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**Increased acute thermal tolerance and little change to hematology following acclimation to warm water in juvenile Striped Bass, *Morone saxatilis***

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**Running title:** Acclimation & CT<sub>max</sub> in Striped Bass

**Keywords:** Striped Bass; CT<sub>max</sub>; Acclimation; Hematology

**Data Availability Statement**

Data from this manuscript will be archived on the University of New Brunswick's Dataverse Research Data Repository.

**Conflict of Interest Statement**

The authors are not aware of any conflicts of interest.

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## Abstract

20 Striped Bass naturally inhabit ~~in~~ a wide range of temperatures, yet little is known about  
21 the ~~physiology and~~ processes that control their acute and chronic temperature limits. The  
22 objective of this study was to determine the effect of temperature acclimation on acute thermal  
23 maxima and physiology of juvenile Striped Bass. Juvenile fish were acclimated to 15, 25 or 30°C  
24 for 4 weeks, then split into two sampling groups: ~~at post~~-acclimation ~~alone~~; ~~after and post~~-critical  
25 thermal maximum trials. We found that fish survived ~~even the highest in all acclimation~~  
26 temperatures; with little change to underlying ~~hameatology~~ hematology, and that critical thermal  
27 maximum (CT<sub>max</sub>) increased with increasing acclimation temperature. At CT<sub>max</sub>, fish acclimated  
28 to 30°C had elevated plasma cortisol, lactate and potassium levels. These results suggest that,  
29 while 30°C is likely to be outside their thermal optima, Striped Bass can survive ~~and perform at~~  
30 high temperatures. This ability to cope with warm temperatures may provide an advantage with  
31 ~~changing increasing~~ global ~~environment~~ temperatures.

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33

## Introduction

34 Temperature has profound effects on the biology of ~~ectothermie~~ poikilothermic fishes  
35 ~~(Fry 1947; Brett 1971).~~ (Fry 1947; Fry 1967; Brett 1971). As ectothermic aquatic animals, fish  
36 must be able to cope with the physiological ramifications of ~~the~~ varying temperatures in their  
37 environment. The effects of temperature are wide-ranging and can be seen from changes in  
38 whole-animal metabolism to specific molecular responses ~~(Bœuf and Payan 2001; Hochachka~~  
39 ~~and Somero 2002; Evans 2008; Currie and Schulte 2013; Schulte 2015).~~ (Bœuf and Payan 2001;  
40 Hochachka and Somero 2002; Evans 2008; Currie and Schulte 2013; Schulte 2015). For

41 example, temperature has been shown to effect rates of enzymatic activities (Hochachka and  
 42 Somero 2002) through to behavioral effects of determining optimal habitats (Bettoli 2005). The  
 43 response to temperature may not be consistent across fish species, as ~~different~~ species may have  
 44 evolved different mechanisms to counter the same problem. Even within a species, populations  
 45 may be locally adapted to different thermal conditions and therefore have different physiological  
 46 responses (~~Fangue et al. 2006; Losos 2011; Schulte et al. 2011; Conte et al. 2012; Whitehead~~  
 47 ~~2012~~)(Fangue et al. 2006; Losos 2011; Schulte et al. 2011; Conte et al. 2012; Whitehead 2012).  
 48 ~~Thus~~For example, different populations of Striped Bass have been shown to different growth  
 49 rates at the same temperature (Secor et al. 2000). Additionally, within populations of a single  
 50 species, individuals ~~with~~may exhibit different life history strategies ~~may~~and react differently  
 51 (~~Morgan and Iwama 1991; Pavey et al. 2015~~)to temperatures (Morgan and Iwama 1991; Pavey et  
 52 al. 2015).

53

54 The ubiquitous effects of temperature on fish biology are well documented, with  
 55 temperature being considered the “master ecological factor” for ectothermic organisms,  
 56 including fish (e.g., Brett 1971). Methods for quantifying temperature tolerance in fishes in an  
 57 ecologically relevant manner has been widely discussed (~~Fry 1967; Brett 1971; Becker and~~  
 58 ~~Genoway 1979; Lutterschmidt and Hutchison 1997b; Lutterschmidt and Hutchison 1997a;~~  
 59 ~~Beitinger and Bennett 2000~~)(Fry 1967; Brett 1971; Becker and Genoway 1979; Lutterschmidt  
 60 and Hutchison 1997b; Lutterschmidt and Hutchison 1997a; Beitinger and Bennett 2000). One  
 61 such standard method is the Critical Thermal Maximum (“CT<sub>max</sub>”) test, whereby temperature  
 62 tolerance limits are estimated by exposing a fish to a linear increase of temperature, up to the  
 63 point of loss of equilibrium (LOE) (~~Lutterschmidt and Hutchison 1997a; Beitinger and Bennett~~

64 ~~2000~~;(Lutterschmidt and Hutchison 1997a; Beitinger and Bennett 2000). While fishes in the  
65 natural environment rarely experience such an acute change in temperature, extreme events are  
66 more likely to offer more selection pressure than “normal” temperatures (Lutterschmidt and  
67 Hutchison 1997b). This method also allows for a quantitative comparison of acute temperature  
68 tolerance between different groups of fishes and across various acclimation temperatures (see  
69 Beitinger and Bennett (2000) for an extensive comparison across North American fishes), which  
70 can facilitate understanding of the evolution of temperature tolerance across geography and  
71 species groups.

72  
73 Geographically wide-ranging fishes provide an interesting model for exploring  
74 temperature biology. Owing to their life history, these fishes may inhabit rivers with drastically  
75 different temperature regimes. The Striped Bass, *Morone saxatilis* (Walbaum, 1792), is a large  
76 anadromous fish, that ~~possesses~~occupies a large putative geographical range along the east coast  
77 of North America. Following a population collapse in the 1980's, stocks have widely recovered,  
78 yet the continued loss of multiple historical Canadian spawning locations recently led to  
79 classification of the Bay of Fundy population of Striped Bass as endangered (~~COSEWIC 2012a;~~  
80 ~~COSEWIC 2012b~~);(COSEWIC 2012). Given the implications for Striped Bass aquaculture to  
81 supplement wild populations, the effects of temperature on growth has long been of interest  
82 (~~Otwell and Merriner 1975; Cox and Coutant 1981; Cook 2003; Cook et al. 2010~~)(Otwell and  
83 Merriner 1975; Cox and Coutant 1981; Cook 2003; Cook et al. 2010). Other temperature  
84 research has demonstrated effects of temperature on disease (~~Carlson et al. 1995; Lapointe~~  
85 ~~2014~~)(Carlson et al. 1995; Lapointe 2014), angling or handling stresses (~~Bettoli and Osborne~~  
86 ~~1998; Wilde 2000; Bradley et al. 2018~~)(Bettoli and Osborne 1998; Wilde 2000; Bradley et al.

87 2018), and movements of wild fish (~~Coutant et al. 1984; van den Avyle and Evans 1990; Secor~~  
88 ~~and Houde 1995; Bettoli 2005; Secor and Piccoli 2007; Duston et al. 2018)~~.(Coutant et al. 1984;  
89 van den Avyle and Evans 1990; Secor and Houde 1995; Bettoli 2005; Secor and Piccoli 2007;  
90 Duston et al. 2018), with adverse effects typically occurring above and/or below more  
91 moderate/optimum temperatures. Less is known about the limits and underlying processes of  
92 temperature tolerance in Striped Bass.

94 While temperature change is inherent and common in fishes' natural environments, it still  
95 can be a stressful stimulus. The cascade of effects of stressors on fishes have been well  
96 documented and may span many levels of biological organization ranging from hormonal  
97 changes through to whole-animal or even population-level changes (Barton 2002). Primary  
98 responses to stress in fishes may be seen through elevation in stress hormones (e.g. cortisol),  
99 followed by secondary changes to metabolism (Kir et al. 2017; Kır and Demirci 2018; Kır 2020)  
100 and other disturbances to homeostasis (e.g. ability to osmoregulation) before tertiary responses  
101 are seen in the animal as a whole. To date, the data on Striped Bass temperature biology has  
102 focused on whole-animal endpoints (e.g. CT<sub>max</sub> or growth (Otwell and Merriner 1975; Cook  
103 2003; Cook et al. 2006; Cook et al. 2010)). While whole-animal studies offer practical insight  
104 into absolute limits, investigation into the primary and secondary stress responses (e.g. cortisol,  
105 metabolism, changes in plasma ions) may indicate disturbances not captured in whole-animal  
106 approaches.

107

108 To date, few studies have assessed critical thermal limits and temperature physiology of  
109 Striped Bass. Cook et al. (2006) published a thorough investigation into the thermal limits of

110 Shubenacadie Striped Bass across a range of acclimation temperatures, using both incipient and  
 111 critical thermal tests. Cook et al. (2006) also suggest that thermal tolerance may be inversely  
 112 related to body size in ~~Striped~~Striped Bass, not directly related, as is common for many fishes.  
 113  $CT_{max}$  values for Oklahoma Striped Bass are also reported by Lutterschmidt and Hutchison  
 114 (1997a) at a single acclimation temperature (10°C) with a small sample size (N = 3). Some  
 115 thermal limits ( $CT_{max}$  or incipient) have also been published for hybrid Striped x White Bass  
 116 hybrids (Woiwode and Adelman 1992; Kelly and Kohler 1999). Davis and Parker (1990)  
 117 demonstrated variations in various hematological ~~to endpoints (e.g cortisol, plasma chloride)~~  
 118 ~~following a stressor in southern Striped Bass acclimated to different temperatures.~~endpoints (e.g.  
 119 cortisol, plasma chloride) following a stressor in southern Striped Bass acclimated to different  
 120 temperatures. It has also been suggested that Northern populations of Striped Bass possess a  
 121 broader temperature tolerance range than Southern counterparts (Cook et al. 2006). While these  
 122 studies provide some insight into the range and variation of temperature tolerance in Striped  
 123 Bass, there is an absence of studies exploring underlying physiological processes with multiple  
 124 acclimation temperatures and at extreme thermal limits, such as  $CT_{max}$ . Understanding these  
 125 processes could shed light on the limiting factors which limit the thermal tolerance ranges of  
 126 Striped Bass and help define traits important for selection.

127

128 The objective of this study is to determine the effect of acclimation temperature on the  
 129 upper thermal limit ( $CT_{max}$ ) of juvenile Striped Bass: from Nova Scotia, Canada. Furthermore,  
 130 we aim to determine whether acclimation and  $CT_{max}$  trials also cause changes to ion regulation  
 131 (plasma ions), indicators of ~~anerobie~~anaerobic metabolism (lactate) and stress (cortisol). Lastly,  
 132 we ~~look~~-test the putative inverse relationship withof thermal tolerance and body weight. We

133 ~~hypothesis~~hypothesize that  $CT_{max}$  will increase with increasing acclimation temperature and that  
134 increased temperature will result in ~~more physiological disturbances.~~significant changes in  
135 plasma hematology.

136

137

## Materials and Methods

### 138 *General husbandry*

139 All fish were juvenile Striped Bass (mass =  $74.5 \pm 3.4$ g; fork length =  $19 \pm 0.4$  cm; mean  
140  $\pm$  standard error), raised from wild-caught eggs, spawned naturally in the Shubenacadie River  
141 (Nova Scotia, Canada). Fish were maintained in circular, fiberglass tanks (1 m in diameter; ~750  
142 L) with a constant flow through of fresh (0‰), dechlorinated municipal water; and prior to any  
143 temperature trials, all fish were maintained at 15°C. Water quality was routinely monitored for  
144 constant pH, chlorine and salinity. The photoperiod was kept constant at 14 h light: 10 h dark  
145 throughout acclimation and experiments. Fish were fed daily to satiation on commercial Trout  
146 pellets (3mm Vita Pellets; EWOS). Prior to trials, food was withheld for 24 hours. ~~Prior to any~~  
147 ~~temperature trials, all fish were maintained at 15°C.~~All procedures were approved by the  
148 University of New Brunswick Saint John's Animal Care Committee according to Canadian  
149 Council of Animal Care standards.

### 150 *Experimental design*

151 In their rearing tanks, fish were acclimated to one of three temperatures: 15°C, 25°C or  
152 30°C. Three replicate tanks were used to acclimate fish to each of these temperatures.  
153 Temperatures were maintained by the use of multiple 800-watt submersible heaters (Aquatop  
154 Titanium 800W; TH800W) with external temperature controllers (Inkbird; ITC-308). Fish were

155 acclimated to the respective temperatures at a rate of 1-1.5°C per day. Once at the acclimation  
156 temperature, fish were maintained at this temperature for 4 weeks prior to thermal maxima trials  
157 and/or ~~tissue~~blood sampling.

158 Fish were sampled as part of one of two treatment groups: (1) acclimation control or (2)  
159 at critical thermal maximum ( $CT_{max}$ ). Acclimation control fish underwent no further temperature  
160 change nor manipulation other than acclimation to one of the three temperatures (15, 25 or 30°C)  
161 and were removed directly from acclimation tanks for sampling.  $CT_{max}$  group fish were sampled  
162 immediately following the  $CT_{max}$  trial (described below). For each treatment and temperature  
163 combination, three fish from the three replicate acclimation tanks were selected to account for  
164 possible tank variation ( $N = 3 \text{ fish} \times 3 \text{ tanks} = 9$  per temperature & treatment). ~~Due to an~~  
165 ~~unforeseen reduced number of fish, not related to temperature acclimation, only 7 fish were~~More  
166 than three fish were housed per holding tank and the three fish were randomly selected at the  
167 sampling time The 30°C acclimation group was not originally planned for in the study design but  
168 was added after we noted the relatively small disturbances at 25°C; therefore only 7 fish from the  
169 same cohort remained to be used for the 30°C acclimation/ $CT_{max}$  group. New fish were not  
170 added to the experiment to avoid confounding source variation.

#### 171 *Critical temperature maximum ( $CT_{max}$ ) chamber*

172 All  $CT_{max}$  trials were performed on individual fish, in the same experimental system and  
173 monitored by the same observer, to avoid inter-observer bias. The experimental system was  
174 comprised of an insulated test tank (56 x 28 x 17.75 cm; 27.7 L) and ~~header~~heater tank (48.25 x  
175 43 x 24 cm; 50.3L). The water in the test tank was pumped to the ~~header~~heater tank at ~1100 L ·  
176 h<sup>-1</sup> (Marineland Maxi-Jet Pro 1200; ML90509), where it was heated before being returned to the  
177 holding tank via passive overflow from the ~~header~~heater tank. The water in the ~~header~~heater tank

178 was heated by two 800-watt submersible heaters (Aquatop Titanium 800W; TH800W) with  
179 external temperature controllers (Inkbird; ITC-308) and circulated with a powerhead (Hydor  
180 Koralia Evolution Powerhead; HY-KOR03A) for efficient heating. The temperature in both  
181 chambers were monitored and recorded electronically to ensure consistent heating rates  
182 (MyDevices IoT Cayenne via Raspberry Pi 3 Model B with DS18B20 temperature probes). Both  
183 the header and experimental tanks were well aerated using pressurized air and ceramic air stones.  
184 Dissolved oxygen was periodically monitored (YSI [Model 85](#)) to ensure levels did not fall with  
185 increasing temperatures and stayed above 80% saturation.

#### 186 *Critical temperature maximum (CT<sub>max</sub>) trials*

187 An individual fish was removed from the acclimation tanks and quickly moved to the  
188 previously described experimental holding tank, containing water of the respective acclimation  
189 temperature. The fish was left to adjust to the experimental tank for 1 hr prior to any  
190 experimentation. Following this 1 hr period, the temperature in the system was increased at a rate  
191 of rate of  $0.3^{\circ}\text{C} \cdot \text{min}^{-1}$  ( $18^{\circ}\text{C} \cdot \text{hour}^{-1}$ ), which is commonly used for CT<sub>max</sub> tests in fishes  
192 (~~Woiwode and Adelman 1992; Lutterschmidt and Hutchison 1997b; Lutterschmidt and~~  
193 ~~Hutchison 1997a; Beitinger and Bennett 2000; Cook et al. 2006~~); (~~Woiwode and Adelman 1992;~~  
194 ~~Lutterschmidt and Hutchison 1997b; Lutterschmidt and Hutchison 1997a; Beitinger and Bennett~~  
195 ~~2000; Cook et al. 2006~~). Loss of equilibrium (LOE) was used as the endpoint for CT<sub>max</sub>, as  
196 defined as the point where the fish could not maintain a dorso-ventral position for 10 seconds  
197 (~~Becker and Genoway 1979; Lutterschmidt and Hutchison 1997b; Zhang and Kieffer~~  
198 ~~2014~~); (~~Becker and Genoway 1979; Lutterschmidt and Hutchison 1997b; Zhang and Kieffer~~  
199 ~~2014~~). When LOE was reached, the temperature was recorded, then the fish was immediately  
200 removed from the chamber and sampled. To ensure any changes found in the CT<sub>max</sub> treatment

201 groups were not artefacts of the experimental design, a pilot study was run using an additional  
202 control group of fishes which underwent a sham CT<sub>max</sub> trial. These sham pilot trials included  
203 transferring fish to the CT<sub>max</sub> chamber, and holding them for a similar length of time of a typical  
204 CT<sub>max</sub> trial but without any further temperature increases before sampling. We did not find any  
205 significant changes to plasma endpoints when compared to the acclimated control fish.

206 *Sampling and ~~Haematologica~~Hematological analysis*

207 At the appropriate sampling period (acclimation or CT<sub>max</sub>), fish were anesthetized in 100  
208 mg · ml<sup>-1</sup> of tricaine methanesulfonate (TMS; Sigma-Aldrich E10521) buffered with sodium  
209 bicarbonate (200 mg · ml<sup>-1</sup>). Once the fish was anesthetized, a ~0.7mL blood sample was  
210 collected via caudal puncture using a 23g needle and 1 mL syringe. The fish was then  
211 euthanized, weighed and measured (total length; T<sub>L</sub>). The blood sample was spun in a mini  
212 centrifuge at 8500g for 5 min. The plasma was separated into duplicate 1.5mL tubes then frozen  
213 in a metal rack on dry ice, and stored at -80°C for future analyses.

214  
215 From one duplicate tube of plasma, plasma ions and lactate were measured.  
216 Concentrations of plasma sodium, chloride and potassium (Na<sup>+</sup>, Cl<sup>-</sup> and K<sup>+</sup>) were quantified  
217 using an electrolyte analyzer (Smartlyte; Diamond Diagnostics) and plasma lactate was  
218 quantified using a medical lactate meter (Lactate Statstrip; Nova Biomedical). From the  
219 remaining duplicate vial, plasma cortisol levels were measured via enzyme-linked  
220 immunosorbent assay (“ELISA”; Neogen kit # 402710). To expedite the solvent evaporation  
221 step, 40 samples were extracted at once, and a stream of nitrogen was provided to each sample  
222 with a length of PE50 catheter tubing connected to a 23g needle (barrel removed) which was  
223 pierced into silicone tubing header containing pressurized nitrogen gas (T. McCormack, Mount

224 Allison University, Canada; pers comm.). ELISAs were run according to manufacturer's  
 225 directions (using the ethyl acetate and HCl stop solution options), aside from the following  
 226 modification: to allow for smaller volume samples, starting volume of samples and all reagents  
 227 were decreased by half up to the point of the 1:100 dilution. Samples that were below the  
 228 detection limit were re-analyzed at a lower dilution (1:10) as available. ELISA plates were read  
 229 using a plate reader (Syngery HTX with Gen5 software; Biotek); sample concentrations were  
 230 calculated with the reader's software, using the plate standards, blank adjustment and accounting  
 231 for background absorbance. All standards, controls and samples were run in duplicate and  
 232 averaged- (duplicate variation was 16% ± 2% (mean ±SEM)).

### 233 *Statistical analyses*

234 The effect of temperature  $CT_{max}$  was assessed using a linear mixed effect model with  
 235 originating rearing tank as a random factor to avoid pseudoreplication (e.g.  $CT_{max} \sim$   
 236  $Acclimation\_Temperature + (1|Tank)$ ) (Bates et al. 2015). Similarly, the effects and interaction  
 237 of acclimation temperature and -treatment (i.e. acclimation control or at  $CT_{max}$ ) on plasma ions,  
 238 lactate and cortisol were also assessed using linear mixed effects models with originating tank as  
 239 a random factor (e.g.  $Sodium \sim Acclimation\_Temperature * Treatment + (1|Tank)$ ). For all tests  
 240 where interactions were significant, the effects of each fixed factor were then assessed in  
 241 isolation (Logan 2010). Where significant effects of a factor were found, differences across the  
 242 levels of each factor (e.g. 15 vs 25 vs 30°C for acclimation temperature) were determined post-  
 243 hoc with Tukeys tests (Hothorn et al. 2009). The effects of acclimation temperature and body  
 244 weight on  $CT_{max}$  were tested using and an ANCOVA (acclimation temtemperature = categorical  
 245 factor; body weight = continuous factor)(Logan 2010). The effect of body weight on  $CT_{max}$   
 246 within each temperature treatment was also assessed using linear regressions. The acclimation

247 response ratio (ARR) was also calculated for  $CT_{max}$  differences as  $ARR = \Delta CT_{max} / \Delta T_a$   
 248 Acclimation Temperature according to Claussen (1977). Furthermore, to determine the  
 249 relationship of acclimation temp and thermal tolerance, linear ( $CT_{max} = \text{Acclimation\_temp}$   
 250 Temperature) and hyperbolic ( $CT_{max} = I(\text{Acclimation\_temp Temperature}^2)$ ) regressions were  
 251 ~~fit~~run and then compared with an ANOVA- for best fit. All data were ~~assess~~assessed for  
 252 normality and outliers (Grubbs test). ~~For all~~All statistical ~~test an alpha of 0.05 was used and~~  
 253 ~~performing tests were performed~~ using R (Version 4.0.1) (R 2008)- and an alpha of 0.05.

254

## 255 **Results**

### 256 *Critical thermal maximum*

257 Critical thermal maxima ~~was~~were significantly different at each acclimation temperature,  
 258 with higher  $CT_{max}$  occurring at high warm acclimation temperatures (Fig 1;  $p < 0.001$  for linear  
 259 mixed effects model ~~and all post hoc comparisons~~). Overall, the mean  $CT_{max}$  in 25 and 30°C  
 260 acclimated fish were 2.7 and 5.6°C higher than  $CT_{max}$  at 15°C. Mean difference between  
 261 observed  $CT_{max}$  and respective acclimation temperatures were 16.7, 9.4 and 7.25°C for the 15, 25  
 262 and 30°C acclimation groups, respectively. The acclimation response ratio was highest when  
 263 comparing 25°C and 30°C acclimated fish (Table 1). The relationship between  $CT_{max}$  and  
 264 acclimation temperature was determined to be non-linear ( $CT_{max} = -0.55 * \text{Acc\_temp} + 0.021 * \text{Acc\_temp}^2 + 35.35$ ;  $p < 0.001$ ; Adjusted  $R^2 = 0.8714$ ). When assessed together with  
 265 acclimation temperature, body weight did not affect  $CT_{max}$  (Fig 2;  $p = 0.5$  for body weight;  $p =$   
 266  $0.015$  for acclimation temperature). Linear regressions of  $CT_{max}$  vs body weight within each  
 267 temperature group were also non-significant ( $p = 0.97, 0.16$  and  $0.53$  for 15, 25 and 30°C,  
 268 respectively; Figure 2).

269

270 *Plasma analyses*

271 Overall, the effects of acclimation temperature and treatment (acclimation control vs  
 272  $CT_{max}$ ) varied across plasma variables (Fig 3 and 4). No interactions between acclimation  
 273 temperature and treatment group were found for plasma sodium and chloride ( $p = 0.56$  and  $0.24$ ,  
 274 respectively), though interactions were found for potassium, lactate and cortisol ( $p = 0.03$ ,  
 275  $<0.001$  and  $0.04$ , respectively). Acclimation temperature was found to have significant  
 276 ~~effecteffects~~ on plasma sodium ( $Na^+$ ), potassium ( $K^+$ ), cortisol and lactate ( $p = 0.0013$ ,  $0.0016$ ,  
 277 for sodium and potassium and  $p < 0.001$  for lactate & cortisol) whereas temperature did not  
 278 affect chloride ( $Cl^-$ ) ( $p = 0.37$ ).

279 Within each plasma endpoint, the patterns of change across temperature and treatment  
 280 also differed. Plasma sodium (Fig 3i) was lower in  $25^{\circ}C$  acclimated fish compared to 15 and  
 281  $30^{\circ}C$  fish ( $p < 0.001$ ). Plasma chloride (Fig 3ii) did not vary significantly by temperature nor  
 282 treatment group. ~~Plasma potassium was increased following  $CT_{max}$ . In fish acclimated to~~ 15 and  
 283  $30^{\circ}C$ , ~~plasma potassium levels were significantly elevated at  $CT_{max}$~~  ( $p = 0.006002$  and  $p < 0.001$ ,  
 284 ~~25 vs 15 and 2530~~ $^{\circ}C$  respectively). Furthermore, ~~plasma potassium was highest~~ in fish  
 285 ~~acclimated to  $30^{\circ}C$  at  $CT_{max}$  was higher from acclimation group at both~~ ( $p = 0.006$  and  $p <$   
 286 ~~0.001, 30c vs 15 and 25~~ $^{\circ}$  ( $p = 0.001$  and  $p < 0.001$ , ~~C~~ respectively). Plasma lactate (Fig 4i)  
 287 increased with each temperature increase within both the acclimated and post- $CT_{max}$  fish ( $p <$   
 288  $0.001$  for all except  $p = 0.002$  for 25 vs  $30^{\circ}C$  ~~in~~ acclimated fish). Lactate was significantly  
 289 increased following  $CT_{max}$  at both 25 and  $30^{\circ}C$  compared to acclimated control fish ( $p < 0.001$   
 290 for both). Cortisol was increased at  $25^{\circ}C$  (Fig 4ii) and elevated in all temperature groups  
 291 following  $CT_{max}$  ( $p = 0.02$  at  $15^{\circ}C$ ,  $p < 0.001$  at 25 and  $30^{\circ}C$ ). Cortisol was highly variable in  
 292 fish acclimated to  $25^{\circ}C$  at  $CT_{max}$ ; this pattern persisted when analyses were re-run removing one

293 outlier ( $p < 0.001$ ), however ~~the~~ removing the outlier did not change the pattern of significant  
294 changes. The fold change in cortisol levels at  $CT_{max}$  from acclimated values was greatest at 25°C  
295 (Fold changes = 1.1, 5.71, 2.16 for 15, 25 and 30°C acclimation groups, respectively).

296

297

## Discussion

298

299 Juvenile Striped Bass ~~were able to survive~~survived in warm temperatures up to four  
300 weeks, ~~with little~~without disruption to stress hormones and plasma ions. Qualitatively, fish  
301 acclimated to 30°C did not have any ~~notable~~ changes to behaviour during acclimation and  
302 holding, nor were there mortalities in any acclimation groups. Furthermore, ~~little~~no change in  
303 resting hematology was found across acclimation temperatures, even following a four week  
304 acclimation period to 30°C. In contrast, Davis and Parker (1990) found increases in plasma  
305 chloride and cortisol when juvenile Southern Striped Bass were acclimated to 30°C for 8 days.  
306 Such changes in cortisol and plasma ions are typical in fish following stressful events (Barton  
307 2002). The lack of stress response found at this high temperature may suggest that Northern  
308 Striped Bass used in this study are better equipped to adapt to higher temperatures. ~~Similar trends~~  
309 ~~of greater thermal tolerance breadth in Northern populations has been documented in other~~  
310 ~~species and may be related to the typically greater seasonal temperature variation found at more~~  
311 ~~poleward latitudes (Sunday et al. 2011).~~Sunday et al. (2011) demonstrated that while thermal  
312 tolerance breadth increases, absolute upper thermal tolerance levels tend to decrease in more  
313 Northern population, which may be contrary to our findings. Future research should aim to  
314 investigate potential differences in tolerance limits between North and South Striped Bass. It  
315 should be noted that plasma lactate was elevated in 25 and 30°C fish (both acclimated and at

316  $CT_{max}$ ), and thus there was a possible switch to anaerobic processes, suggesting these  
317 temperatures may be outside the ideal temperature range for these fish: as they were not able to  
318 meet energy demands with aerobic processes. This aligns with ~~previous studies, which Cox and~~  
319 Coutant (1981) who found ideal growth temperatures to be more moderate (14-22°C) ~~(Cox and~~  
320 ~~Coutant 1981). Nonetheless, it-),~~ though this optimum may differ if fish were not provided a  
321 fully satiating diet.

322  
323 One potential confounding aspect of comparing tolerance limits within and across species  
324 is ~~obvious that~~ the length of acclimation. In the present study, fish were acclimated for at least 28  
325 days, while other studies on Striped Bass ~~can survive for many~~ used shorter acclimations,  
326 ranging from 8 days (Davis and Parker 1990) to 2 weeks at high temperatures (30°C). (Cook et  
327 al. 2006). In their discussion and critique of utilizing  $CT_{max}$ , Lutterschmidt and Hutchison  
328 (1997b) acknowledge that rate and length of acclimation is important to the resultant  $CT_{max}$  but  
329 suggest only that in ectotherms, thermal acclimation is rapid, occurring with hours to a few days.  
330 Beitinger and Bennett (2000) suggest acclimation of new thermal tolerance limits suggest this  
331 may range from 1 to 20 days. While the absolute definition of what precisely defines acclimation  
332 has long been discussed (Lagerspetz 2006), it generally thought of as physiological or  
333 behavioural changes related to a new environment (e.g. higher temperature tolerance), but the  
334 time course to acclimation may depend on which system is being investigated. There is no “gold  
335 standard” of how long fishes should be acclimated for thermal tolerance testing. Still, many  
336 studies in other fish species use longer acclimation times more similar to our period than shorter  
337 times used in other aforementioned Striped Bass papers (for example see acclimation time in  
338 Table 1). The difference acclimation time may explain some of the differences seen between the

339 present study and others. For example, the increased cortisol by Davis and Parker (1990), may be  
340 an artefact of the shorter (8 day) acclimation period if this was insufficient time to acclimate.  
341 Although it should be noted that similar tolerance limits to ours were found by Cooke *et al.*  
342 2006, suggesting that the 2 weeks used by Cooke *et al.* was sufficient acclimation to adjust  
343 thermal tolerance in these Northern fish. Without further information on the timeline of thermal  
344 acclimation in Striped Bass, it is difficult to determine whether the differences between Northern  
345 and Southern fish are valid or an artefact of methodology.

347 In addition to their ability to acclimate to warm waters, the upper acute thermal tolerance  
348 of juvenile Striped Bass increased with increasing acclimation temperatures. While  $CT_{max}$  is  
349 widely used in fishes, few other studies have used it to test thermal limits in Striped Bass, though  
350 our values generally coincide with these ~~previous~~previously published values. While the authors  
351 did not report mean  $CT_{max}$  directly, our  $CT_{max}$  values are close to the estimated values from  
352 regressions published by Cook et al. (2006), ~~though may be slightly lower at 30°C (Table 1).~~ As  
353 the fish used by Cook et al. (2006) are from the same population and rearing facility, similar  
354 thermal tolerances were expected. The slightly lower values at the higher temperatures may be  
355 due to experimental set-up or an artefact of differing statistical methods (regression values vs  
356 means). The pattern of change in  $CT_{max}$  across acclimation temperatures found in this study is  
357 surprising. Typically, the upper thermal limit will increase linearly as acclimation temperature  
358 increases, and then plateau as acclimation temperature approaches thermal maxima (Beitinger  
359 and Bennett 2000). Thus, we would expect the acclimation response ratio to decrease as the  
360 acclimation temperature passes the linear portion and approaches thermal maximum (see Table 1  
361 for ARR values from other fishes). ~~Yet our~~The fish species selected in this table represent a wide

362 range of taxonomic and ecological diversity, from ancient lineages of temperate sturgeon  
363 (*Acipenser brevirostrum*), to tropical modern Perciformes (*Therapon jarbua*), to a similar  
364 temperate sea-bass (*Dicentrarchus labrax*). Yet only the Striped Bass and Striped Bass hybrid  
365 show increased ARR at the highest temperature pairs. Our data show that the ARR is largest  
366 between the two highest temperatures, suggesting the scope for increase in acute maxima is still  
367 increasing. The ARR's found at higher temperatures in this study are also on the high end for a  
368 mid-latitude fish (Morley et al. 2019). Other studies examining *Morone* spp. thermal tolerance  
369 across similar temperatures used in this study have found linear relationships of  $CT_{max}$  to  
370 acclimation temperature, thus implying a constant ARR (Table 1), unlike our data. Given that  
371  $CT_{max}$  continued to increase with each increase in acclimation temperature (i.e. the relationship  
372 has not plateaued/nonlinear), it is possible that further increases to acute thermal maximum may  
373 be possible at even higher acclimation temperatures.

374

375 While upper thermal tolerance did increase with acclimation, indicators of physiological  
376 stress and acid-base changes at  $CT_{max}$  were present in fish acclimated to warmer temperatures.  
377 During a stressful event, such as a  $CT_{max}$  trial, disturbances to ion regulation following the  
378 cascade of stress hormones are common (~~Barton 2002; Farrell 2011~~);(Barton 2002; Farrell 2011).  
379 Thus, the increases in cortisol found at  $CT_{max}$  are to be expected, given the stressful nature of  
380 such an acute test. The modest fold change in cortisol pre/post  $CT_{max}$  found at 25°C was within  
381 the range of fold changes reported before/after confinement stress (~3 to 5 fold change) (~~Davis~~  
382 ~~and Parker 1990; Secor et al. 2000~~)(Davis and Parker 1990; Secor et al. 2000), but lower fold-  
383 changes were found at the other two acclimation temperatures. When compared to other types of  
384 stressors, much higher fold changes (~86-fold) in cortisol have been documented in Striped Bass

385 following acute salinity transfer (Madsen et al. 1994), though this may be related to the  
 386 additional role of cortisol in seawater readiness in addition to stress response (Evans et al. 2005).  
 387 It is surprising that the highest  $CT_{max}$  cortisol values were found in 25°C acclimated fish, not the  
 388 highest acclimation-~~highest~~ temperature, which we would expect to be the most stressful. This  
 389 may be an artefact of the variable nature of cortisol response found, especially at 25°C, or there  
 390 may ~~actually~~ be a depression of cortisol response occurring at the highest temperatures combined  
 391 with an acute stress. Furthermore, the highly variable cortisol at 25°C coincides with the highest  
 392 variability in  $CT_{max}$ , which could suggest increased variation across individuals at this moderate  
 393 temperature treatment. The cortisol response following stress at high temperatures may be tied to  
 394 an overall reduction to metabolism, which has been seen in other fishes (Kir et al. 2017; Kir and  
 395 Demirci 2018; Kir 2020), should be investigated further.

397 Evidence of plasma ion disruption was also illustrated in the increase seen in plasma  
 398 potassium at  $CT_{max}$ , particularly in fish acclimated to 30°C. The apparent hyperkalemia at 30°C  
 399 ~~reflects~~ is mirrored by the ~~increase in~~ increased lactate ~~found at~~  $CT_{max}$  at the same acclimation  
 400 temperature. This pairing of changes ~~are~~ is common in fishes following stressful or strenuous  
 401 activities, in which the increase in lactate production from anerobic metabolism results in plasma  
 402 acidosis, -which in turn leads potassium to migrate into the plasma in order to close the anion gap  
 403 ~~(Wood et al. 1983; Kieffer et al. 1994)~~ (Wood et al. 1983; Kieffer et al. 1994). This was not seen  
 404 at  $CT_{max}$  in the lower temperature groups, which again suggests that while fish may survive at  
 405 these elevated temperatures for many weeks, it is likely to be less ideal than moderate  
 406 temperatures. While the other  $CT_{max}$  studies in striped bass did not measure plasma ions  
 407 ~~(Lutterschmidt and Hutchison 1997a; Cook et al. 2006)~~ (Lutterschmidt and Hutchison 1997a;

408 Cook et al. 2006), chloride fluctuation has been documented during recovery from confinement  
409 stress in Striped Bass and their hybrids (~~Davis and Parker 1990; Davis 2004~~)(Davis and Parker  
410 1990; Davis 2004). We did not find chloride fluctuations immediately following CT<sub>max</sub>, though  
411 ion disruption may not occur until many hours after the stressor (~~Davis and Parker 1990; Davis~~  
412 ~~2004~~)(Davis and Parker 1990; Davis 2004), thus it is possible changes in chloride may have  
413 been seen if fish were allowed to recover and monitored. Future studies should aim to investigate  
414 the time course of ion perturbation following temperature stress.

415

416 In contrast to Cook et al. (2006), we did not find any indication that thermal tolerance  
417 was inversely related to body size in juvenile Striped Bass. Generally, CT<sub>max</sub> would be expected  
418 to increase with body mass, likely owing to the lag of temperature transfer to the interior of a  
419 larger bodied animal (~~Beitinger and Bennett 2000; Ziegeweid et al. 2008~~)(Beitinger and Bennett  
420 2000; Ziegeweid et al. 2008). Cook et al. (2006) has suggested an inverse relationship of body  
421 weight to thermal tolerance. We found no indication that temperature tolerance is related to body  
422 size. Compared to Cook et al. (2006), our paper assessed the patterns of thermal tolerance change  
423 on a continuous basis across a wider range of sizes and generally larger size juveniles (52 – 102g  
424 in the present study; small = 21.8 g vs large = 34.4g by Cook et al. (2006)). Zhang and Kieffer  
425 (2014), found a positive-a relationship between thermal tolerance and body weight *within* small  
426 and large groups of Shortnose Sturgeons, *Acipenser brevirostrum* (Lesueur 1818 non Heckel  
427 1836), yet the smaller fish as a group had higher tolerance when compared to large fish  
428 categorically. It is possible that the inverse relationship exists in smaller juvenile Striped Bass as  
429 previously described, but the direction changes in larger juveniles. As previously mentioned, our  
430 CT<sub>max</sub> values were slightly lower than those found by Cook et al. (2006), which may support this

431 notion. ~~It has been suggested that thermal preference in wild Striped Bass may vary on a longer~~  
432 ~~seasonal (Coutant et al. 1984) and/or life history stage scale (Coutant 1986). Notwithstanding,~~  
433 ~~both the current study and Cook's previous work on body size assess these trends over a small~~  
434 ~~subsection of juvenile Striped Bass sizes; future investigation into this phenomenon should~~  
435 ~~assess trends across a larger size range.~~

436  
437 As a wide-ranging, migratory fish, the natural thermal regimes of Striped Bass vary  
438 greatly across their range. In addition to changes in thermal regimes, Northern Striped Bass are  
439 more migratory and typically utilize saline stretches of their environment more than their  
440 Southern counterparts (Secor et al. 2000; Conroy et al. 2015). The Shubenacadie Striped Bass  
441 used in the present study are one of the most Northern populations of the species. Furthermore,  
442 the Shubenacadie River is a tidal bore system (COSEWIC 2012) and is connected to the Bay of  
443 Fundy, which boasts the highest tides in the world (Desplanque and Mossman 2001). As such,  
444 this population faces extremes in both temperature and salinity fluctuations on and hourly and  
445 seasonal scale. The temperatures used in this study span a range of temperatures that may be  
446 found naturally in this system: spring spawning occurs around 15°C (COSEWIC 2012) and  
447 summer temperatures in the estuary are expected to be around 22°C (Duston et al. 2004). In  
448 2020, this population experienced an abnormal summer fish kill, purportedly related to unusually  
449 warm temperatures (Withers 2020). With rising global temperatures, understanding the  
450 processes with limit tolerance to these fluctuations will become increasingly important.

451  
452 The ability of juvenile Striped Bass to acclimate to increased temperatures is  
453 demonstrated in this paper. Few physiological disruptions were found at acclimation, although

454 ~~increase~~increased stress hormones and likely anerobic metabolism occurred at  $CT_{max}$  at the  
455 highest temperatures. Nonetheless, juveniles were able to live at 30°C for multiple weeks, which  
456 is only 1.7°C away from the  $CT_{max}$  measured of fish acclimated to 15°C. This impressive ability  
457 for a temperate fish to withstand warm temperatures may have ecological ramifications as it  
458 provides a competitive edge in the face of global temperature change. Better understanding is  
459 needed to determine the mechanisms which underpin temperature tolerance, including the  
460 potential of heritable genetic traits. Furthermore, as a species which inhabits a wide range of  
461 temperature regimes, future research should aim to explore the difference in temperature  
462 tolerance across their range.

463

464

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- 648

649

**Table**

650

**Table 1.** Comparison of Acclimation Response Ratios (ARR) across multiple fish species and acclimation temperatures ( $T_a$ ).

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“Relationship” describes the published (or implied) relationship of thermal maximum to acclimation temperatures. Multiple rows

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within a study represent ARR for different temperature intervals (as denoted by the “upper” and “lower”  $T_a$ ). Where not stated in

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original publication, ARR was calculated from selected  $T_a$  and  $CT_{max}$  values as  $ARR = \Delta CT_{max} / \Delta T_a$  according to Claussen (1977).

654

Species	Relationship (CT <sub>max</sub> α T <sub>a</sub> )	Acclimation (weeks)	N (fish/ group)	T <sub>a</sub> (°C)		Δ T <sub>a</sub> (°C)	Δ CT <sub>max</sub> (°C)	ARR	Study
				Lower	Upper				
<i>Morone saxatilis</i>	Non-linear	4	7 to 9	15	25	10	2.69	0.27	This study
				25	30	5	2.89	0.58	
<i>Morone saxatilis</i>	Linear	2	5	15	25	10	4.2	0.42	Cook et al. (2006) †
				25	30	5	2.1		
<i>Morone saxatilis</i> x <i>M. chrysops</i>	Linear	5 to 7	2	12.2	23	10.8	5.3	0.49	Woiwode and Adelman (1992)
				23	29.2	6.2	3.2	0.52	
<i>Dicentrarchus labrax</i>	Linear	5	15	15	20	5	1.6	0.32	Dülger et al. (2012)
				20	25	5	1.1	0.22	
<i>Fundulus heteroclitus</i> (Northern)	Non-linear	3	15	12.4	26.5	14.1	6.8	0.48	Fangue et al. (2006)
				26.5	32.1	5.6	1.4	0.25	
<i>Fundulus heteroclitus</i> (Southern)	Non-linear	3	15	12.4	26.5	14.1	6.5	0.46	Fangue et al. (2006)
				26.5	32.1	5.6	1	0.18	
<i>Acipenser brevirostrum</i>	Linear	4	8	10	15	5	3.9	0.78	Zhang and Kieffer (2014)
				15	20	5	1.3	0.26	
<i>Etroplus suratensis</i>	Linear	Unknown	Unknown	20	25	5	0.75	0.15	Rajaguru (2002)
				25	30	5	2.5	0.5	
				30	35	5	0.5	0.1	
<i>Therapon jarbua</i>	Linear	Unknown	Unknown	20	25	5	0.75	0.15	Rajaguru (2002)
				25	30	5	1.5	0.3	
<i>Therapon jarbua</i>	Linear	Unknown	Unknown	30	35	5	0.75	0.15	Rajaguru (2002)
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† Authors did not include mean CT<sub>max</sub> values per T<sub>a</sub>, thus the slope of the linear regression is used to represent the overall ARR

## Figure Captions

657

658

659 **Fig1** Box plot of critical thermal maxima ( $CT_{max}$ ) of juvenile Striped Bass, *Morone*  
 660 *saxatilis*, acclimated to different temperatures. Letter designations denote significant differences  
 661 between temperature acclimation groups.

662

663 **Fig2** Critical thermal maxima ( $CT_{max}$ ) of juvenile Striped Bass, *Morone saxatilis*,  
 664 acclimated to various temperatures according to body weight. Fish were acclimated to 15C ( $\circ$ ),  
 665 25°C ( $\blacksquare$ ), or 30°C ( $\nabla$ ) for 4+ weeks prior to undergoing  $CT_{max}$  trials. Lines reflect linear  
 666 regressions within each temperature, though none of these regressions were found to be  
 667 significant. Effects of acclimation temperature (“Temp”), Body Weight and their interaction  
 668 (“Int”) were determined via ANCOVA.

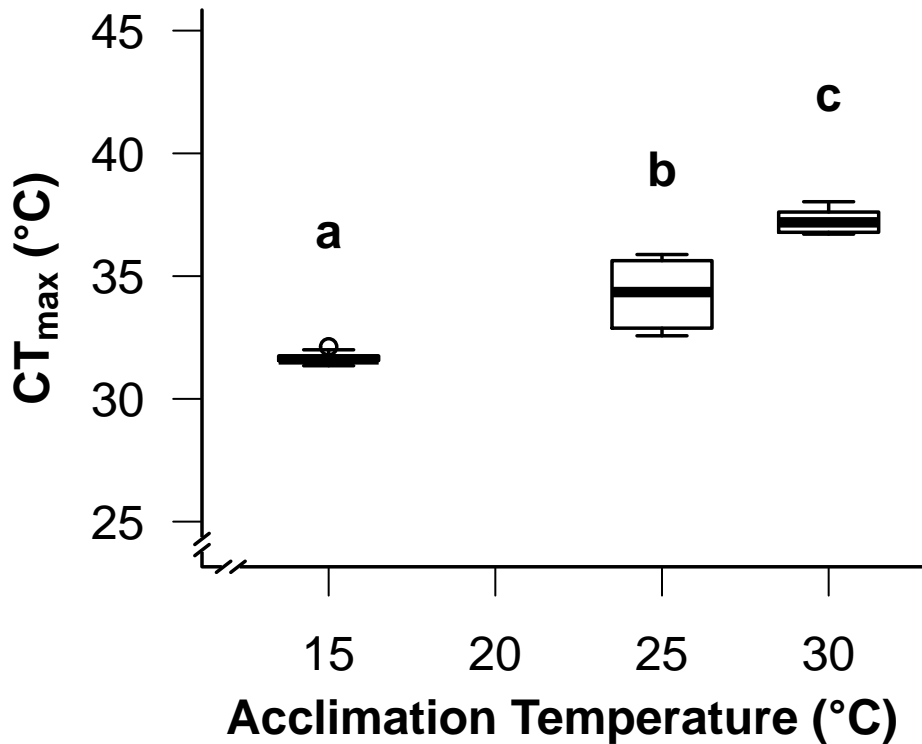
669

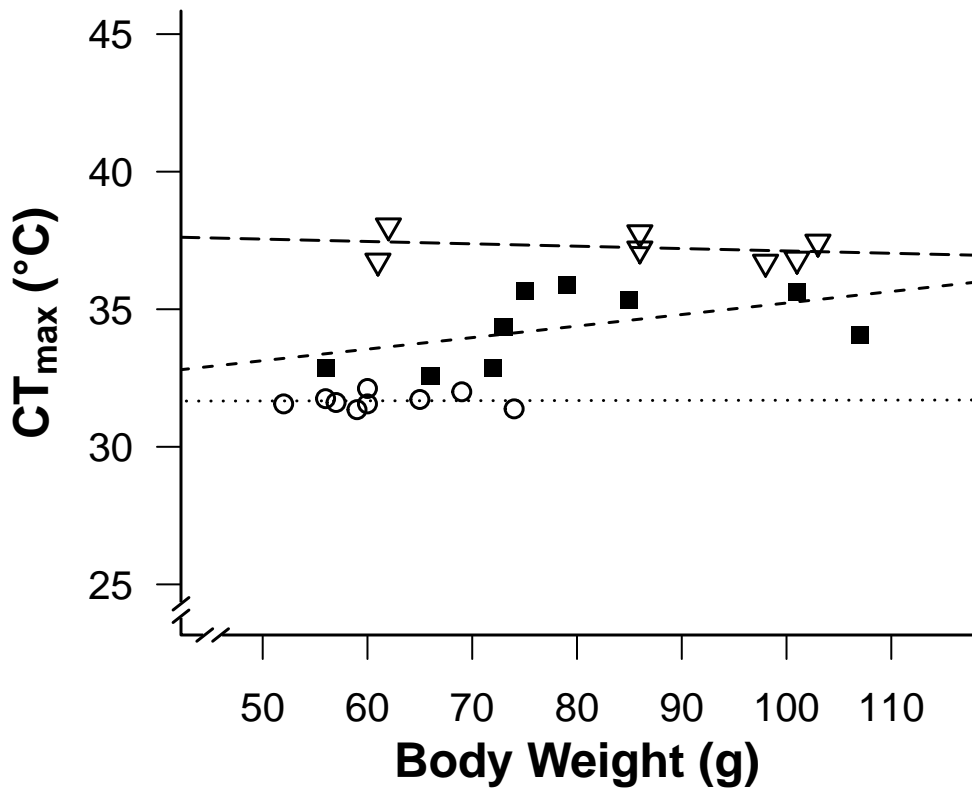
670 **Fig3** Mean ( $\pm$ SEM) plasma ion concentrations (sodium, chloride & potassium in i, ii, &  
 671 iii, respectively) in juvenile Striped Bass, *Morone saxatilis*, acclimated to various temperatures.  
 672 Fish were sampled as following: after 4 weeks acclimated to appropriate temperature ( $\triangle$ )( $\blacktriangle$ ) or  
 673 immediately following critical thermal maxima ( $CT_{max}$ ) trial ( $\blacksquare$ ). ~~Letter~~( $\square$ ). Lowercase letter  
 674 designations denote significant differences across temperature within the  $CT_{max}$  trial group.  
 675 Uppercase letter designations designate differences across temperature, regardless of  $CT_{max}$   
 676 treatment (no interaction). An asterisk (\*) denotes a significant difference in  $CT_{max}$  from  
 677 respective acclimation control. ~~A pound sign (#) denotes an overall difference between~~  
 678 ~~acclimation temperatures.~~ Statistical differences were determined via linear mixed effects models  
 679 followed by pairwise comparisons determined via Tukey’s post hoc tests as appropriate.

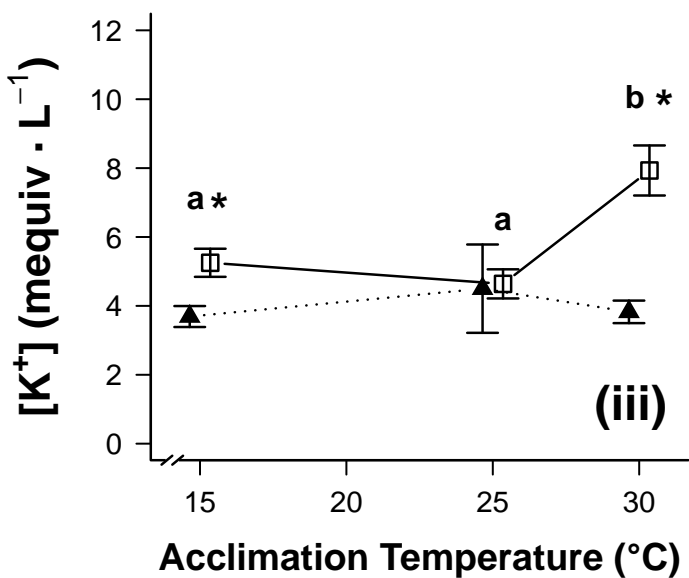
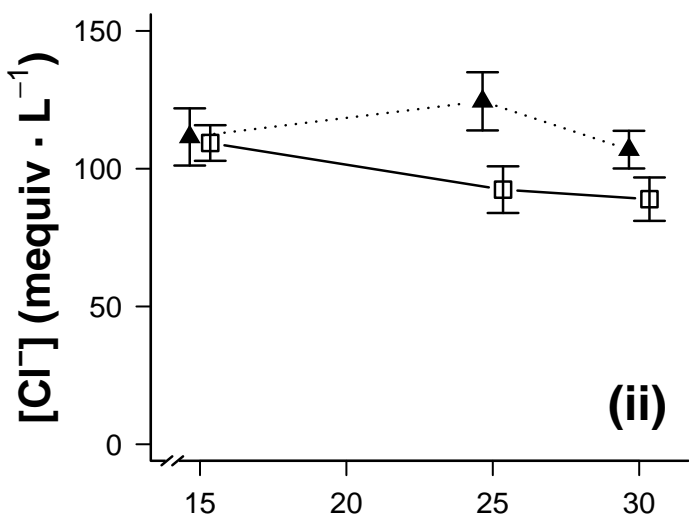
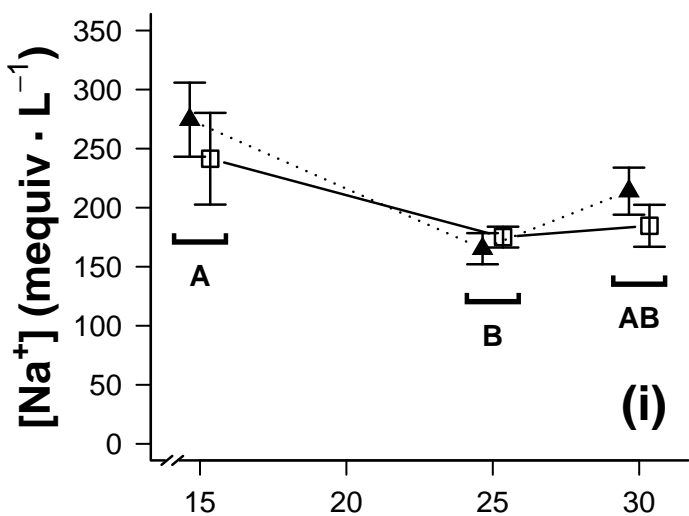
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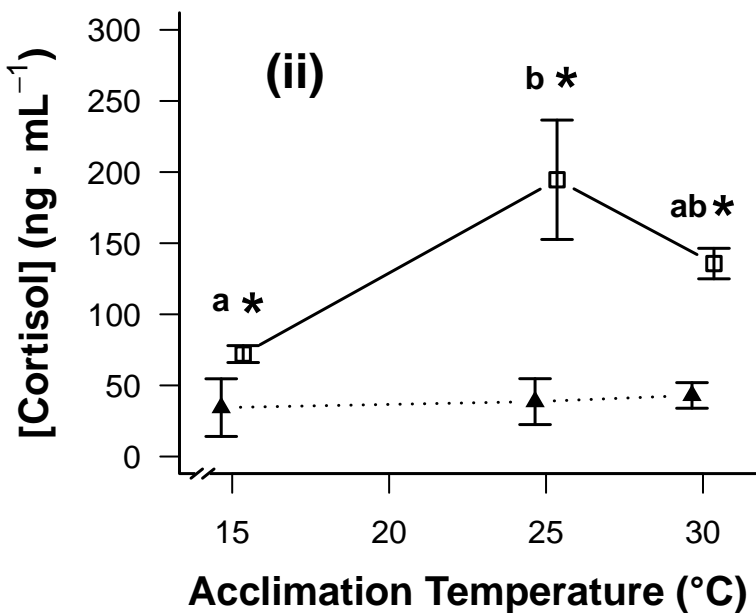
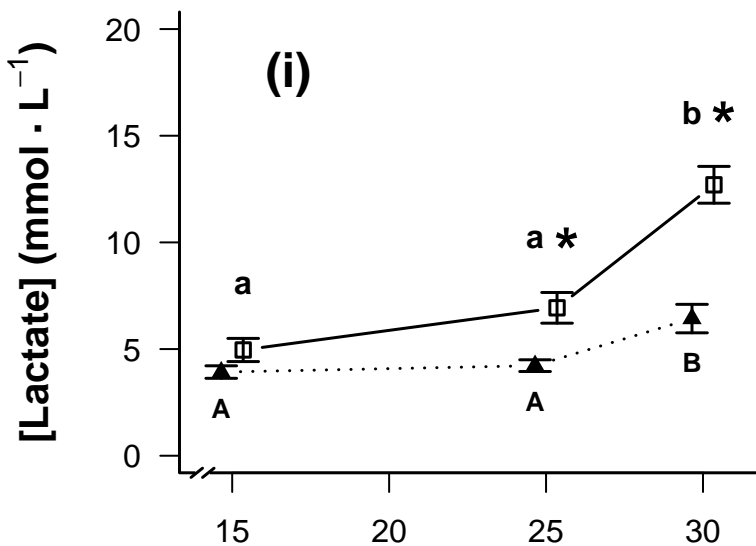
681 **Fig4** Mean ( $\pm$ SEM) plasma lactate and cortisol (i & ii, respectively) in juvenile Striped  
682 Bass, *Morone saxatilis*, acclimated to various temperatures. Fish were sampled as following:  
683 after 4 weeks acclimated to appropriate temperature ( $\triangle$ )( $\blacktriangle$ ) or immediately following critical  
684 thermal maxima (CT<sub>max</sub>) trial ( $\blacksquare$ ). ~~Upper case letter designations denote significant differences~~  
685 ~~across temperature within both acclimation control and CT<sub>max</sub> trial groups individually. Lower~~  
686 ~~case( $\square$ ). Lowercase~~ letter designations denote significant differences across temperature within  
687 the CT<sub>max</sub> trial group. ~~Uppercase letter designations denote significant differences across~~  
688 ~~temperature within the acclimation trial group.~~ An asterisk (\*) denotes a significant difference in  
689 CT<sub>max</sub> from respective acclimation control. Statistical differences were determined via linear  
690 mixed effects models followed by pairwise comparisons determined via Tukey's post hoc tests  
691 as appropriate.

692









1

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**Penny, F. M.; Pavey, S. A.**

**2021-04-21**

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