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Slow heating rates increase thermal tolerance and alter mRNA HSP expression in juvenile white sturgeon (*Acipenser transmontanus*)

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ABSTRACT

Freshwater fish such as white sturgeon (*Acipenser transmontanus*) are particularly vulnerable to the effects of anthropogenically induced global warming. Critical thermal maximum tests (CT_{max}) are often conducted to provide insight into the impacts of changing temperatures; however, little is known about how the rate of temperature increase in these assays affects thermal tolerance. To assess the effect of heating rate (0.3 °C/min, 0.03 °C/min, 0.003 °C/min) we measured thermal tolerance, somatic indices, and gill Hsp mRNA expression. Contrary to what has been observed in most other fish species, white sturgeon thermal tolerance was highest at the slowest heating rate of 0.003 °C/min (34.2 °C, and CTmax of 31.3 and 29.2 °C, for rates 0.03 and 0.3 °C/min, respectively) suggesting an ability to rapidly acclimate to slowly increasing temperatures. Hepatosomatic index decreased in all heating rates relative to control fish, indicative of the metabolic costs of thermal stress. At the transcriptional level, slower heating rates resulted in higher gill mRNA expression of *Hsp90a, Hsp90b,* and *Hsp70*. *Hsp70* mRNA expression was increased in all heating rates relative to controls, whereas expression of *Hsp90a* and *Hsp90b* mRNA only increased in the two slower trials. Together these data indicate that white sturgeon have a very plastic thermal response, which is likely energetically costly to induce. Acute temperature changes may be more detrimental to sturgeon as they struggle to acclimate to rapid changes in their environment, however under slower warming rates they demonstrate strong thermal plasticity to warming.

1. Introduction

Climate change is predicted to increase mean water temperatures and the frequency of extreme weather events, with northern latitudes being disproportionately affected by these changes (Hoegh-Guldberg et al., 2018; Vincent et al., 2015). The impacts of anthropogenic global warming are especially significant for ectotherms, such as fish, because their body temperature is directly influenced by the temperature of their environment. For ectotherms, temperature is considered an "abiotic master factor" as it plays a critical role in determining population range, physiology, community dynamics, and survival (Boltaña et al., 2017; Crozier et al., 2008; Li et al., 2015; O'Gorman et al., 2016; Pankhurst and Munday, 2011; Schulte, 2015). As such, it is vital for conservation efforts to better evaluate how ectotherms respond to the magnitude and rate of temperature change.

Critical thermal tests were developed to assess the thermal limits of organisms (Cowles and Bogert, 1944); however, their ecological relevance has been questioned as the rates of temperature increase used often exceed those seen in nature, with the conventional rate for critical thermal maximum test (CT_{max}) being 0.3 °C/min (Beitinger and Lutterschmidt, 2011). When slower heating rates are used, organisms often have a lower thermal tolerance than has been predicted by conventional CT_{max} studies (Illing et al., 2020; Kovacevic et al., 2019; Mora and Maya, 2006). This discrepancy could indicate that current approximations of thermal tolerance based upon conventional CT_{max} estimates may overestimate thermal tolerance in nature (Becker and Genoway, 1979; Illing et al., 2020; Kovacevic et al., 2019; Mora and Maya, 2006). When organisms undergo conventional CT_{max} tests, upper thermal tolerance is thought to be limited by either cardiac or neurological failure as a result of limited oxygen supply (Andreassen et al., 2022; Christen et al., 2018). At slower heating rates, upper thermal tolerance may be limited by the gradual onset of oxidative stress and damage from mitochondrial dysfunction that occurs when organisms spend too long at temperatures where the rate of cellular damage exceeds the rates of repair (Rezende

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et al., 2014). The potentially contrasting physiological impacts and responses to different heating rates are especially relevant when considering the acute (e.g. extreme weather events like heatwaves) or chronic (e.g. mean temperature increases) effects of climate change, as laboratory measurements are used to help inform conservation efforts.

Modifications to heat shock protein (Hsp) mRNA expression are an indicator of stress at the transcriptional level. Hsps act as chaperones, helping cells cope with the effects of thermal stress by binding to proteins to stabilize them and refolding or breaking down damaged proteins, to mitigating damage caused by environmental stressors (Chen et al., 2018; Garbuz and Evgen'ev, 2017; Tomanek and Somero, 2000). The magnitude of Hsp upregulation often positively correlates with thermal tolerance (Krebs and Feder, 1997; Tomanek and Somero, 2000; Bugg et al., 2020). Together these metrics provide an indication of how fishes respond to thermal stress at the whole-organism, organ, and transcriptional level and may highlight the effects of different heating rates.

Responses to environmental change can vary between species, populations, and individuals. Thus, understanding inter-individual variation is crucial for determining how populations may respond to changing climates (Schulte, 2014). Identifying the tolerances and interindividual variation of fish to environmental changes, like thermal stress, and their respective physiological characteristics, can highlight what molecular mechanisms may promote increased survival. Examining physiological responses to environmental change in the gill, at a key interface between the organism and the external environment, can provide insights into individual performance as environments conditions rapidly shift (Akbarzadeh et al., 2018; Gilmour and Perry, 2018; Alfonso et al., 2021; Bugg et al., 2023). Ultimately, by measuring these molecular and physiological changes in individuals with various tolerance levels during a thermal stressor, like CT_{max}, we can highlight phenotypes that will help fish persist in the face of environmental change, particularly important for species of conservation concern.

In Canada, many populations of white sturgeon (*Acipenser transmontanus*) are endangered (COSEWIC, 2003; Fisheries and Oceans Canada, 2023), but despite their perilous conservation status, little is known about how they respond to thermal stress. Anthropogenic climate change further hampers recovery efforts as white sturgeon will increasingly experience temperatures that have the potential to induce thermal stress. Moreover, there are no known studies that have used slower, potentially more ecologically relevant heating rates, to assess their upper thermal tolerance (Hildebrand et al., 2016). White sturgeon are particularly susceptible to anthropogenic impacts due to their life history and requirement for different habitats across their life stages (Auer, 1996; Boreman, 2005; Jager et al., 2001; Parsley and Kappenman, 2000). Throughout their early life stages there are multiple bottlenecks to survival and consequently high rates of mortality, making survival in these critical periods particularly important for population persistence (Hildebrand et al., 2016). As such, minimizing mortality during the first year of life is critical to white sturgeon population recovery (Gross et al., 2002). To do so, it is paramount to gain a better understanding of environmental factors such as temperature that affect white sturgeon during this precarious period.

To address these knowledge gaps, the thermal tolerance of juvenile white sturgeon was examined using three different heating rates during CT_{max} trials. Currently, juvenile white sturgeon experience acute warming rates of up to 0.005 °C/min with the potential for increases in the severity and duration of heatwaves as climates intensify (Earhart et al., *In Review*; watteroffice.ec.gc.ca, Hydrometric Data for Nechako River at Vanderhoof (08JC001)). Thus, of the three heating rates that were tested, one was the conventional rate used often in previous thermal tolerance research (0.3 °C/min), one mimicked natural temperature changes that the white sturgeon would experience in their native river system (0.003 °C/min), and the other was an intermediate rate (0.03 °C/min; Beitinger, 2000).

We hypothesized that white sturgeon thermal tolerance would be affected by heating rate. As has been observed in other fish species, the thermal tolerance of juvenile white sturgeon was predicted to decrease at slower heating rates (Becker and Genoway, 1979; Kovacevic et al., 2019). As somatic indices may be impacted by temperature with physiological consequences, this study sought to characterize the effects of different warming rates on condition factor, hepatosomatic index (HSI), cardiosomatic index (CSI), and craniosomatic index. Finally, gill samples were taken from the bottom eight and the top eight performers (individuals with the lowest and highest CT_{max} values, respectively) during each of the CT_{max} trials to measure mRNA expression of Hsps (*Hsp 47*, *90a*, *90b,* and *70,*) as it was hypothesized that heating rate would affect Hsp expression. Due to their role in thermal tolerance and thermal stress responses, Hsp expression was predicted to be upregulated following the CT_{max} trials relative to controls, and in higher performing individuals versus low performers.

2. Methods

2.1. White sturgeon broodstock and holding

White sturgeon were acquired through wild broodstock spawning at the Nechako White Sturgeon Recovery Initiative (NWSRI) in Vanderhoof, BC. At 60 days post hatch (corresponding to August 2020), 300 individuals were transported to the Department of Zoology, University of British Columbia. Upon arrival, fish were randomly divided and held for two weeks in two large flow-through tanks (700L) maintained under constant conditions (16:8h light-dark cycle; 15 °C). Fish were fed bloodworms (Hikari Bio-Pure Frozen Bloodworms) twice a day until satiation. For the duration of the holding period and during the CT_{max} experiment, water was kept oxygen-saturated (DO% > 90), water quality was monitored daily and water changes were conducted when nitrate levels exceeded 10 ppm and/or ammonia levels exceeded 0.5 ppm. This study was conducted in accordance with the guidelines set by The University of British Columbia Animal Care and Use Committee and approved under the permit number A19-0284.

2.2. CT_{max} trials and sampling procedures

White sturgeon were randomly selected from the two holding tanks where they were held at a density of approximately 0.5 fish per liter. In each of the CT_{max} trials, temperature was increased at a constant rate of either 0.3 °C/min, 0.03 °C/min, or 0.003 °C/min until a loss of equilibrium (LOE) occurred, which was defined as inability to right themselves after two consecutive tail prods or when dorsal lateral orientation was lost (Bugg et al., 2020; Deslauriers et al., 2016). CT_{max} was recorded as the water temperature at LOE. Once fish attained LOE, they were removed from the aquarium and euthanized with 200 mg/L MS-222 buffered with 400 mg/L sodium bicarbonate.

Prior to the first trial, we randomly selected both control (20) and CT_{max} (45) sturgeon from their holding tanks, and transported both groups to the experimental area where CT_{max} was conducted. Following transport, experimental fish were placed into the CT_{max} arena. Immediately (for control fish) and following CT_{max}, sturgeon were euthanized as described above and sampled blotting them dry with kimwipes to remove excess water and then measuring total mass (to nearest 0.01 g; Mettler Toledo, New Classic SG) and length (to nearest 1 mm). Gill samples were then immediately removed from control sturgeon as well as the top eight (the last eight fish to reach CT_{max}) and bottom eight performers (the first eight fish to reach CT_{max}) from each each CT_{max} trial and placed into RNA*later* (Thermofisher Scientific, Waltham, USA. Brain, liver, and heart were dissected out and weighed (to nearest 0.001 g; Sartorius CP124S) and the somatic index for each was calculated ([tissue mass/body mass] x 100). Condition factor was calculated (Fulton, 1904; Nash et al., 2006) using the following formula:

$$Condition\ factor = \frac{Whole\ body\ mass\ (g) \times 10^5}{Total\ length\ (mm)^3}$$

Five trials (45 fish per trial) were run sequentially over the course of two weeks. Three CT_{max} heating rates were used where temperature was increased at 0.3 °C/min (taking 1 h total), 0.03 °C/min (taking 8 h total), and 0.003 °C/min (taking 4.5 d total). Because of the possibility of diurnal effects on thermal tolerance, an additional two trials (second 0.3 °C/min and third 0.3 °C/min) were conducted at different times of the day to ensure CT_{max} and Hsp expression did not differ (Healy and Schulte, 2012; Lankford et al., 2003).

For all trials, fish were randomly selected and evenly divided between three aquaria (15L) that were placed within a larger water table (200L). For the first three trials (first 0.3 °C/min, 0.03 °C/min, and 0.003 °C/min), 45 fish were used (15 fish per replicate tank). For the final two trials (second 0.3 °C/min and third 0.3 °C/min) 30 fish were used (10 fish per replicate tank). Fish were weighed (nearest 0.01g; Mettler Toledo, New Classic SG) and total length was measured (nearest mm) prior to the start of CT_{max}. The fish were given 1 h to recover from handling after being placed in the replicate tanks before the CT_{max} trial started. The water in the water table was aerated using air stones and it was circulated using multiple water pumps (VicTsing 400GPH) to insure adequate mixing. For the 0.3 °C/min trials, the water in the water table was heated directly by immersed heater sticks (finnex TITANIUM 300+) and the heating rate was monitored using multiple temperature probes (HANNA checktemp 1). For the 0.03 °C/min and 0.003 °C/min trials, an additional sump (200L) was used to double the total water volume of the system, to insure water quality over the longer CT_{max} trials and to allow for slower heating rates. Temperature controllers (Fisher Scientific Traceable Digital Temperature Controller) were used in conjunction with heater sticks to slowly increase temperature at the respective rates. Temperature was monitored using temperature probes and temperature logger (HOBO Tidbit MX2203) in each aquarium. For the 0.003 °C/min trial, an infrared security camera (geeni GNC-CW020) was used to monitor the fish 24 h a day. Prior to the start of the trials, food was withheld for 12 h, however if the trial lasted more than 12 h (i.e. the 0.003 °C/min trial) then bloodworms were offered two times a day.

2.3. mRNA expression

Gill samples in RNA*later* were left at room temperature (20 °C) for 24 h and then transferred to a −80 °C freezer. Sample order was randomized for all RNA analysis. Tissues were homogenized using a Bullet Blender® 24 (Next Advance, Inc.), and RNA was isolated using a Qiagen RNeasy® Mini Kit in accordance with the manufacturer's instructions (Qiagen RNeasy® Mini Handbook). A Nanodrop (Nanodrop, 2000, Thermofisher Scientific, Waltham, USA) was used to determine total RNA quantity and quality with a threshold of 1.8, for both 260/230 and 260/280 measurements. Total RNA was stored at −80 °C until further analysis.

Complementary DNA (cDNA) was synthesized from 1 μg of DNAse-treated RNA using a qScript cDNA synthesis kit (Quantabio; Beverly, Massachusetts) according to the manufacturer's instructions. Synthesis was conducted using a SimpliAmpThermal Cycler (Thermo Fisher; Waltham, Massachusetts) with cycling conditions of 1 cycle of 22 °C for 5 min, 1 cycle of 42 °C for 30 min and 1 cycle of 85 °C for 5 min and hold at 4 °C. Following synthesis, cDNA samples were stored at −20 °C.

Expression of mRNA in the samples was measured using real time quantitative polymerase chain reaction (qPCR). Forward and reverse primers were designed for the reference genes *RPS18* and *RSP6*, and the target genes *Hsp47, Hsp90a, Hsp90b, Hsp70* (Table 1) based on an annotated white sturgeon liver transcriptome (Doering et al., 2016). *RSP6* and *RPS18* were selected as reference genes as they displayed stable expression across all treatments. All reactions contained 0.075 μL of both the forward and reverse primers, 7.5 μL of SYBR Green Mastermix (Thermofisher Scientific), 5.85 μL of ultrapure H_2O, and 2 μL of cDNA. For both reference genes, cDNA was undiluted 1:1 with nuclease-free water and for the target Hsp genes a 1 cDNA:10 nuclease-free water dilution was used. The qPCR was performed with a BIO-RAD CFX96 Real-Time System on a 96-well plate. To measure mRNA expression, the following protocol was used: denaturation at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 15s, annealing at 60 °C for 30s, and extension at 72 °C for 30s. The qPCR was followed by conducting a melt curve to ensure that only one product was produced. Expression of the genes of interest were normalized to the reference genes (*RSP6* and *RPS18*) using the Vandesompele method and expressed as a relative change compared the controls (Vandesompele et al., 2002).

2.4. Statistical analysis

All statistical analyses were performed using R Studio (Version 1.1.423) and alpha was set at 0.05 throughout (R Core Team, 2022). Prior to analyzing the dataset, the effect of aquaria on CT_{max} for each heating rate was assessed using a one-way ANOVA with tank ID (A, B, or C) as the fixed factor. There was no effect of tank on CT_{max} for any of the heating rates, so effect of aquaria was excluded as a random effect. The CT_{max}, Hsp expression, and somatic indices of the three 0.3 °C/min trials were assessed using a one-way ANOVA. There was no difference between the three 0.3 °C/min trials, conducted to assess diurnal effects, so the results of the trials were pooled.

Each of the above measurements were analyzed using a one-way ANOVA, followed by a *post-hoc* Tukey HSD with heating rate as the factor (control, 0.3 °C/min, 0.03 °C/min, or 0.003 °C/min). Additionally, each variable (except CT_{max}) was analyzed using a two-way ANOVA, followed by a *post-hoc* Tukey HSD, with CT_{max} performance (high performer or low performer) and heating rate (0.3 °C/min, 0.03 °C/min, or 0.003 °C/min) as factors. Assumptions of normality and homogeneity of variances were assessed using the Shapiro-Wilks and Levene's tests to assess the normality of data and homogeneity of variance, respectively. Further, residual plots were visually inspection to assess the distribution of data and Hsp data was log 2 transformed. All data met assumptions for these analyses. All figures were created using ggplot2 (Wickham, 2016).

3. Results

3.1. Effect of heating rate on thermal tolerance

There were no diurnal effects on CT_{max} when fish were heated at 0.3 °C/min ($p > 0.05$). Thermal tolerance was significantly affected by heating rate ($F_{2,135} = 515.2$, $p < 0.0001$; Table 2; Fig. 1), with slower

Table 1
Forward and reverse primers and their efficiencies in gill tissue in white sturgeon.

Gene	Forward	Reverse	Efficiency
RPS6	GGACAGGTTGAAGAGCTTGC	ATCATCAAGAAGGGCGAGAA	93%
RSP18	TCTCTCCAGATCCTCACGCA	AAGGACGGCAAATACAGCCA	86%
Hsp47	GACTCCAACGCCTTCAAGAG	TGTGATCATGGCTGAGAAGC	100%
Hsp90a	GCAGAGGTTCTCGAACTTGG	AGACCCTGGTGTCTGTGACC	93%
Hsp90b	GCAACTTGGTCCTTGCTCTC	AGCTCTCAGTCTGGGGATGA	89%
Hsp70	GAGAGGCTCATTGGAGATGC	AAACAGTGTTGCTGGGGTTC	100%

Table 2
Somatic indices of juvenile white sturgeon in control conditions or one of three heating rates (0.3 °C/min 0.03 °C/min and 0.003 °C/min). The average of each somatic index with standard error and sample size are provided. Treatments that differ from controls are indicated with an asterisk (*) at alpha <0.05.

Heating rate	
Condition factor	
Control (n = 20)	0.4124 ± 0.010 (n = 20)
0.3 (n = 105)	0.4440 ± 0.006 (n = 105)
0.03 (n = 45)	0.4148 ± 0.008 (n = 45)
0.003 (n = 45)	0.3920 ± 0.008 (n = 45)
Craniosomatic index	
Control	0.0076 ± 0.0004 (n = 20)
0.3	0.0071 ± 0.0003 (n = 45)
0.03	0.0076 ± 0.0003 (n = 45)
0.003	0.0074 ± 0.0003 (n = 45)
Cardiosomatic index	
Control	0.0034 ± 0.0003 (n = 20)
0.3	0.0031 ± 0.0001 (n = 45)
0.03	0.0033 ± 0.0002 (n = 45)
0.003	0.0029 ± 0.0002 (n = 45)
Hepatosomatic index	
Control	0.0199 ± 0.0013 (n = 20)
0.3	0.0160 ± 0.0007 (n = 45) *
0.03	0.0164 ± 0.0007 (n = 45) *
0.003	0.0152 ± 0.0007 (n = 45) *

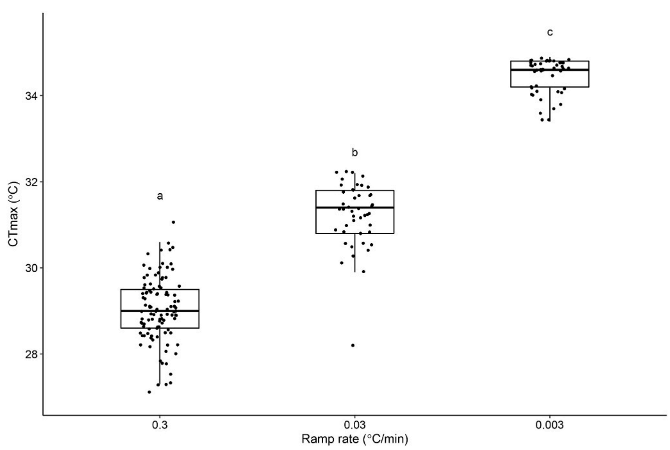

Fig. 1. CT_{max} values of 15 °C acclimated juvenile white sturgeon subjected to three different heating rates (0.3 °C/min, 0.03 °C/min, and 0.003 °C/min). Black dots represent individual data points and the boxplot represents group data. The horizontal line in the boxplot is the median, while the whiskers represent the maximum and minimum. Letters that differ indicate statistically significant at alpha = 0.05. N = 105 for 0.3 °C/min and N = 45 for 0.03 °C/min and 0.003 °C/min.

heating rates yielding significantly higher CT_{max} results. Average CT_{max} increased by about 5 °C between the fast and slowest heating rates. Average CT_{max} in the conventional heating rate was 29.2 °C ± 0.24 °C and the trial lasted approximately 1.5 h. Average CT_{max} in the 0.03 °C/min trial was 31.3 °C ± 0.24 °C and the trial lasted approximately 8 h. Average CT_{max} in the 0.003 °C/min trial was 34.2 °C ± 0.15 °C and the trial lasted approximately 108 h (4.5 days). There was no mortality observed in any ramp rate during trials.

3.2. Effect of heating rate and performance on somatic indices

Averages for somatic indices are summarized in Table 2. There was no significant effect of heating rates on craniosomatic index or CSI ($p > 0.05$; Table 3). For the top and bottom performers, there was no significant effect of heating rate or performance – or an interaction effect between the two – for condition factor, craniosomatic index, CSI, or HSI

Table 3
Summary of a one-way ANOVA on the effect of heating rate on CT_{max}, somatic indices, and Hsp expression in juvenile white sturgeon. Asterisk (*) denotes significance at alpha <0.05.

Measurement	d.f.	F	p-value
CT_{max}	3	631.9	<0.001*
Condition factor	3	9.406	<0.001*
Craniosomatic index	3	0.726	0.538
Cardiosomatic index	3	1.011	0.39
Hepatosomatic index	3	4.547	0.00443*
Hsp47	3	3.256	0.0251*
Hsp90a	3	31.8	<0.001*
Hsp90b	3	55.83	<0.001*
Hsp70	3	298.9	<0.001*

($p > 0.05$ for all; Table 4). HSI differed significantly between all heating rates and controls ($F_{3,121} = 4.48$, $p < 0.01$) – controls had significantly greater HSI than sturgeon that experienced heating rates of 0.03 °C/min ($p < 0.05$) and 0.003 °C/min ($p < 0.01$; Table 3; Fig. 2). Condition factor was significantly affected by heating rate ($F_{2,153} = 8.23$, $p < 0.0001$; Fig. 3), with the lowest condition factor in the slowest warming group. However, condition factor did not differ significantly between controls and sturgeon exposed to different heating rate ($p > 0.05$ for all).

3.3. Effect of heating rate and performance on hsp mRNA expression

There were no diurnal effects on the expression of any Hsps measured when fish were heated at 0.3 °C/min ($p > 0.05$).

3.3.1. Hsp47

mRNA expression of *Hsp47* was significantly affected by heating rate ($F_{3,94} = 3.26$, $p < 0.05$; Fig. 4A). Sturgeon in the 0.003 °C/min group

Table 4
Summary of a two-way ANOVA on the effect of heating rate and performance on various somatic indices and Hsp expression in juvenile white sturgeon. Asterisk (*) denotes significance at alpha = 0.05.

Measurement	Variable	d. f.	F	p-value
Condition factor	Heating rate	2	6.677	0.002*
	Performance	1	0.031	0.860
	Heating rate × performance	2	1.631	0.203
Craniosomatic index	Heating rate	2	1.332	0.275
	Performance	1	1.418	0.241
	Heating rate × performance	2	0.285	0.753
Cardiosomatic index	Heating rate	2	1.032	0.365
	Performance	1	0.716	0.402
	Heating rate × performance	2	0.123	0.885
Hepatosomatic index	Heating rate	2	1.273	0.290
	Performance	1	0.001	0.979
	Heating rate × performance	2	0.635	0.535
Hsp47	Heating rate	2	6.474	0.003*
	Performance	1	2.484	0.119
	Heating rate × performance	2	2.703	0.074
Hsp90a	Heating rate	2	69.13	<0.001*
	Performance	1	0.266	0.608
	Heating rate × performance	2	1.62	0.205
Hsp90b	Heating rate	2	67.585	<0.001*
	Performance	1	1.029	0.314
	Heating rate × performance	2	0.213	0.809
Hsp70	Heating rate	2	285.806	<0.001*
	Performance	1	46.836	<0.001*
	Heating rate × performance	2	5.887	0.004*

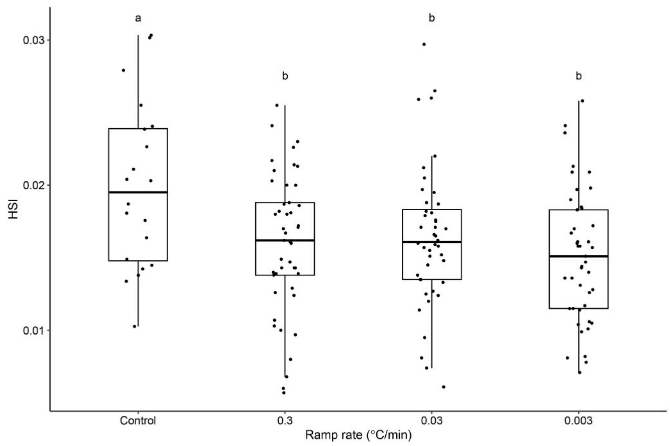

Fig. 2. Hepatosomatic Index (HSI) of juvenile white sturgeon following CT_{max} trials at different heating rates (control, 0.3 °C/min, 0.03 °C/min, and 0.003 °C/min). Black dots represent individual data points, while the boxplot represents group data. The horizontal line in the boxplot is the median, while the whiskers represent the maximum and minimum. Letters that differ indicate statistical significance at alpha = 0.05. N = 20 for control and N = 45 for 0.3 °C/min, 0.03 °C/min, and 0.003 °C/min.

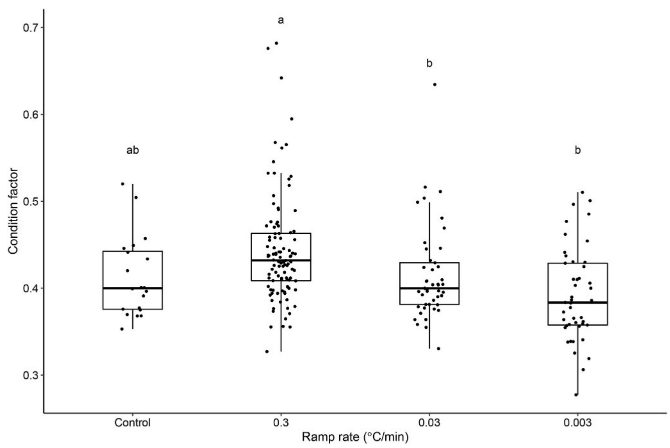

Fig. 3. Condition factor of juvenile white sturgeon following CT_{max} trials at different heating rates (control, 0.3 °C/min, 0.03 °C/min, and 0.003 °C/min). Black dots represent individual data points, while the boxplot represents group data. The horizontal line in the boxplot is the median, while the whiskers represent the maximum and minimum. Letters that differ indicate statistical significance at alpha = 0.05. N = 20 for control; N = 105 for 0.3 °C/min; and N = 45 for 0.03 °C/min, and 0.003 °C/min.

had significantly higher *Hsp47* expression than sturgeon in the 0.03 °C/min ($p < 0.05$). When performance was included, *Hsp47* expression was affected by heating rate ($F_{2,72} = 6.47$, $p < 0.01$; Fig. 5A). In the 0.3 °C/min group, high performers had significantly lower *Hsp47* expression than low performers ($p < 0.05$). Within high performers, *Hsp47* expression was significantly greater in sturgeon heated at 0.003 °C/min than those heated at 0.03 °C/min ($p < 0.01$) and 0.3 °C/min ($p < 0.05$).

3.3.2. Hsp90a

Hsp90a mRNA expression was significantly affected by heating rate ($F_{3,94} = 31.8$, $p < 0.0001$; Fig. 4B). *Hsp90a* expression was significantly elevated in fish that were in the 0.03 °C/min ($p < 0.01$ and $p < 0.0001$) and 0.003 °C/min trials (both $p < 0.0001$) compared to controls and fish heated at 0.3 °C/min. When performance was included, *Hsp90a* expression was significantly affected by heating rate ($F_{2,72} = 69.13$, $p < 0.0001$; Fig. 5B). *Hsp90a* expression in high performing sturgeon heated at 0.3 °C/min was significantly lower than those heated at 0.03 °C/min ($p < 0.001$) and 0.003 °C/min ($p < 0.001$). *Hsp90a* expression of low performing sturgeon increased significantly between each heating rate ($p < 0.05$), with the slowest heating rate having the highest expression.

3.3.3. Hsp90b

Hsp90b mRNA expression was significantly affected by heating rate ($F_{3,94} = 55.83$, $p < 0.0001$; Fig. 4C). Controls and sturgeon heated at 0.3 °C/min did not have significantly different *Hsp90b* expression. *Hsp90b* expression increased significantly as heating rate slowed (all $p < 0.0001$). When performance was included, *Hsp90b* expression was significantly affected by heating rate ($F_{2,72} = 67.59$, $p < 0.0001$; Fig. 5C). *Hsp90b* expression in high performing sturgeon heated at 0.3 °C/min was significantly lower than those heated at 0.03 °C/min ($p < 0.001$) and 0.003 °C/min ($p < 0.0001$). *Hsp90b* expression in high performers was significantly lower in the 0.03 °C/min than the 0.003 °C/min trial ($p < 0.05$). *Hsp90b* expression of low performing sturgeon increased significantly between each heating rate, with the slowest heating rate having the highest expression (all $p < 0.001$).

3.3.4. Hsp70

mRNA expression of *Hsp70* was significantly affected by heating rate ($F_{3,94} = 298.9$, $p < 0.0001$; Fig. 4D). *Hsp70* expression of sturgeon in the 0.03 °C/min and 0.003 °C/min trials did not differ significantly ($p > 0.05$), but both had significantly greater *Hsp70* expression than controls and sturgeon in the 0.3 °C/min trial (all $p < 0.0001$). Fish heated at 0.3 °C/min had significantly greater expression than control fish ($p < 0.0001$). When performance was included, there was a significant effect of heating rate ($F_{2,72} = 285.81$, $p < 0.0001$) and performance ($F_{1,72} = 46.84$, $p < 0.0001$) as well as an interaction effect between the two ($F_{2,72} = 5.89$, $p < 0.005$; Fig. 5D). High performers had greater *Hsp70* expression than low performers in the 0.3 °C/min and 0.003 °C/min trials ($p < 0.0001$ and $p < 0.01$, respectively). For both high and low performers, sturgeon heated at 0.3 °C/min had significantly lower *Hsp70* expression than those heated at 0.03 °C/min ($p < 0.001$ for both) and 0.003 °C/min ($p < 0.001$ for both).

4. Discussion

The results of this study characterized the relationship between heating rate and somatic indices, upper thermal tolerance, and Hsp mRNA expression in the gills of juvenile white sturgeon. Contrary to the prediction that thermal tolerance would be lower when slower heating rates were used, thermal tolerance was found to be significantly higher as warming rate decreased, and under all warming rates a decrease in HSI was observed. This suggests the sturgeon were able to acclimate during the duration of the trial to the warming temperatures (Alfonso et al., 2021), increasing their thermal maximum. It is possible this may be in part because of the regulation of Hsp mRNA expression (Iwama et al., 1998; Iwama and Vijayan, 1999). As predicted, gill mRNA expression of *Hsp90a, Hsp90b,* and *Hsp70* are upregulated in accordance with the duration of heat stress. There were also differences in the expression of *Hsp70* between high and low performers who experienced the same rate of heating, which may indicate its importance in thermal tolerance (Metzger et al., 2016). These findings suggest that white sturgeon increased their thermal performance as heating rates decreased by adjusting to their environment and upregulating Hsps in the gill at an energetic cost, as indicated by diminished HSI.

4.1. Thermal tolerance and warming rate

Unlike the majority of fish species studied to date, juvenile white sturgeon were able to rapidly adjust their thermal tolerance resulting in higher thermal tolerance in the slower heating rates (Becker and Genoway, 1979; Illing et al., 2020; Kovacevic et al., 2019). Zebrafish (*Danio rerio*) is the only fish species studied to date to show the same pattern as sturgeon in this study. Zebrafish fish display different trends

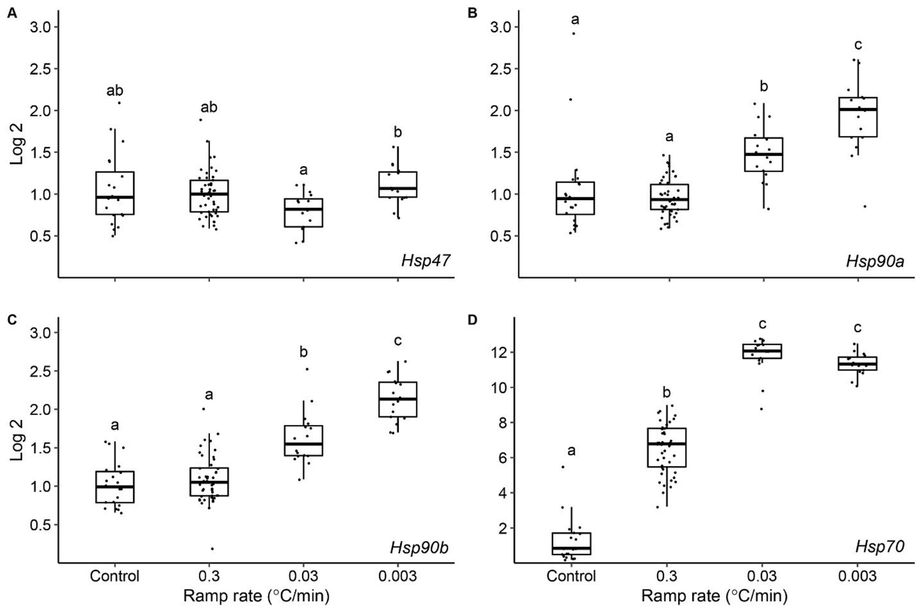

Fig. 4. Relative expression of (A) *Hsp47,* (B) *Hsp90a*, (C) *Hsp90b,* and (D) *Hsp70* (log 2 scale) of juvenile white sturgeon following CT_{max} trials at different heating rates (control, 0.3 °C/min, 0.03 °C/min, and 0.003 °C/min). Black dots represent individual data points, while the boxplot represents group data. The horizontal line in the boxplot is the median, while the whiskers represent the maximum and minimum. Letters that differ indicate statistical significance at alpha = 0.05. N = 20 for control; N = 48 for 0.3 °C/min; and N = 16 for 0.03 °C/min, and 0.003 °C/min.

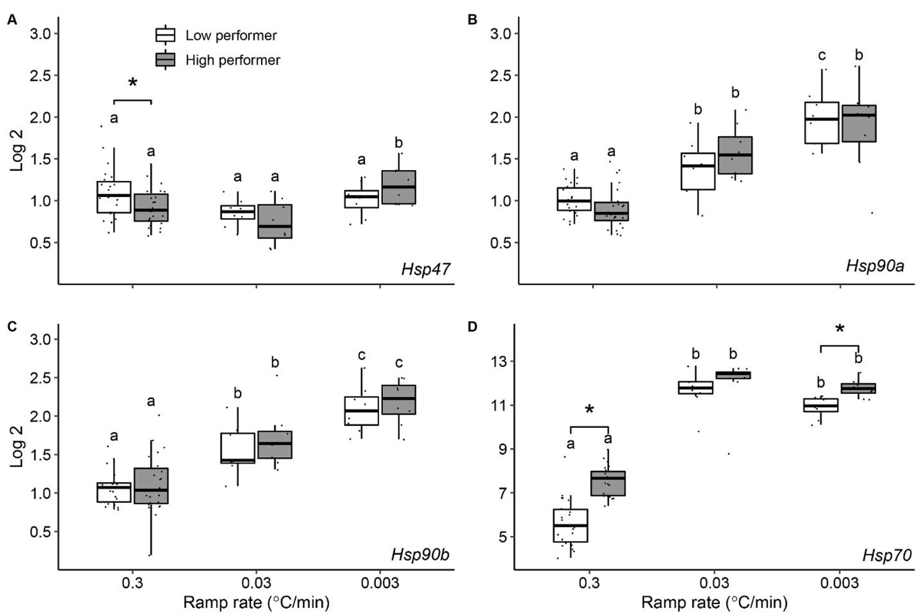

Fig. 5. Relative expression of (**A**) *Hsp47,* (**B**) *Hsp90a*, (**C**) *Hsp90b,* and (**D**) *Hsp70* (log 2 scale) of low and high performing juvenile white sturgeon following CT_{max} trials at different heating rates (0.3 °C/min, 0.03 °C/min, and 0.003 °C/min). Black dots represent individual data points, while the boxplot represents group data. The horizontal line in the boxplot is the median, while the whiskers represent the maximum and minimum. Letters that differ indicate statistically significance a given performance group. Asterisk (*) denotes a statistically significant difference between high and low performers with the same heating rate at alpha = 0.05. N = 24 in the 0.3 °C/min trial and N = 8 in the 0.03 °C/min, and 0.003 °C/min trials per performance level (low and high).

depending on their initial acclimation temperature – when acclimated to supra-optimal temperatures (34 °C), they had higher thermal tolerance when heated at 0.3 °C/min relative to 0.025 °C/min, whereas zebrafish acclimated to sub-optimal temperatures (22 °C) were able to increase their thermal tolerance when heated at 0.025 °C. In 22 °C acclimated zebrafish, CT_{max} was 1.53 °C higher in the slower trial (Åsheim et al., 2020). In comparison, in the current study sturgeon were able to increase their thermal tolerance by 2.1 °C when the heating rate was reduced from 0.3 °C/min to 0.03 °C/min. The slowest trial took 4.5 days and in it the sturgeon increased their thermal tolerance by 5 °C relative to the conventional heating rate of 0.3 °C/min. This 5 °C increase in CT_{max} is similar to the increase in CT_{max} (0.3 °C/min) when sturgeon are acclimated from 15 °C to 24 °C for 2 weeks (Penman, 2022) indicating that sturgeon are able to rapidly acclimate and increase thermal tolerance during warming. These findings suggest that sturgeon may be able to tolerate higher temperatures with a real-time river warming scenario more so than what a conventional CT_{max} may suggest.

In conventional CT_{max}, thermal tolerance may be limited by a neurological and/or cardiac malfunction (Andreassen et al., 2022; Christen et al., 2018). However, at slower heating rates, thermal tolerance is assumed to be limited by the accumulation of heat damage as a result of organisms spending more time at temperatures where the rate of cellular damage exceeds the rate of repair (Rezende et al., 2014). Interestingly, the thermal tolerance of white sturgeon was more variable under acute warming rates, while variability was decreased under slower rates, potentially suggesting lower variability in physiological limitations (as described above) under slower warming conditions. Further, of the somatic indices studied, HSI was unique as it was the only one to be affected by heating rate, and in each of the CT_{max} trials was significantly lower (approximately 20%) than in the control group, indicating that white sturgeon exhibited increased energy consumption, depleting some hepatic reserves during the thermally stressful trials (Hung et al., 1990; Chellappa et al., 1995; Bugg et al., 2020; Morrison et al., 2020). These results suggest that white sturgeon possess physiological or biochemical mechanism(s) reliant on the rapid mobilization of energetic reserves which may promote acute and chronic acclimation (Bugg et al., 2020; Morrison et al., 2020).

4.2. Transcriptional responses to warming rate

Modulation of Hsp mRNA expression is one mechanism by which ectotherms are thought to be able to regulate their thermal tolerance in response to changing environments (Feder and Hofmann, 1999; Hochachka and Somero, 2002; Somero, 2020). Hsps are preferentially transcribed during periods of thermal stress and their presence, which can increase by multiple orders of magnitude in a matter of minutes, has been linked to increased thermotolerance (Tomanek and Somero, 2000). In insects, studies have shown that inhibition of Hsp expression suppressed thermal tolerance, while enhanced expression increased thermal tolerance (Feder et al., 1996; Lu et al., 2016; Rinehart et al., 2007). Similarly, in this study white sturgeon displayed a highly plastic Hsp mRNA response with gill mRNA expression of *Hsp90a*, *Hsp90b*, and *Hsp70* significantly elevated in the slower rate of heating trials under which thermal tolerance was increased.

Of the four Hsps examined in the current study, gill expression of *Hsp47* mRNA was the only one that remained relatively unchanged between controls and different heating rates, while *Hsp90a*, *Hsp90b*, and *Hsp70* were responsive to increasing temperatures. *Hsp47* expression is less conserved and more species-specific than other Hsps – in response to acute heat shock. For instance, when presented with thermal stress, juvenile Atlantic sturgeon upregulated expression of *Hsp47*, while juvenile brook trout did not (Mackey et al., 2021; Mohanty et al., 2018; Yebra-Pimentel et al., 2020). Unlike *Hsp47*, expression of *Hsp90a*, *Hsp90b,* and *Hsp70* mRNA were all elevated in response to heat stress as hypothesized. Upregulation of these Hsps can help renature damaged proteins and prevent cellular apoptosis (Beere, 2004). Acute (*Hsp90a* and *Hsp70*) and constitutive (*Hsp90b*) expression depends on the severity and duration of the stressor (Chen et al., 2018; Somero, 2020). Interestingly, both isoforms of *Hsp90* had very similar induction profiles across performer groups, with upregulation observed following the two longer trials (0.03 °C/min and 0.003 °C/min. Isoforms of *Hsp90* were not upregulated in the conventional CT_{max} (0.3 °C/min) and it could be that their activation is time dependent and that the 0.3 °C/min trial was too acute for upregulation to occur. This is different from what is observed in lake sturgeon where *Hsp90a* increased in response to a conventional CT_{max}, suggesting there may be species-specific induction thresholds for Hsps during thermal stress (Bugg et al., 2020). Alternatively, it could be that they are temperature dependent and the sturgeon in the conventional CT_{max} did not experience high enough temperatures to trigger *Hsp90* expression. To tease apart these potential mechanisms, future studies should consider continuous sampling throughout CT_{max} trials.

The speed and extent to which juvenile white sturgeon upregulated gill *Hsp70* mRNA expression, highlights their plasticity and may help explain how they were able to physiologically adjust and demonstrate the opposite CT_{max} pattern to what was predicted. *Hsp70* expression underwent the greatest increase of all Hsps in every trial, however upregulation plateaued between the two slower trials (0.03 °C/min and 0.003 °C/min). This may be a result of the extent of *Hsp70* upregulation, as cells may have been saturated with *Hsp70* meaning further upregulation would have provided no additional advantage. *Hsp70* production is also energetically costly so further upregulation may have been metabolically constrained (Han et al., 2011). Finally, while Hsps are preferentially synthesized during periods of stress and can be upregulated in a matter of minutes, it could be that under slower warming rates, sturgeon are able to enlist further long-term physiological process(es) that may help them cope with heat stress (Tomanek and Somero, 2000).

Hsp70 was the only Hsp to be differentially expressed between high and low performers in the gill of juvenile white sturgeon. High-performing sturgeon in both the 0.3 °C/min and 0.003 °C/min trials upregulated *Hsp70* expression significantly more than low performers. Additionally, while not statistically significant, the same trend was apparent in the 0.03 °C/min trial. Importantly, although less likely in conventional CT_{max}, both time and temperature are possible confounds in all trials as high performers were exposed to higher temperatures and heat stress for longer durations than low performers. Thus, differences between the groups could be due to the temperature and/or duration of heat exposure. However, time and temperature may not explain the variation between performance, thus this finding suggests that greater upregulation of *Hsp70* mRNA likely contributes to increased recruitment of protective mechanisms during thermal stress and to the observed differences in thermal tolerance at the individual level.

4.3. Physiological impacts of warming rate

Condition factor, craniosomatic index, and CSI were not affected by heating rate while HSI decreased from control levels for all heating rates. Studies that have found significant differences in condition, craniosomatic and CSI metrics have been associated with longer exposure durations of stress, which may explain why these alterations were not observed in the current study (weeks – months; Gilbert and Farrell, 2021; Raspopov et al., 2017; Soengas and Aldegunde, 2002). HSI in all three trials was significantly lower than in the controls, but it did not differ between heating rates. Lower HSI may indicate the metabolic costs of coping with heat stress. Decreases in HSI have been observed in lake sturgeon following a 30-day temperature acclimation, and in white sturgeon and three-spined sticklebacks decreases in HSI have been linked to decreased glycogen reserves (Bugg et al., 2020; Chellappa et al., 1995; Hung et al., 1990). Glycogen reserves stored in the liver are a readily available source of energy that can be utilized under acute and chronic thermal stress (Viant et al., 2003; Rossi et al., 2017). Thus, a decrease in HSI likely suggests that exposure of white sturgeon to thermal stress, at any of the warming rates used in this study, can induce a mobilization of energetic reserves which promote acclimation.

4.4. Study limitations

The transcriptional analysis in this study was solely focused on the regulation of Hsps in gill tissue, as a key interface between the external and internal environment, but may be a limiting factor as there could be diverse responses across a variety of tissue types. Further the observed Hsp responses are at the transcriptional level, and as translation and protein production is an energetically demanding process, if there are indeed energetic constraints, these mRNA level modifications may not represent protein level responses (Lewis et al., 2016; Mottola et al., 2020). Heat shock proteins additionally have pleiotropic roles, impacting many different processes throughout the tissue, thus their induction may have multiple physiological outcomes.

Additionally, observed changes in HSI across CT_{max} warming rates may be considered relatively small (approximately 20% reduction across warming rates), which could lead to minimal physiological impacts and could perhaps be related to increases in metabolic rates with elevating temperature. Additionally, glycogen is bound to water, so as glycogen reserves are diminished, water is released (King et al., 2018). It is possible that the observed decrease in HSI is in part due to water loss caused by glycogen catabolism, further reducing overall liver mass. Measuring changes in glycogen reserves as well as changes in dry mass are possible avenues for future research. However, in this instance we would expect to observe increased depletion of energetic reserves as warming rates decreased and trial duration increased, instead HSI was consistently decreased across warming rates. Further, changes in HSI could be potentially explained by the Arrhenius principal, however relying solely on this principal for interpretation can lead to an over-simplification of the factors effecting overall organismal responses across biological levels of organization (Schulte, 2015). As the presented study examines the physiological responses of white sturgeon under different warming rates in a laboratory setting, future research may focus on how white sturgeon respond to different thermal regimes and warming rates behaviorally in their natural environment.

5. Conclusions

In this study we observe impacts of warming rate across levels of biological organization in white sturgeon. In contrast to most fishes, white sturgeon were better able to thermally acclimate during the slowest warming rate, which increased their thermal tolerance when compared to faster warming rates. During the CTmax trials, sturgeon responded to thermal stress by increasing Hsp mRNA levels which ultimately impacted overall performance. As such, fish with higher maximum thermal tolerance exhibited greater mRNA expression of these key thermal stress genes. Further, their acclimatory capacity was much higher and more rapid than other studied species, thus an understanding of these underlying physiological principles may provide broad insights into the mechanisms underlying effective thermal acclimation. Our findings demonstrate the importance in considering ecological relevance under which which endangered species live when measuring thermal tolerance, however, we do not know the lasting physiological impacts of responding to thermal stress as there could be negative implications of these energetically costly responses later in life. Further, there is impressive thermal plasticity observed in these white sturgeon during a key developmental time, suggesting that white sturgeon may be able to cope with rapid increases in river temperature. This is encouraging as, with an increase in the severity and frequency of heatwaves, sturgeon will need to rapidly induce plastic responses to environmental change as demonstrated in this study. As such, the tremendous plasticity demonstrated in this study suggests that, during acute thermal stress, juvenile sturgeon can respond across multiple levels of biological organization and thus, these sturgeon will hopefully be able to cope with our warming climate.

Data availability

Data for this study can be found on Dryad data depository:

Penman, Rachael; Earhart, Madison (2023), Slow heating rates increase thermal tolerance and alter mRNA HSP expression in juvenile white sturgeon (Acipenser transmontanus), Dryad, Dataset, https://doi.org/10.5061/dryad.w0vt4b8x5.

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CRediT authorship statement

RJP, CJB, BRK, WSB, and MLE, designed and conducted the research. RJP, BRK, WSB, and MLE conducted CT_{max} trials. WSB designed primers for molecular assays. RJP, BRK, MLE conducted molecular assays. RJP wrote the first draft of the manuscript and all authors provided comments on the final version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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