The effects of water temperature on the resting and post-exercise physiology in juvenile Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*)

by

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ABSTRACT

The effects of cold-water temperature on the resting and post-exercise physiology were investigated in juvenile Atlantic sturgeon. Hematocrit, protein, glucose, osmolality, and lactate were measured after four weeks of acclimation, at rest (at 3 °C, 6 °C, or 9 °C), or following five minutes of exercise (at 3 °C or 9 °C). At 3 °C, fish appeared motionless and were positioned at the bottom of the tank. At 9 °C, fish were visibly more active. Resting glucose, protein, lactate, and hematocrit levels were similar across temperatures, and resting osmolality values were lowest at 9 °C. Post-exercise lactate and glucose levels were similar at 3 and 9 °C. Post-exercise osmolality and hematocrit values increased at 9 °C only, and levels of protein decreased at both temperatures. Overall, sturgeon can survive at cold temperatures, but don't show the typical physiological response to this type of exercise (i.e., increased glucose, lactate and osmolality).

DEDICATION

I'd like to dedicate this work to my mother and father who have provided me with so much throughout my life, and who have always supporting me with all of my endeavors.

ACKNOWLEDGEMENTS

I'd like to thank first and foremost Dr. Jim Kieffer for accepting me as an honours student. His guidance and support throughout this process has been crucial and imperative. I'd also like to thank him for his ability to push me towards being the writer and researcher I can be and helping me to strive towards my potential. His commitment to helping me better myself through mentoring is extremely valued and indispensable.

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Finally, I would like to thank the MADSAM fish group and all its members for the support and guidance while caring for the animals.

STATEMENT OF RESEARCH CONTRIBUTION

For this research, all experimentation and data collection was performed by myself under the guidance and supervision of Dr. Kieffer. While the fish were acclimating to the various temperatures, I performed the husbandry of all animals. For the experiments, I manually chased all the fish and assisted Dr. Kieffer with blood sampling and preparation for metabolite assays. All analytical procedures were performed by myself either alone or with assistance from Dr. Kieffer. Statistical analysis was performed on all factors by myself following the analytical procedures.

Acclimation of the resting, non-exercising sturgeon took place from January of 2018 until February 2018. Following this, blood sampling required approximately 3 hours in February of 2018. Acclimation of the exercised fish took place from mid-September of 2018 until mid-October 2018. Following this, the forced exercise and blood collection of these fish took approximately 4 hours. Analytical procedures of hematological parameters took place between January and February of 2019. Statistical analysis of these parameters required approximately 3-4 hours, marking all data collection and interpretation complete in February of 2019.

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Chapter 1

Introduction

Considering water temperature has been shown to influence many biological functions of fish (Brett, 1971; Fry, 1971), it is important to understand the broad implications of a wide temperature gradient on their biology. While there is a general understanding of the effects of optimal and/or high temperatures on fish physiology, the focus of cold temperature on aspects of fish physiology hasn't been as well studied (Szekeres et al., 2016). Lower water temperatures are commonly experienced naturally by numerous fish species during winter months (4 °C Rogue River Oregon; Mayfield and Cech Jr., 2004; 0 °C Saint John River; Deslauriers and Kieffer 2012; 0 °C Winnipeg; Deslauriers et al., 2018). It is well known that a change in water temperature strongly cues migration in fish (Fry, 1971; Sims et al., 2004; Brodersen et al., 2011). A reduction (acute or chronic) in water temperature could, potentially, stimulate this cue, putting a migrating fish at a greater risk of mortality as they are already limited to a specific environment in early life stages by other factors such as salinity and predation (Hilton et al., 2016). Temperature also affects a fish's ability to swim, and studies have shown that swimming ability (e.g. critical swimming speed) and the response to exercise is negatively affected by colder temperatures (Beamish, 1981; Kieffer et al. 1994; Galloway and Kieffer 2003; Adams et al., 2003; Guan et al., 2008; Deslauriers & Kieffer, 2012; Yang et al., 2013; Mandal et al., 2016). Thus, a lower swimming capacity, and/or recovery, at colder temperatures can have vast effects on feeding behaviour and predator avoidance patterns of fish during periods of extended cold-water exposure or over-wintering phases, particularly if the river currents are increased (Adams

et al., 2003; Mayfield and Cech, 2004; Deslauriers and Kieffer, 2012). Therefore, as cold temperature impacts swimming in fish, studies should be performed investigating the organism's ability to swim/exercise at lower temperature; this is an area of fish physiology that hasn't been given the necessary attention, particularly under cold conditions (<5 °C).

Sturgeon populations, in general, have been affected by habitat destruction through human disturbance, over fishing, and climate change (Hilton et al., 2016; Yuan et al., 2017). As of 2012, all populations of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) worldwide were listed, under the US Endangered Species Act and the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), as either endangered or threatened (Hilton et al., 2016). Due to these listings, conservation and population growth studies are becoming increasingly important for this species, and considering numerous sturgeon research programs seek to develop methods to increase population growth and to adopt better conservation strategies (Cheong et al., 2006; Yuan et al., 2017; Porter and Schramm Jr., 2018); the ability of a sturgeon to avoid mortality in multiple scenarios (i.e. lower temperature, exercise at lower temperatures), and reach sexual maturity should therefore be of importance. More specifically, in order to better develop these types of plans for Atlantic sturgeon, the factors which heavily affect the species must be better understood (Atlantic States Marine Fisheries Commission, 2015).

Due to the Atlantic sturgeon's anadromous lifestyle, temperature is an important factor to study, as it has been shown to be one of the most significant abiotic factors that impacts variables such as the distribution, physiology, and behaviour of fishes (Brett, 1971; Fry, 1971; Beitinger and Lutterschmidt, 2011; Zhang et al., 2017). If the goal of some Atlantic sturgeon research is to increase populations, and the number of individuals within those populations, physiology of individuals at progressively colder temperatures should be investigated to examine the potential

metabolic costs of living at these temperatures. The need for this information is extremely important, as surviving its first overwintering phase is a great predictor for if a sturgeon will survive and reach sexual maturity (Deslauriers et al., 2018). Finally, due to cold temperatures affecting numerous internal processes research should also examine how cold temperatures specifically affect cellular, molecular, and physiological parameters in fish, as these factors dictate how an organism will respond to reduced temperatures (Szekeres et al., 2016).

Due to these important aspects of cold temperature and its effects on sturgeon, I will examine the effect of cold temperatures on the physiology of resting Atlantic sturgeon, by comparing plasma glucose, protein, lactate, and osmolality at three acclimation temperatures (3, 6, and 9 °C). These blood parameters are considered secondary stress indicators of fish, and the measurable response can aid in identifying which physiological processes are affected by temperature (Wendelaar Bonga, 1997; Donaldson et al., 2008). Furthermore, to assess the need for research on cold temperatures affecting exercise ability in fish, I will be exploring the effects of exhaustive exercise (i.e., a five-minute chasing stress) at colder temperatures (3 and 9 °C) in Atlantic sturgeon. This will also allow me to compare the hematological parameters between rest and exercise at colder temperatures. To date, most work on exhaustive exercise responses in sturgeon have been conducted at temperatures at or above 10 °C (Lankford et al., 2003; Baker et al., 2005 b; Preston et al., 2017).

To assess the effects of stress (i.e. cold temperature, exercise at cold temperature) on fish, researchers can utilize numerous physiological parameters, which help to define primary, secondary, and/or tertiary stress responses (Donaldson et al., 2008). Primary stress responses involve the release of numerous hormones (e.g., adrenaline; cortisol) which can eventually cascade a secondary stress response (e.g., changes in lactate, glucose, hematocrit, ions), which in

turn can cause a tertiary stress response which involves whole organism changes such as growth (Donaldson et al., 2008). For this study, the measurable variables will be that of a secondary stress response. Specifically, plasma osmolality, glucose, lactate, protein, and hematocrit will be measured prior to, and following five minutes of manual chasing (exhaustive exercise).

The measure of plasma lactate is useful as a measure of exhaustive exercise limits (Kieffer, 2000), plasma protein and glucose are used to assess alterations in metabolic patterns as they are used for energy metabolism (Mazeaud et al., 1977; Wendelaar Bonga, 1997), while hematocrit and osmolality can be used to show if osmoregulation has been altered (Mazeaud et al., 1977; Wendelaar Bonga 1997). Under stressful conditions (i.e. temperature change; exercise), plasma glucose and lactate have been shown to increase, while osmolality and hematocrit have been shown to both increase and decrease from optimal levels, although hematocrit more frequently shows an increase (Mazeaud et al., 1977; Wendelaar Bonga, 1997). Finally, plasma protein has been shown to increase under stress due to the recruitment of stress proteins or the use of proteins as an energy source (Mazeaud et al., 1977; Wendelaar Bonga, 1997).

Considering the patterns of the secondary stress indicators in animals under stress, and the fact cold temperatures and exercise are stressors in many species (Mazeaud et al., 1977; Wendelaar Bonga, 1997), it is likely cold temperatures and exercise at these temperatures alter the secondary stress indicators in Atlantic sturgeon. Therefore, as cold temperatures and exercise are anticipated to be stressful, an increase in plasma glucose and lactate (only following exercise), and hematocrit are expected. Osmolality is expected to change, but the trend is difficult to predict as levels have been reported to either increase or decrease under stress.

Finally, protein is expected to increase if these events are stressful as the animal attempts to recruit protective proteins into its blood.

Chapter 2

Methods

2.1 Animal Husbandry

Young of the year Atlantic sturgeon were obtained from Acadian Sturgeon and Caviar Inc. (http://www.acadian-sturgeon.com). The fish were raised from eggs retrieved from St John river sturgeon stocks. The sturgeon were held in (60 L) cylindrical tanks supplied with a flow-through of fresh, aerated, dechlorinated, municipal tap water at 15 °C. Preceding the experiments, fish were acclimated to one of three temperatures (see below for details). To reach the acclimation temperatures, the incoming water was cooled at 1 °C/day using industrial chillers (Frigid Units, Inc., Toledo, Ohio) until the acclimation temperature was reached (Zhang and Kieffer, 2017). Water temperature inside the tank was continuously monitored by electronic temperature probes (Loligo Systems, Viborg, Denmark). Additionally, the water was continuously aerated by two aerators. The fish were acclimated for four weeks, while being fed daily to apparent satiation (EWOS micro 1.2mm, 53% protein) but were fasted 24 hours prior to experimentation (Baker et al., 2005 b; Zhang and Kieffer, 2017; Brown and Kieffer, 2018). A photoperiod of 12-h light: 12-h dark was maintained throughout the study.

2.2 Resting Hematology

Sturgeon (~120g, 32.5 cm total length) were acclimated to 2.5-3 °C (n=6), 5.5-6 °C (n=6), or 8-9 °C (n=7) water as described above. Following the four-week acclimation period, an individual sturgeon was netted, withdrawn from the relevant acclimation tank, and placed in an anaesthetic bath at acclimation temperature consisting of a buffered mixture of $NaHCO_3$ and

MS-222, (250mg/L). Care was taken not to disturb other fish in the tank when removing the sturgeon being tested (Brown and Kieffer 2018). Once fully anaesthetized, the sturgeon was withdrawn from the bath and blotted dry, after which the mass (nearest g) and length (nearest cm) were recorded. Blood samples (~about 1ml) were drawn from the caudal vessels (Zhang and Kieffer, 2014; Brown and Kieffer, 2018), through use of a lithium heparin-coated needle and syringe (Baker et al., 2005 b; Penny and Kieffer 2014; Brown and Kieffer, 2018), and placed in centrifuge tubes where they were treated (as described below). The total process from netting the fish to processing the fish's blood required approximately 20 minutes.

2.3 Post-Activity Hematology:

Sturgeon (~70g, 29.5 cm total length) were acclimated to 2.5-3 °C or 8-9 °C water, as described above. The 5-6 °C group of fish was not tested for post-activity hematology due to an issue with acquiring sufficient fish of an appropriate size. An individual sturgeon was netted, withdrawn from the relevant acclimation tank, and placed in an exercise tank (approximately 45cm in diameter and 60 cm deep) half filled with water at acclimation temperature. Like that of the resting hematology process, care was taken not to disturb other fish in the tank when removing the sturgeon being tested. Immediately following being placed in the swimming tank, the sturgeon was forced to exercise for five minutes using manual chasing (Kieffer et al., 2001; Baker et al., 2005 b; Brown and Kieffer, 2018). Fish were exercised to a behavioural state of exhaustion (i.e., no longer responding to the manual chasing) rather than quantifying the activity, as five minutes of exercise is typically enough time for the fish to reach exhaustion, and the intensity of the physiological responses (i.e. through changes in blood parameters) were characterized (Kieffer et al., 1994, Kieffer et al. 2001, Brown and Kieffer, 2018). Upon finishing

the five-minute exercise period, the sturgeon was removed from the swimming tank and placed in an anesthetic bath (as noted above). The blood collection process was identical to the blood collection process for the resting hematology fish (see above). The total process from netting the fish to processing the blood was about 25-30 minutes.

2.4 Analytical Protocols

From the whole blood sample, duplicate 70 µl aliquots were used to calculate hematocrit (Kieffer et al., 1994; Brown and Kieffer, 2018). The remaining sample of whole blood was centrifuged at 6700g for 2 minutes. The plasma supernatant of the sample was pipetted into tubes, then immediately stored at -20 °C until data analysis occurred. From these plasma samples, the lactate, protein, osmolality and glucose concentrations were measured. Lactate concentrations were calculated using a standard colorimetric assay and appropriate standards (lactate reagent: 735–10; lactate standard: 735–11; Trinity Biotech; Brown and Kieffer, 2018) and relating the samples to the standards using a lactate standard curve. Plasma glucose was measured using a OneTouch Ultra glucometer (OneTouch Ultra 2; Code 25 test strips; www. onetouch.ca; Penny and Kieffer 2014; Downie et al. 2018; Brown and Kieffer, 2018), and osmolality using the Advanced Micro Osmometer (Advanced Instruments Inc., U.S.A) (Brown and Kieffer, 2018). Total plasma protein concentrations were measured using a standard colorimetric assay and appropriate standards (Total Protein Reagent, Biuret Method & Protein Standard Set P5495; Sigma Canada) and relating the samples to the standards using a protein standard curve (Brown and Kieffer, 2018).

2.5 Statistical Analysis

The data was statistically analyzed for normality and equal variance using the Shapiro-Wilk's test and Levene's test, respectively (Baker et al., 2005 b). A series of two-way ANOVAs (α=0.05, factors being rest/exercise; acclimation temperature, and their interaction) were used to analyze the measured hematological parameters between resting and exercised fish at the two acclimation temperatures (3 vs 9 °C). A one-way ANOVA was also used to examine the effects of acclimation temperature (3, 6 or 9 °C) on the resting hematological parameters (Baker et al., 2005 b; Zhang and Kieffer, 2017). Holm-Šidák multiple comparison post hoc tests (α= 0.05) were used to compare mean values between groups.

Chapter 3

Results

3.1 Resting Hematology

Average plasma glucose concentration (~3.8 mmol/L) (Figure 1a), plasma lactate concentration (~0.83 mmol/L) (Figure 1b), plasma protein amounts (~14.8 mg/ml) (Figure 2a), and hematocrit values (~18%) (Figure 2b), were similar across all three temperatures at rest (Table 1). However, values for resting osmolality (Table 1) were higher at 3 °C in comparison to 9 °C (Table 2) (Figure 3).

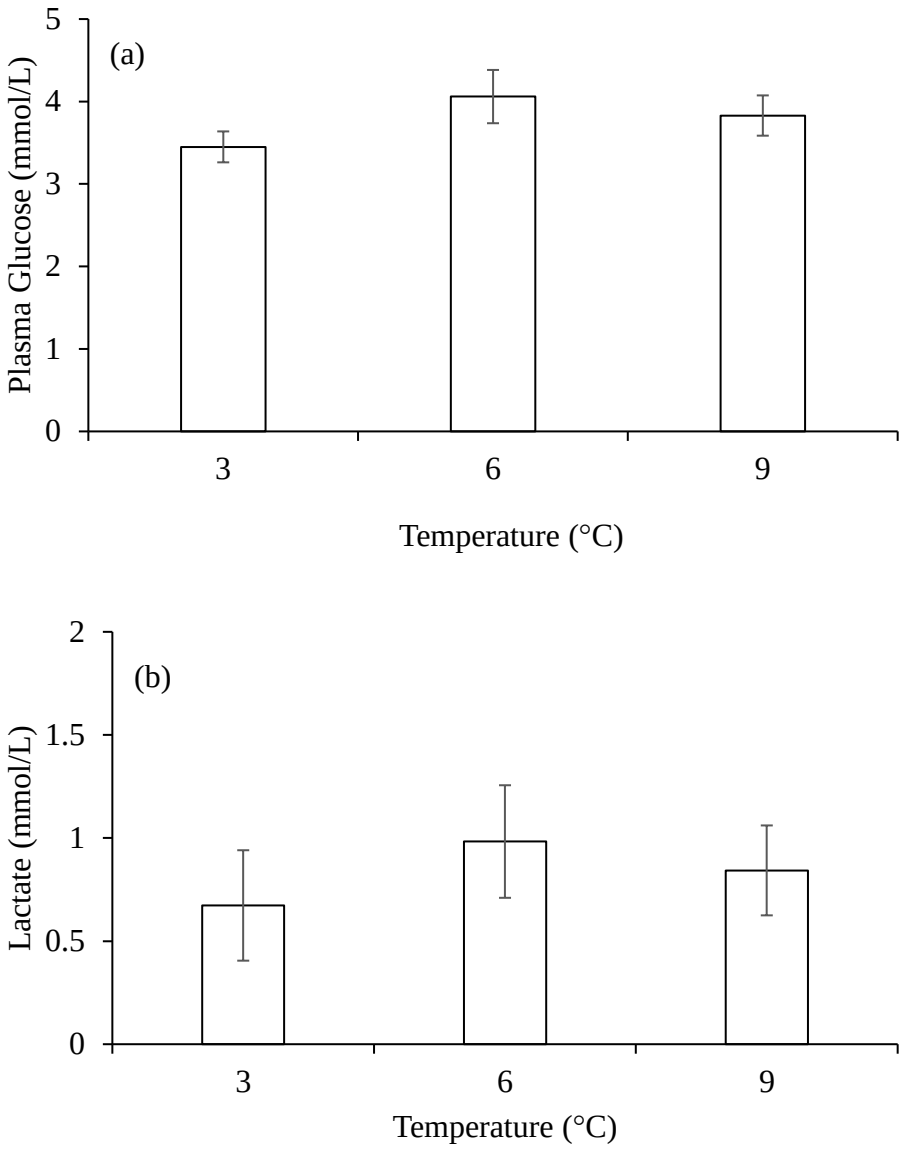

Figure 1. Average (± S.E.) plasma glucose concentration (mmol/L) (a), and plasma lactate concentration (mmol/L) (b) of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) at varying temperatures at rest.

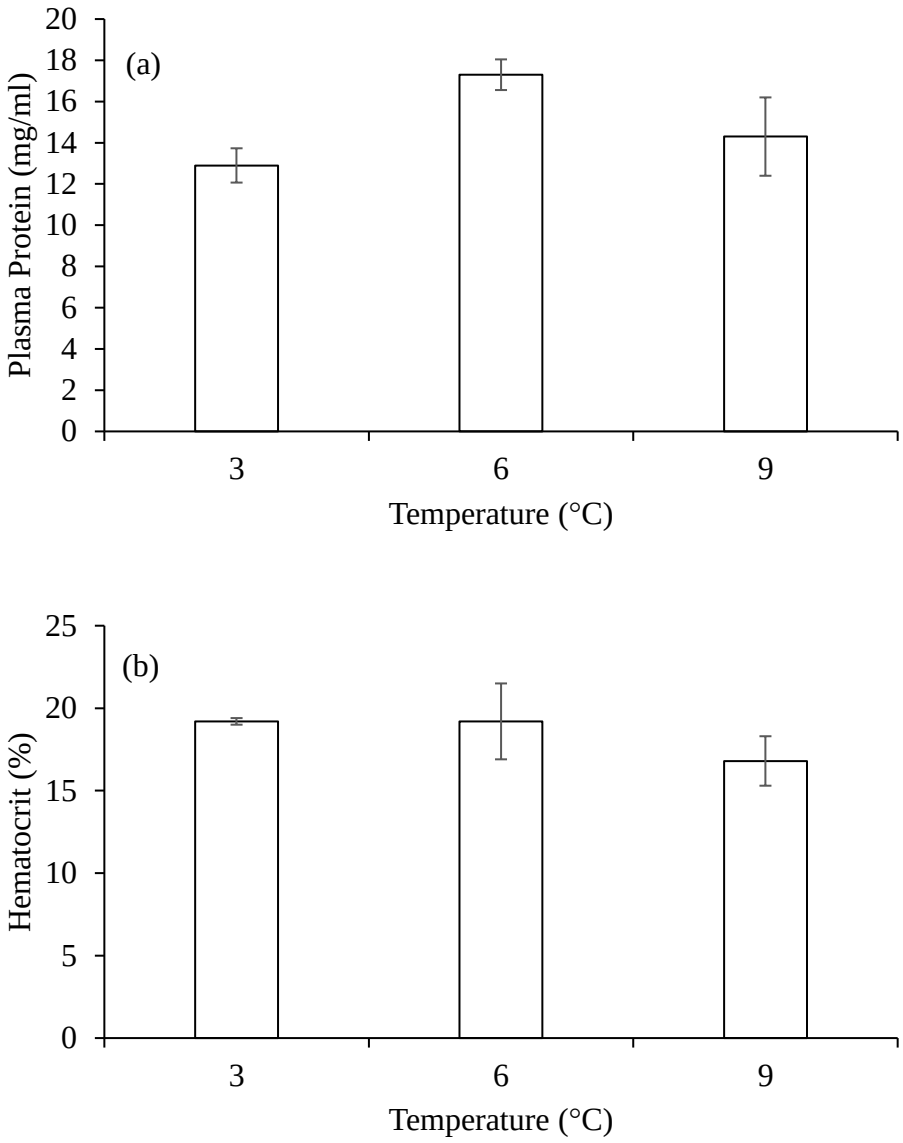

Figure 2. Average (± S.E.) plasma protein (mg/ml) (a), and hematocrit (%) (b) of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) at varying temperatures at rest.

Table 1. Mean (± S.E.) hematological variable value for resting juvenile Atlantic sturgeon acclimated to varying temperatures.

Variable	Temperature (°C)	Value
Plasma Glucose (mmol/L)	3	3.4 ± 0.2
	6	4.1 ± 0.3
	9	3.8 ± 0.2
Plasma Lactate (mmol/L)	3	0.67 ± 0.27
	6	0.98 ± 0.27
	9	0.84 ± 0.22
Hematocrit (%)	3	19 ± 0
	6	19 ± 0
	9	17 ± 0
Plasma Protein (mg/ml)	3	12.9 ± 0.8
	6	17.3 ± 0.7
	9	14.3 ± 1.9
Osmolality (mOsmol/kg)	3	269 ± 8
	6	251 ± 10
	9	226 ± 13

Table 2 One-way ANOVA results for hematological variable values at varying temperatures for resting juvenile Atlantic sturgeon. Significant differences noted with *.

Variable	SS	F	P
Plasma Glucose	1.163	1.39	0.278
Plasma Lactate	0.7502	2.57	0.108
Hematocrit	0.002577	0.78	0.476
Plasma Protein	60.89	2.57	0.108
Osmolality	5969	3.84	**0.043***

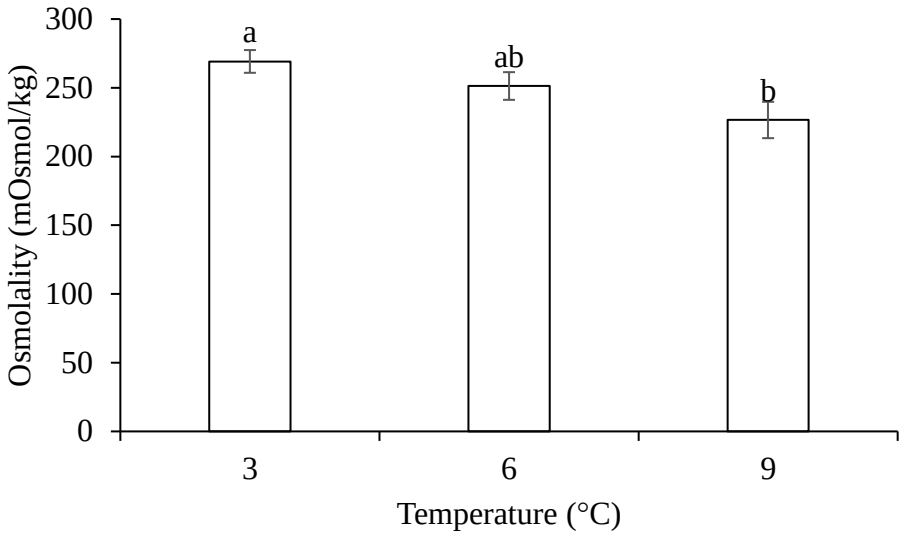

Figure 3. Average (± S.E.) osmolality (mOsmol/kg) of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) at varying temperatures at rest. Different letters represent significant difference (P<0.05) between temperatures.

3.2 Post-Activity Hematology

Hematocrit values (Table 3) following forced activity were similar to resting hematocrit values at 3 °C but showed a significant increase (Table 4) following exercise at 9 °C (Figure 4). Results for plasma lactate concentrations (Table 3) at 3 °C were similar between treatments while exhibiting a non-significant increase at 9 °C from rest to exercise (Figure 5a). Plasma glucose, values (Table 3) at 9 °C stayed similar between treatments, while at 3 °C resting values were lower than levels following exercise (Figure 5b). Plasma protein concentrations showed significant decreases (Table 4) following forced activity in comparison with resting values (Table 2) at both temperatures (Figure 6a), while values for osmolality (Table 2) showed different responses at each temperature. At 3 °C osmolality decreased following exercise in comparison to the resting values, while at 9 °C there was a significant increase (Table 4) in osmolality following exercise when compared with resting values (Figure 6b).

Table 3 Mean hematological variable value (± S.E.) for juvenile Atlantic sturgeon acclimated to varying temperatures at rest and following exhaustive exercise.

Variable	Temperature (°C)	Rest Value	Post-exercise Value
Plasma Glucose (mmol/L)	3	3.4 ± 0.2	4.7 ± 0.6
	9	3.8 ± 0.24	3.8 ± 0.5
Plasma Lactate (mmol/L)	3	0.67 ± 0.27	0.71 ± 0.14
	9	0.84 ± 0.22	1.19 ± 0.22
Hematocrit (%)	3	19 ± 0	21 ± 1
	9	17 ± 0	22 ± 2
Plasma Protein (mg/ml)	3	12.9 ± 0.8	7.08 ± 1.2
	9	14.3 ± 1.9	10.4 ± 1.3
Osmolality (mOsmol/kg)	3	269 ± 8	257 ± 6
	9	226 ± 13	267 ± 8

Table 4. Two-way ANOVA (factors: temperature and exercise condition) for hematological variable values for sturgeon prior to and immediately following exhaustive exercise at varying temperatures for juvenile Atlantic sturgeon. Significant differences noted with *.

Variable	Factor	SS	F	P
Plasma Glucose	Temperature	0.3972	0.23	0.638
	Treatment	2.6664	1.52	0.228
	Temperature x Treatment	2.6976	1.54	0.226
Plasma Lactate	Temperature	0.7726	2.46	0.129
	Treatment	0.2659	0.85	0.366
	Temperature x Treatment	0.1801	0.57	0.456
Hematocrit	Temperature	0.000403	0.26	0.614
	Treatment	0.008452	5.46	**0.028***
	Temperature x Treatment	0.001978	1.28	0.269
Plasma Protein	Temperature	38.853	2.84	0.104
	Treatment	170.387	12.47	**0.002***
	Temperature x Treatment	6.448	0.47	0.498
Osmolality	Temperature	1913	3.06	0.093
	Treatment	1440	2.30	0.142
	Temperature x Treatment	4920	7.86	**0.010***

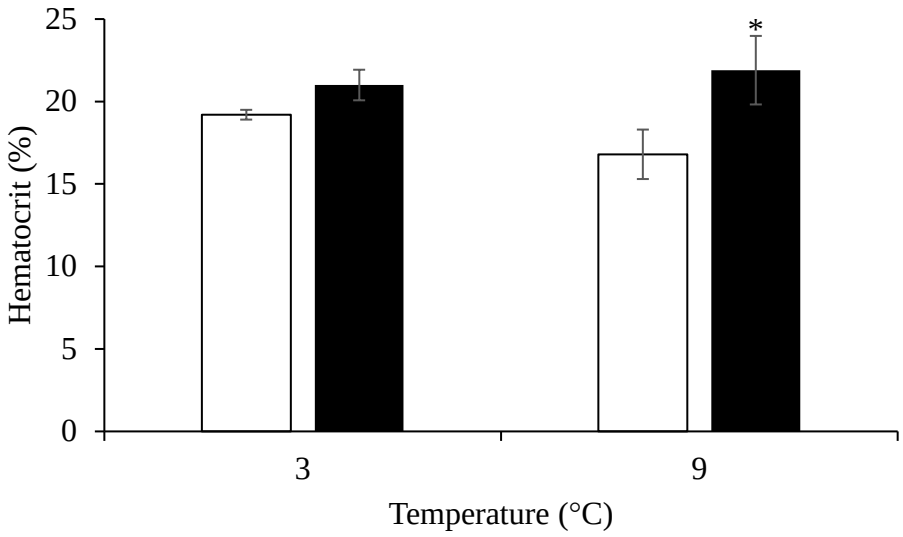

Figure 4. Average (± S.E.) hematocrit (%) of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) at varying temperatures at rest (□) and following 5 minutes forced activity (■). *, significant difference (P<0.05) from the resting value at the same temperature.

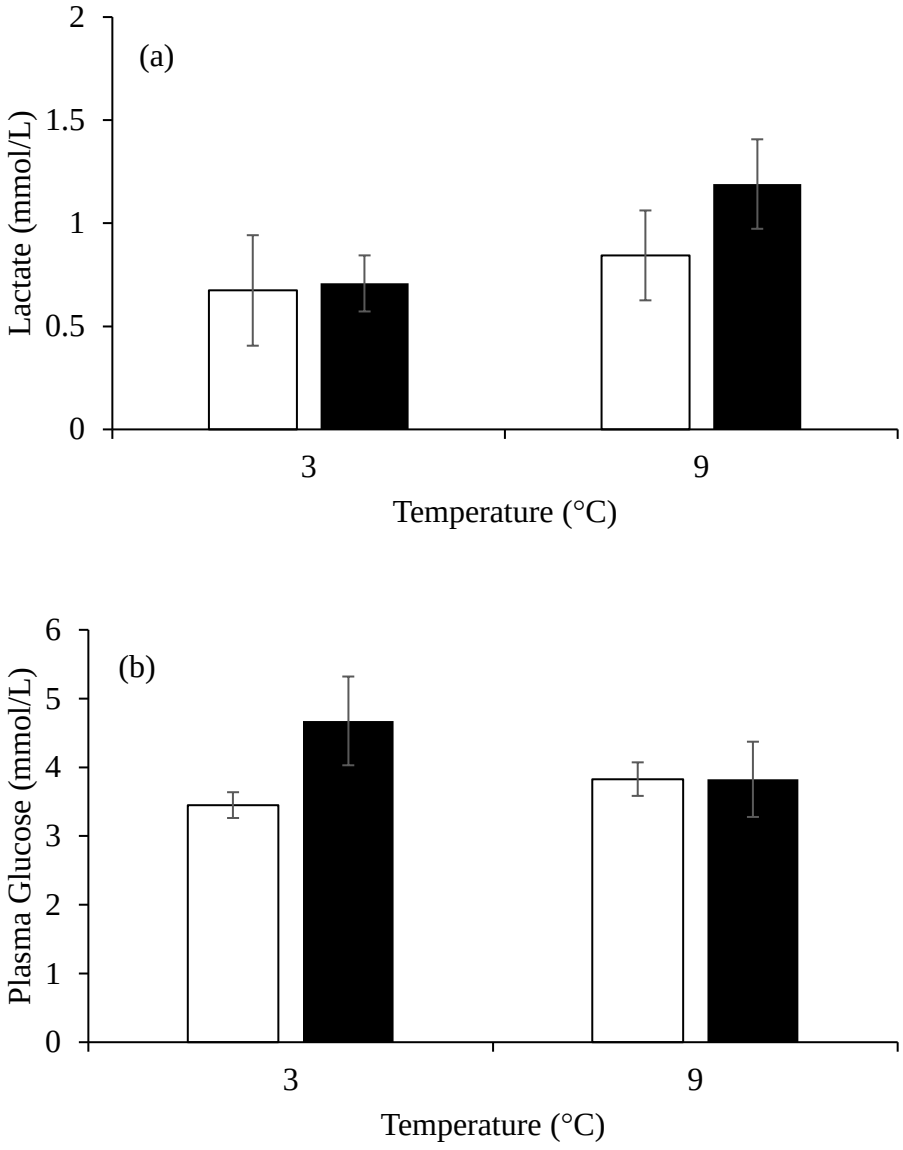

Figure 5. Average (± S.E.) plasma lactate concentration (mmol/L) (a), and plasma glucose concentration (mmol/L) (b) of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) at varying temperatures at rest (□) and following 5 minutes forced activity (■).

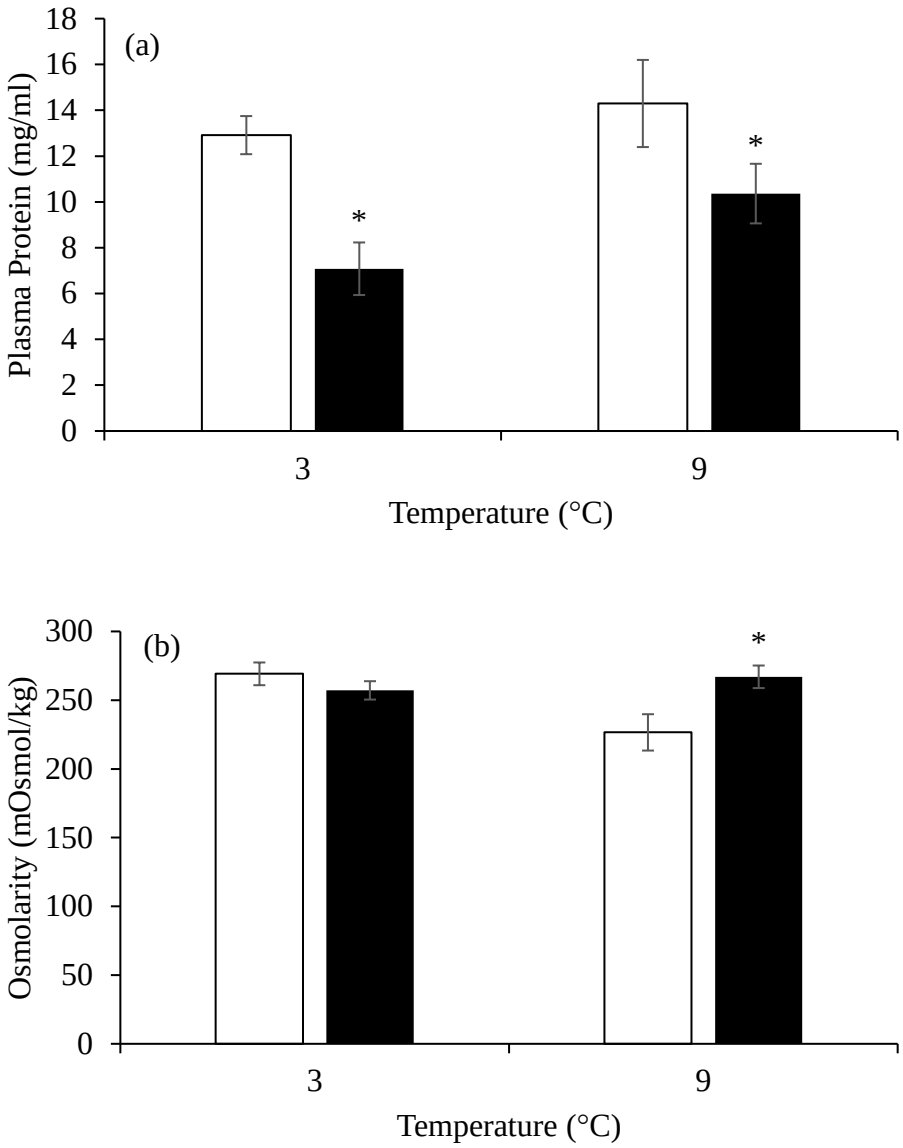

Figure 6. Average (± S.E.) plasma protein (mg/ml) (a), and osmolality (mOsmol/kg) (b) of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) at varying temperatures at rest (□) and following 5 minutes forced activity (■). *, significant difference (P<0.01) from the resting value at the same temperature.

Chapter 4

Discussion

4.1 General Observation of Activity

In general, fish held at the coldest temperature (3 °C) remained relatively inactive in comparison to fish at 9 °C; this lack of activity at 3 °C was relatively maintained even during feeding. A similar observation of decreased activity at colder temperatures has been documented in studies conducted on juvenile lake sturgeon (*Acipenser fulvescens*) (Peake, 1999; Deslauriers et al., 2018), green sturgeon (*Acipenser medirostris*) (Mayfield and Cech, 2004), walleye pollock (*Theragra chalcogramma*) (Sogard and Olla, 1998), and white sturgeon (*Acipenser transmontanus*) (Crocker and Cech, 1997). This reduced activity at lower temperatures can possibly be attributed to the reduced need for foraging, which would be required to meet the increased energy requirements for growth and metabolism observed at increased temperatures (Mayfield and Cech, 2004). Additionally, reduced activity helps to conserve energy at lower temperatures. Since critical swimming speed in fish is significantly reduced at lower temperatures (Koumoundouros et al., 2002; Deslauriers and Kieffer, 2012; Mandal et al., 2016; Yuan et al., 2017), foraging would therefore become more of an energetic expense than benefit. To offset this reduced intake of resources, it has been reported that sturgeon significantly suppress their metabolic rate, as much as ten-fold, in response to a drastic reduction in temperature (Deslauriers et al., 2018).

4.2 Resting Hematology

Hematocrit values at rest did not change between temperatures, suggesting that reduced temperatures (i.e. < 10 °C) does not alter the oxygen carrying capacity of Atlantic sturgeon at rest. Considering fish have a wide range of species-specific hematocrits (10-35%), the value for Atlantic sturgeon in the present study of 20%, likely reflects the lower oxygen carrying capacity needed by sturgeon (Baker et al. 2005 b). Previous work in our lab has shown that resting hematocrit levels for Atlantic sturgeon were significantly higher at 15 °C compared with 5 °C acclimation (Kieffer et al. 2011). This contrasts what was found for shortnose sturgeon (*Acipenser brevirostrum*); that no differences existed in the hematocrit levels across temperatures (5-25 °C; average hematocrit was approximately 21%; Kieffer et al. 2014; Zhang and Kieffer, 2014). These species differences may be related to the higher activity levels of shortnose sturgeon and differences in their ability to respond to, and recovery, from stressful scenarios (Baker et al. 2005 a, b).

At rest, glucose levels were similar across all temperatures (3, 6 and 9 °C) suggesting that lower temperatures may not act as a stressor to the mechanisms which control glucose levels in Atlantic sturgeon. It has been noted that due to the importance glucose plays in an organism's survival (i.e. supplying tissues with the necessary materials to perform basic survival processes), animals may alter mechanisms to ensure glucose levels remain stable. More specifically, common physiological processes altered include; reducing the rate of glucose use, increasing gluconeogenic and glycogenolytic potentials, and/or increasing liver glucose export capacity (Costas et al., 2011; Deslauriers et al., 2018). However, these mechanisms to stabilize glucose levels may in fact be triggered by a reduction in temperature, like that of colder temperatures reducing activity (see above). Under different stressors such as temperature increase (Spear and

Kieffer, 2016), or anoxia (Kieffer et al., 2011), plasma glucose levels can increase suggesting the controls, or initiating step, of these glucose maintaining mechanisms may have to do more with reduced temperatures than any other temperature or stressor. Additionally, other species of sturgeon, such as shortnose, show consistent glucose levels at colder temperatures (5 - 10 °C). However, at the same temperatures, glucose levels can be increased if a secondary stressor (i.e., salinity) is introduced (Downie et al., 2018). These findings further suggesting glucose stabilization is easier to perform at reduced temperatures in the absence of a secondary stressor, but in the presence of one (e.g. salinity, anoxia, exhaustive exercise) stabilization can be difficult. Furthermore, these mechanisms appear to be better developed in sturgeon compared with teleost fish, such as rainbow trout (*Oncorhynchus mykiss*) (Connors et al., 1978), Senegalese sole (*Oncorhynchus mykiss*) (Costas et al., 2011), or silver catfish (*Rhamdia quelen*) (Lermen et al., 2004), as plasma glucose levels in these fish can vary heavily between a reduced and optimal temperature.

As lactate is a product of anaerobic exercise, resting levels would not be expected to vary between temperatures unless a certain temperature warranted a large increase in anaerobic activity. In this current and previous studies on Atlantic sturgeon (Baker et al., 2005 a, b; Kieffer et al., 2011; Spear and Kieffer, 2016) there is a general lack of relationship between resting lactate levels across temperatures. As not all sturgeon species elicit this same stabilization across varying temperatures (Lankford at al., 2003; Leal et al., 2018), this suggests Atlantic sturgeon are not overly active at any temperature (or don't rely on anaerobic metabolism at rest) and are well suited for acclimation at varying temperatures.

Results of plasma protein at rest suggest that cold temperatures are not a stressful experience for Atlantic sturgeon as the levels did not vary between temperatures. Considering

many fish species release shock proteins under stress (i.e. temperature change) to protect cells from various damages (Wendelaar Bonga, 1997), it suggests Atlantic sturgeon are better developed to deal with the colder temperatures.

Resting osmolality values significantly decreased from 3 °C to 9 °C which would suggest that osmoregulatory processes in Atlantic sturgeon are altered at cold temperatures. This is supported by similar finding by Kieffer et al. (2011) where osmolality values significantly increased at 5 °C in comparison to 15 ° C; however, it should be noted that the exact values between each experiment varied (about 50 mOsmol kg^{-1}). Considering osmolality is a measure composed of a total of multiple ion fluxes, it would be more beneficial to explore the values of all the various ions and components (e.g. Na^{+}, Cl^{-}, K^{+}), as this would illustrate which ion fluxes are being affected more in comparison to others. This in turn would assist in associating which mechanisms are likely altered at lower temperatures. Due to the time frame of this experiment, and the unavailability of the equipment (ion analyzer requires new probes and sensors), these measures could not be performed. In comparison, other fish species such as the bald rockcod (*Pagothenia borchgrevinki*) (Lowe and Davison, 2005), emerald rockcod (*Trematomus bernacchii*) (Hudson et al., 2008), and Atlantic salmon (*Salmo salar*) (Wilkie et al., 1997) all showed an increase in osmolality at higher or lower temperatures (stress temperature) in comparison to an optimal temperature. Due to the altered osmolality values at higher and lower temperature in these species, in comparison to a neutral (or control) temperature, it suggests that osmoregulatory processes are heavily affected by temperature in these species. Furthermore, in other sturgeon species such as Adriatic (Cataldi et al., 1998), levels of osmolality stay quite constant at varying temperatures, suggesting the basic osmoregulatory functions in Atlantic sturgeon are not as well-developed as other sturgeon species to be maintained at varying

temperatures. However, it should be noted that samples are from different animals (and a relatively small sample size) in the current study which could partly explain any differences.

4.3 Post-Activity Hematology

Following exercise, the values for hematocrit stayed relatively similar at 3 °C to the resting levels but showed a significant increase at 9 °C following exercise. This result suggests that at warmer temperatures, where activity increases (see above), there is an attempt to increase red blood cell count (i.e. higher hematocrit), to meet the oxygen demand associated with this increased activity. Some evidence from our lab has previously shown that hematocrit doesn't change immediately following various stressors (exhaustive exercise; Baker et al., 2005 b; hypoxic stress at 15 °C; Kieffer et al., 2011; thermal increase stress; Spear and Kieffer, 2016). However, there have also been studies performed on Atlantic sturgeon in which hematocrit levels vary following a stressor or during the recovery period following the stressor (1 hour following exhaustive exercise; Baker et al., 2005 b; 2 hours following hypoxic stress at 5 °C; Kieffer et al., 2011). The time-frame in the present study is limited (i.e. immediately following exercise). The same varying results can be seen in shortnose sturgeon where hematocrit remains similar between control and stress (Spear and Kieffer, 2016), while under the same stress hematocrit can change significantly (Zhang and Kieffer, 2014). Due to this, it may be that hematocrit is not a sensitive enough indicator, in comparison to other indicators, due to its unreliability to show consistent results within replicates.

Post-activity plasma glucose showed a non-significant increase at 3 °C, while at 9 °C no significant differences were noted. The increase at 3 °C may be a result of multiple stressors (i.e. cold temperature and exercise) overwhelming the mechanisms which control or stabilize glucose

levels. The process behind this involves stressful events causing a release of catecholamines and corticosteroids, which in turn initiate glycogenolysis (Mazeaud et al., 1977; Deslauriers et al., 2018). As glycogenolysis is a strategy used by fish to stabilize glucose levels (see above), a process which alters this regulation would lead to altered glucose levels. Similar situations in which a small and/or reduced temperature change alone does not affect glucose levels, but the presence of a second stressor does, has been noted in Adriatic sturgeon (prolonged handling stress; Cataldi et al.,1998), shortnose sturgeon (acute salinity exposure; Downie et al., 2018), and green sturgeon (air emersion; Lankford et al., 2003). Furthermore, Lankford et al. (2003) saw a significant increase in green sturgeon plasma glucose in a reduced temperature post stressor, compared to a higher temperature post stressor, but no significant difference between control levels at the varying temperatures. Lankford et al (2003) suggest that these findings are a result of stress induced glycogenolysis and gluconeogenesis or decreased glucose utilization, further supporting the notion that multiple stressors may be too much for the glucose level maintaining mechanisms to support.

The fact that lactate levels did not increase post-exercise at any temperature in the present study reflects two important features of sturgeon physiology. The first is that sturgeon have a reduced/dampened physiological response to exhaustive exercise relative to teleost fishes such as salmonids and basses (Wood 1991; Kieffer et al. 1994; Kieffer et al. 2001; Baker et al. 2005 b; Kieffer and Cooke 2009; Brown and Kieffer 2018). The second feature is that blood lactate levels (both levels and the patterns for recovery) for other fishes, such as trout and salmon, are extremely temperature sensitive (Kieffer et al. 1994; Wilkie et al. 1997; Kieffer 2000; Galloway and Kieffer 2003). Lactate levels have been reported to increase in Atlantic sturgeon at 15 °C following exercise (Baker et al. 2005 b). Comparing the results between the current study and

those of Baker et al. (2005 b) suggest the same temperature dependent relationship might exist for Atlantic sturgeon but at a reduced magnitude. Future experiments should consider comparing post-exercise levels across a wider temperature range. Furthermore, temperature can affect diffusion of lactate from muscle into blood following exhaustive exercise (Kieffer et al., 1994). The physiological processes behind this involve colder temperatures reducing the rate of diffusion for metabolic end products (i.e. lactate), and/or blood perfusion to the muscle (Kieffer et al., 1994; Kieffer and Tufts 1996). Due to the reduced diffusion, lactate will remain in the muscle longer and not enter the blood as quickly as it would at higher temperatures (Kieffer and Tufts 1996). In response to this, lactate levels, while not the same in the blood, may be similar in production and/or concentration in the muscle at the varying temperatures. Additionally, it has been noted with *in vitro* research on white perch (*Morone americana*) muscle, kinematic viscosity of cytosolic extracts increases with decreasing temperature (Sidell and Hazel, 1987). As kinematic viscosity is a measure related to diffusive resistance, an increase would result in slower and/or reduced diffusion, meaning that reduced temperatures do in fact reduce diffusion (Sidell and Hazel, 1987). However, another possible explanation could be fish at the warmer temperature exerted themselves more during the forced activity (Lankford et al., 2003). Seeing as the amount of exercise was not quantified in this experiment the real reasoning cannot truly be justified, however a measure of lactate levels in muscle tissue in future studies could help to explain results.

Plasma protein decreasing at both temperatures following exercise did not follow the prediction made for the study. Levels of protein would rise due to the release of shock proteins under most stressful situations (Wendelaar Bonga, 1997), however these levels can only rise if the protein reserves are available. Under longer durations of cold temperatures, fish utilize all of

their glycogen reserves, following this the fish utilizes lipids and proteins (Deslauriers et al., 2018). Therefore, it may be that during exhaustive exercise, to meet energetic demands, Atlantic sturgeon utilize proteins as a fuel source. This would explain a decrease from resting levels as the protein in the plasma would be the first available to the tissues.

Osmolality in most teleosts following stress seems to vary between species and type of stress (Jain and Farrell, 2003; Hudson et al., 2008; Preston et al., 2017). As for sturgeon species, osmolality has been noted in many studies (Cataldi et al., 1998; Kieffer at al., 2001; Baker et al., 2005 b) to not vary as a result of stress. However, there have also been studies, this one included, suggesting that stress can significantly alter osmolality in sturgeon (Kieffer et al., 2011). To this point, stress performed on Atlantic sturgeon (Kieffer et al., 2011), at two different temperatures, elicited only a significant difference in osmolality post-stress at the colder temperature (5 °C). This would be the opposite of the results found in this study, where colder temperature levels stayed consistent while the warmer temperature showed a significant difference. While these studies stressed fish in different manners, it should be concluded that osmolality, as a whole, is a crude measure of stress response in sturgeon. It may be therefore more beneficially and informative to examine the components which make up osmolality (Na^+, K^+, Cl^-, etc.) individually, as certain ion fluxes may be affected more than others. Due to the varying values between stressors and species, it is difficult to pinpoint if and how mechanisms controlling osmolyte fluxes are being affected. As for this study a possible suggestion for increased osmolality in 9 °C following exercise and not 3 °C could be a similar reasoning seen for lactate, in that diffusion is delayed at colder temperatures (see above).

4.4 General Conclusion

Considering the sturgeon between the resting and exercised groups were vastly different in size, it is important to note this likely did not impact results as it has been shown in shortnose sturgeon that body size does not impact levels of hematological parameters (Brown and Kieffer, 2018). While there may be an alteration in the secondary stress responses measured in this study, sturgeon appear to be better suited to dealing with multiple stressors than other fish (Lankford et al., 2003; Baker et al., 2005 b). However, the general reasoning behind these lowered responses is debatable. Various hypotheses as to why sturgeon elicit such a small response to stress have been developed. More specifically life history, physiological design, and morphological characteristics are generally used to explain such results (Baker et al., 2005 b). Regardless of how the Atlantic sturgeon reduces its stress response, more studies are required examining wider ranges of parameters (i.e. temperature). By conducting studies with wide ranges in temperatures, better trends and results can be developed/modelled to show at what point certain mechanisms are altered by the stress imposed on the animal. Additionally, more studies must be conducted examining the acute exposure to reduced temperatures in Atlantic sturgeon as acute temperature drops can occur in the wild through natural or anthropogenic processes (Donaldson et al., 2008).

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Raw Data

Table 5. Raw data of resting fish hematological parameters

Fish (#)	Temperature (°C)	Hematocrit (Proportion/1)	Plasma Glucose (mmol/L)	Plasma Protein (mg/ml)	Plasma Lactate (mmol/L)	Osmolarity (mOsmol/kg)
1	9	0.087	2.8	13.85	1.537	292
2	9	0.2	4.1	24.04	2.668	230
3	9	0.189	3.9	16.58	1.840	232
4	9	0.151	4.9	8.036	0.891	240
5	9	0.189	3.4	13.49	1.497	206
6	9	0.165	4	12.95	1.437	202
7	9	0.194	3.7	11.13	1.235	184
8	6	0.2	4.3	19.49	2.163	240

9	6	0.179	4.2	19.49	2.163	272
10	6	0.211	3.1	16.95	1.881	234
11	6	0.087	5.3	15.85	1.759	273
12	6	0.26	3.3	16.95	1.881	216
13	6	0.215	4.2	15.13	1.679	273
14	3	0.2	3.4	13.67	1.517	261
15	3	0.2	3.4	10.22	1.134	258
16	3	0.182	4.2	12.4	1.376	270
17	3	0.188	3.1	16.4	1.820	273
18	3	0.188	2.9	12.4	1.376	306
19	3	0.194	3.7	12.4	1.376	247

Table 6 Raw data of exercised fish hematological parameters

Fish (#)	Temperature (°C)	Hematocrit (Proportion/1)	Plasma Glucose (mmol/L)	Plasma Protein (mg/ml)	Plasma Lactate (mmol/L)	Osmolarity (mOsmol/kg)
A	9	0.2	2.1	9.71766	1.078	280
B	9	0.2	5.8	7.80176	0.865	235
C	9	0.095	2.9	4.35314	0.483	280
D	9	0.222	4.1	12.59151	1.397	270
E	9	0.273	2.9	8.9513	0.993	255
F	9	0.273	6.4	16.42331	1.822	251
G	9	0.222	3.8	9.90925	1.099	310
H	9	0.266	2.6	13.16628	1.461	255

I	3	0.25	5.6	3.2036	0.355	279
J	3	0.231	4.1	7.61017	0.844	273
K	3	0.166	3.3	9.71766	1.078	280
L	3	0.2	7.9	8.9513	0.993	262
M	3	0.2	6.5	8.9513	0.993	240
N	3	0.2	3	4.35314	0.483	240
O	3	0.2	2.8	2.62883	0.291	233
P	3	0.231	4.2	11.25038	1.248	250

Table 7. Raw data of resting fish mass and length

Fish (#)	Mass (g)	Length (cm)
1	N/A	N/A
2	132	35
3	102	32.5

4	115	33
5	150	35
6	113	32
7	121	34
8	110	32
9	95	31
10	144	34
11	110	31
12	128	32
13	120	34
14	115	31
15	140	32.5
16	130	32
17	125	31.5
18	122	31
19	120	31

Table 8. Raw data of exercised fish mass and length

Fish (#)	Mass (g)	Length (cm)
A	64	26
B	59	28
C	78	30

D	85	30
E	78	30
F	67	26
G	56	27
H	61	26.5
I	117	34
J	64	32
K	71	32
L	69	30
M	58	27
N	71	30
O	81	31
P	83	31