sturgeon through manipulation of the recovery period between the CT_{max} tests. This might give greater insight into the temporal differences in thermal tolerance (e.g., daily or annually). From a broader point of view, it appears sturgeon have the capacity to maintain their thermal tolerance as their thermal environment continues to fluctuate. Understanding the combined effects of various factors (such as fish age, salinity tolerance, ontogeny, hypoxia, reproductive status) on the thermal tolerance should be the focus of future studies.

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Figure Captions:

Fig. 1. Timeline outlining the protocol of the four thermally stressed groups (*N*=8) of juvenile shortnose (*Acipenser brevirostrum*) within the study (Experiment 1).

Fig. 2. Linear regression of repeated critical thermal maxima, $CT_{max}2$ (°C) regressed against $CT_{max}1$ (°C) (i.e., CT_{max} values from Group 4 and 5) ($CT_{max}2$= 7.495 + (0.725 * $CT_{max}1$); R^2 = 0.567), of juvenile shortnose sturgeon. When the data point indicated by the asterisk in Fig. 2 is removed, the linear relationship between $CT_{max}2$ and $CT_{max}1$ becomes $CT_{max}2$= 2.109 + (0.937 * $CT_{max}1$); R^2=0.847. $CT_{max}2$ and $CT_{max}1$ were separated by a 24hr recovery period. The dotted line represents the line of unity and the solid line represents the linear regression equation line.

Figure 3. Mean (±S.E) (a) hematocrit (%) (b) plasma Cl^{-1} (mequiv. L^{-1}) and (c) plasma osmolality (mOsm Kg^{-1}) of juvenile sturgeon in the control group (*N*=8) and thermally stressed groups (*N*=8) Different letters indicate significant differences. (*P*<0.05; Holm-Sidak Multiple comparisons). Open circles represent data for individual sturgeon. The thick horizontal line represents the group means.

Figure 4. Mean (±S.E) (a) plasma lactate (mmol L^{-1}) and (b) plasma glucose (mmol L^{-1}) of juvenile shortnose sturgeon in the control group (*N*=8) and thermally stressed groups (*N*=8). Different letters indicate significant differences. (*P*<0.05; Holm-Sidak Multiple comparisons). Open circles represent data for individual sturgeon. The thick horizontal line represents the group means.

Figure 5. Linear regression of repeated critical thermal maxima, $CT_{max}2$ (°C) regressed against $CT_{max}1$ (°C) ($CT_{max}2$ = (1.154 * CTmax 1) -3.16 (R^2=0.835, *P*<0.001) of juvenile shortnose

sturgeon. $CT_{max}2$ and $CT_{max}1$ were separated by a 1hr recovery period. The dotted line represents the line of unity and the solid line represents the linear regression equation line.

Group 1 (Control)
Does not undergo CTmax protocol (i.e. no thermal stress), placed in test area for 3 hours and sampled (N=8)

Group 2 (CT_{max}1)
Undergoes CTmax protocol and sampled for blood immediately after reaching CTmax (N=8)

Group 3 (CT_{max}1- 24hrs)
Undergoes CTmax protocol, returned to holding tank for 24hrs, then sampled for blood (N=8)

Group 4 (CT_{max} 2)
Undergoes CTmax protocol, returned to holding tank for 24hrs, subjected to a second CTmax test, then sampled for blood. (N=8)

Group 5 (CT_{max} 2-24hrs)
Undergoes CTmax protocol, returned to holding tank for 24hrs, subjected to a second CTmax test, returned again to holding for 24hrs, then sampled for blood. (N=8)

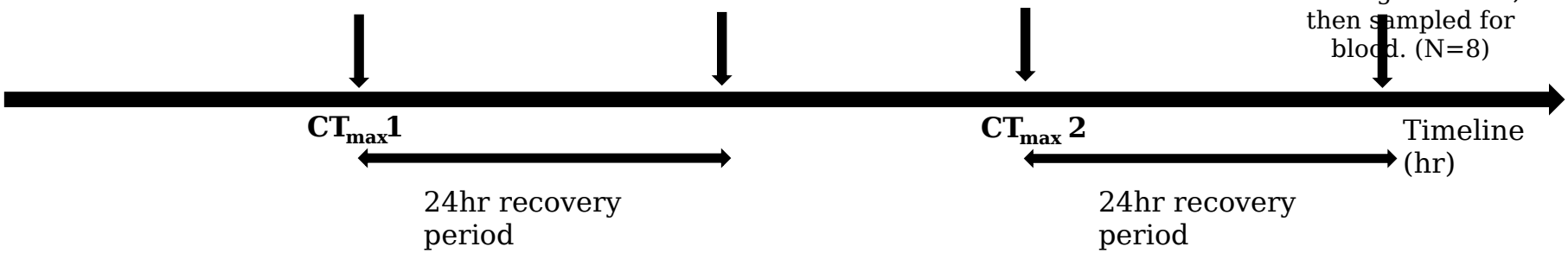

Fig2

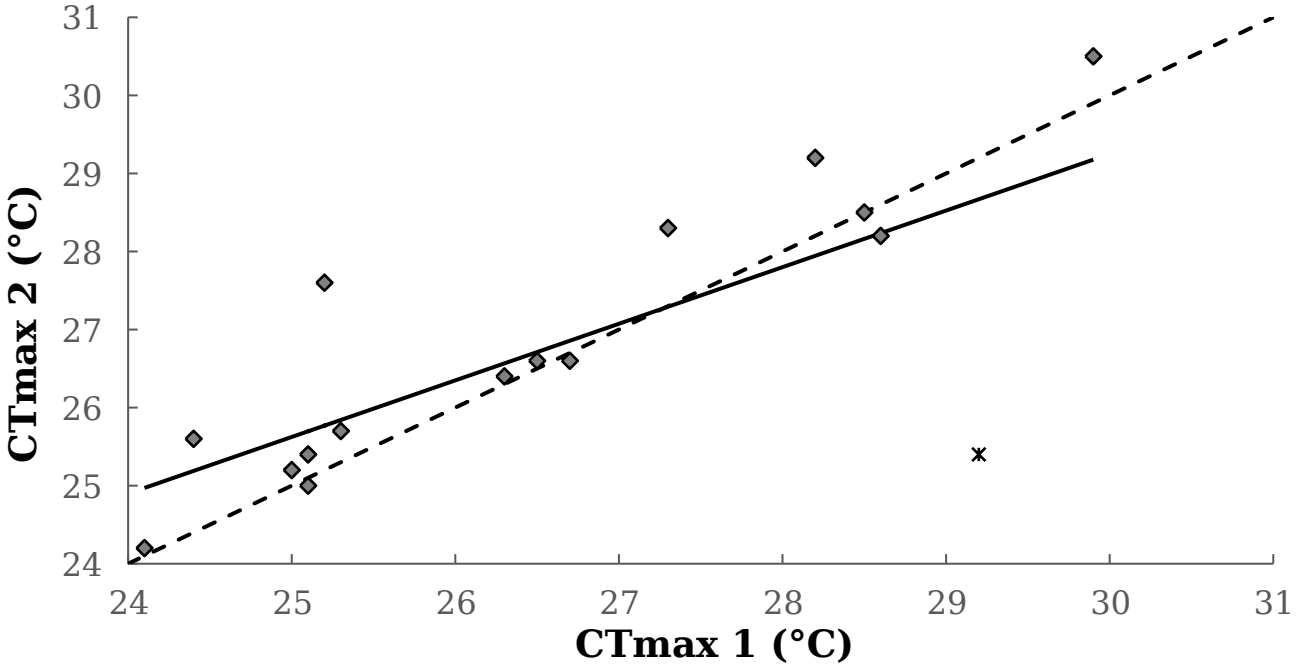

Fig3

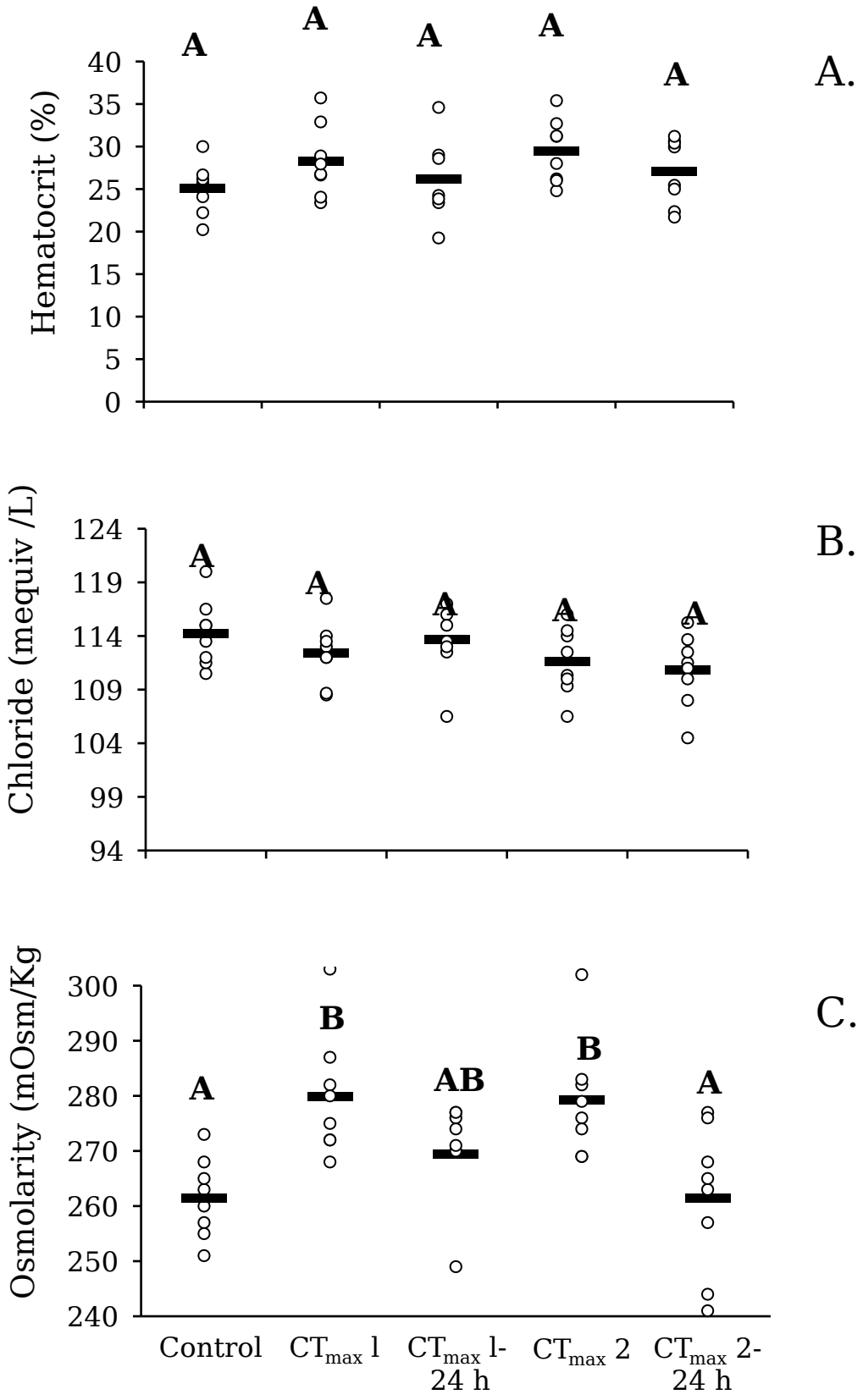
A.
Hematocrit (%)
40
35
30
25
20
15
10
5
0
A
A
A
A
B.
Chloride (mequiv /L)
124
119
114
109
104
99
94
A
A
A
A
A
C.
Osmolarity (mOsm/Kg
300
290
280
270
260
250
240
A
B
AB
B
A
Control
CTmax l
CTmax l-24 h
CTmax 2
CTmax 2-24 h

Fig4

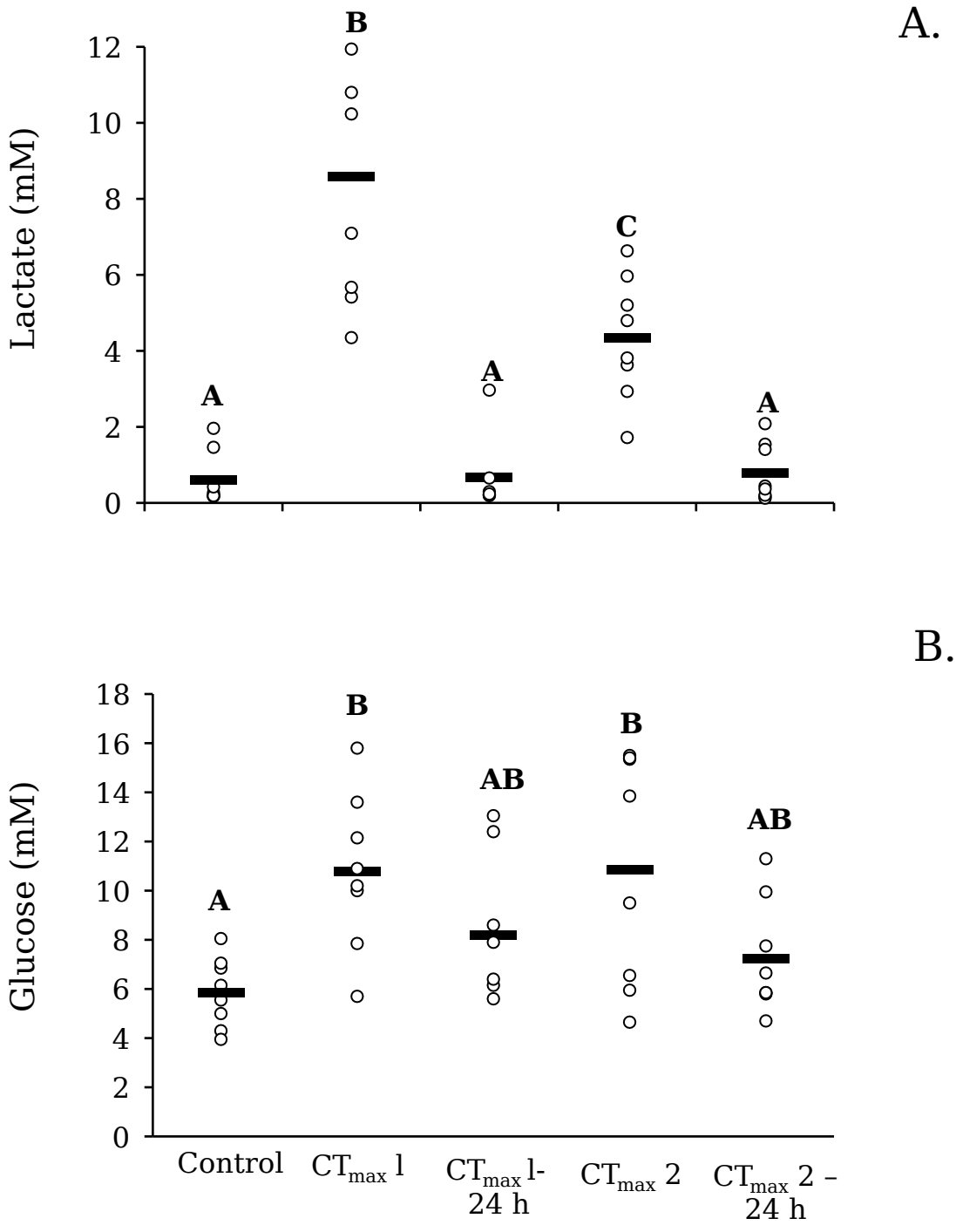
A.
Lactate (mM)
12
10
8
6
4
2
0
B
A
C
A
B.
Glucose (mM)
18
16
14
12
10
8
6
4
2
0
A
B
AB
B
AB
Control
CTmax l
CTmax l-24 h
CTmax 2
CTmax 2 – 24 h

Fig5

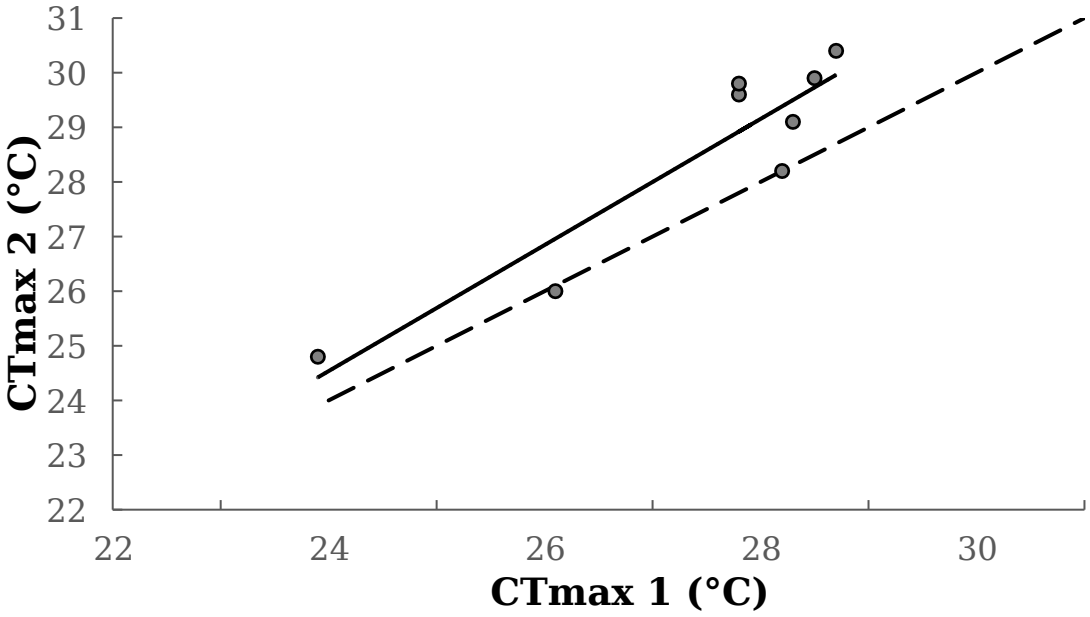

Table 1: Mean mass (±S. E.) and mean length (±S.E.) of juvenile shortnose sturgeon (*Acipenser brevirostrum*) used in the study (*N*=8). No significant differences existed between groups (*P*>0.05, One-way ANOVA).

Group	Weight (*g*)	Length (*cm*)
1 - ($CT_{max}1$)	124.0±9.1	31.6±0.7
2 - ($CT_{max}1$-24h)	138.0±11.2	31.9±1.0
3 - ($CT_{max}2$)	129.8±5.1	32.1±0.3
4 - ($CT_{max}2$ – 24h)	134.5±12.6	32.6±1.1
5 - (Control)	136.0±6.3	31.3±0.5