

The effects of cold on routine activity and food consumption of juvenile
Atlantic sturgeon (*A. oxyrhynchus oxyrhynchus*) and shortnose sturgeon
(*A. brevirostrum*)

by

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ABSTRACT

Fishes living at poleward latitudes are exposed to challenging environmental conditions in the winter, such as cold temperatures that slows physiological processes (e.g., metabolic rate, swimming activity) and poor food availability that constrains energy supply. Atlantic (*A. oxyrhynchus oxyrhynchus*) and shortnose (*A. brevirostrum*) sturgeon co-exist in the Saint John River basin in New Brunswick but have divergent overwintering strategies. During winter, adult Atlantic sturgeon leave the river and move into the warmer seawater of the Bay of Fundy (~6-8°C), while shortnose sturgeon remain in the colder freshwater (~1°C), where they appear to be less active and possibly dormant on the riverbed. Using laboratory experiments, I investigated how acute cold challenge (15°C to 2°C) and low temperature acclimation (2-3°C) affects the routine activity and food consumption of two different juvenile age/size classes (<1 year and >1 year) Atlantic and shortnose sturgeon. During an acute cooling challenge both food consumption and activity declined in juvenile Atlantic and shortnose sturgeon, but never ceased. Atlantic sturgeon showed a drastic decline in activity around 12°C, whereas shortnose had a more gradual change and were more active at lower temperatures. Overall, the behavior of Atlantic sturgeon was more thermally sensitive to cooling compared with shortnose sturgeon, even after cold acclimation, suggesting these two species have distinct responses to cold that may be associated with their divergent overwintering strategies.

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List of Symbols, Nomenclature or Abbreviations

g: Gram

°C: Degrees Celsius

hr: Hour

12L:12D: The photoperiod used for all experiments and holding conditions; 12 hours light, 12 hours dark.

min: Minute

s: seconds

mm: Millimeter

cm: Centimeter

BL: Body lengths

n: Samples size

df: Degrees of freedom

ANOVA: Analysis of variance

GLMM: Generalized linear mixed effects model

Q₁₀: The thermal sensitivity quotient

U_{Burst}: Maximum acceleration

L: Liters

AS: Atlantic sturgeon

SN: Shortnose sturgeon

INTRODUCTION

The impacts of temperature on ectotherms

The environment fundamentally influences the ability of ectotherms, such as fishes, to acquire and use energy for survival, growth, and reproduction (Fry, 1947; Rankin and Jensen, 1994; Schulte 2015). In particular, temperature greatly affects an ectotherm's performance by setting its internal temperatures and consequently the rate and capacity for physiological processes (Rankin and Jensen, 1994; Falahatkar et al., 2013). The effect of temperature is profound for many physiological and behavioural processes, such as metabolism and activity, where, in general, processes are accelerated by warming and slowed by the cold (Rankin and Jensen, 1994; Morgan et al., 2001; Peck et al., 2002; Falahatkar et al., 2013; Kieffer et al., 2014; Safi et al., 2019). Thus, temperature influences fish distribution and, in many respects, it is the most important abiotic factor affecting an organism's biology (Fry 1947; Payne et al., 2015; Schulte, 2015). Because temperature impacts an ectotherm's performance, cooling or warming can challenge their behaviour, physiology, and survival (Rankin and Jensen, 1994; Sogard and Olla, 1998; Morgan et al., 2001; Lee et al., 2003; Richards, 2010; Marchand, 2013; Peck, 2016; Clarke, 2017; McMeans et al., 2020). This was first conceptualized by Fry (1947), who showed that the effects of temperature on fish and other ectotherms, can be modeled using a thermal performance curve (TPC; Figure 1). Ectothermic animals possess a thermal tolerance that is described by an upper (critical maximum, CT_{max}) and lower (critical minimum, CT_{min}) thermal limit to the thermal performance curve beyond which mortality occurs (Fry, 1947; Huey and Stevenson, 1979; Sinclair et al., 2016). With warming above CT_{min} , there is an increase in performance, before reaching an

optimal temperature (T_{opt}), followed by quickly decreasing performance as temperature approaches the CT_{max} (Huey and Stevenson, 1979; Sinclair et al., 2016). Conversely, cooling below T_{opt} will cause reductions in performance, such as swimming activity and food intake, until CT_{min} is reached at which death can occur due to frigid cold (Hathaway, 1927; Brett, 1964; Shrable et al., 1969; Crawshaw et al., 1982; Lemons and Crawshaw, 1985; Fracalossi and Lovell, 1994; Hanson et al., 2007; Deslauriers and Kieffer, 2012; Colchen et al., 2016).

The anchoring components of the thermal performance curve (CT_{max} , CT_{min} and T_{opt}) and the overall shape and breadth of the thermal performance curve may be adjusted depending on the length of the exposure to a new temperature, across acute to evolutionary timescales. When an individual animal is exposed to a change in temperature within hours or days, which can commonly occur in the wild especially in dynamic habitats such as estuaries (Elliott, 2002), they will show an acute response (Alfonso et al., 2020). For example, many fish species when warmed will show a stress response within hours, characterized by an increase in cortisol levels and heat-shock proteins (HSP) to help combat the impacts of acute temperature change (Alfonso et al., 2020). If an individual animal is exposed to a change in temperature over weeks or months their physiology may be adjusted due to compensatory acclimation, which can shift the TPC warmer or colder or change its shape (i.e., thermal plasticity) (Huey and Stevenson, 1979; Sinclair et al., 2016). For example, some fish species can alter their metabolic rate once acclimated to a new temperature, such as by increasing metabolic rate in the cold to compensate for the slowing effect of cold (Norin and Speers-Roesch, 2021). Finally, there is thermal adaptation that happens over generations, which also can

modulate the TPC magnitude, shape, and position along the temperature range. This can occur within and among species and involves genetic change that modifies physiology to become better suited to the prevailing conditions. For example, the common killifish (*F. heteroclitus*) have been found to be locally adapted along their broad latitudinal distribution, resulting in northern (*Fundulus heteroclitus macrolepidotus*) and southern (*Fundulus heteroclitus heteroclitus*) subspecies that differ in various temperature-dependent traits such as CT_{max} (Fangue et al., 2009). This is thought to have occurred because of the cooler versus warmer temperatures generally encountered by the northern and southern subspecies, respectively. Among species, differences in thermal environment over evolutionary time has resulted in the CT_{max} , CT_{min} and T_{opt} becoming considerably different between extratropical and tropical fish (Pörtner and Peck, 2010; Sunday et al., 2010; Payne et al., 2015; Comte and Olden, 2017; Nati et al., 2021).

Fish enduring large seasonal changes in temperature, such as temperate species, usually have the broadest thermal tolerance range (i.e., wider thermal performance curves), compared to tropical fish that experience less variation in ambient water temperatures (Pörtner and Peck, 2010; Sunday et al., 2010; Payne et al., 2015; Comte and Olden, 2017; Nati et al., 2021). In part, this is the result of a strong capacity among temperate species to acclimate to variable temperatures, thus shifting their thermal performance curve seasonally to optimize performance to the new conditions (Fry, 1947; Egginton and Sidell, 1989; Guderley and Gawlicka, 1992; Richardson et al., 1994; Beitinger et al., 2000; Pörtner and Peck, 2010; Allen et al., 2016). Since species have differing thermal performance curves and differing capacities to acclimate, it is important to know their physiological or behavioural responses to changes in environmental

temperature to predict how they will fare in future thermal regimes (Fry, 1947; Crawshaw, 1977; Eaton and Scheller, 1996; Sogard and Olla, 1998; Pörtner and Peck, 2010; McMeans et al., 2020).

Ongoing climate warming has led to most research being done on the biological impacts of higher temperatures (Ficke et al., 2007; Vinagre et al., 2015; Alfonso et al., 2020; McKenzie et al., 2020). It is well known that increasing temperatures impact many physiological processes, such as by increasing metabolic, growth, and heart rates, swim performance, and food and oxygen demands (Burel et al., 1996; Morgan et al., 2001; Peck et al., 2002; Koumoundouros et al., 2002; Kieffer et al., 2014; Prystay et al., 2017; Safi et al., 2019; Volkoff and Ronnestad, 2020). Specifically, much research has been conducted on the upper thermal limits of fish. The CT_{max} of many fish species is well known as an indicator of upper thermal tolerance (Beitinger et al., 2000; Neuheimer et al., 2011); temperatures approaching CT_{max} can diminish performance and cause stress, reducing fitness and survival, while exceeding CT_{max} becomes lethal (Beitinger et al., 2000).

On the other hand, many poleward species are challenged with harsh frigid temperatures during winter (Shuter et al., 1989). Cooling slows biological processes — for instance decreasing metabolic rate, swim performance and food intake — which can limit overall performance and survival (Hathaway, 1927; Brett and Higgs, 1970; Shrable et al., 1969; Crawshaw et al., 1982; Lemons and Crawshaw, 1985; Fracalossi and Lovell, 1994; Hanson et al., 2007; Deslauriers and Kieffer, 2012; Colchen et al., 2016). Certain fish species are more proficient at performance in the cold and acquiring energy in the winter compared to others, which can result in differences in their geographical

distributions or possibly their overwintering strategy (Fry, 1947; Pörtner and Peck, 2010; Shuter et al., 2012; Reeve et al., 2022). Recently, more studies are being conducted on cooling water temperatures as there is recognition that climate change can also have large impacts on cold adapted fish, such as salmonids, during winter (e.g., due to more variable winter temperatures) (Beitinger et al., 2000; Post and Parkinson, 2001; Donaldson, et al., 2008; Deslauriers et al., 2018; Gilbert and Farrell, 2021; Reid et al., 2022). It is important to determine species-specific physiological and behavioural responses to cold as this can help determine their ability to survive with the changing climate and understand their distribution.

The responses to winter cold temperatures in fish species

Deviation from a species' temperature optimum can result in utilization of different physiological or behavioural strategies to cope with temperature changes (Pörtner and Peck, 2010; Soyano and Mushirobira, 2018). During winter for example, fishes at poleward latitudes encounter challenging temperatures that reach their lowest levels of the year (Shuter et al., 2012). There are three overwintering strategies that fishes, and other ectotherms, can use to cope with these harsh winter/cold conditions. First, through behavioural mechanisms, some fish can avoid the harsh environmental conditions by migrating to warmer waters (Marchand, 2013; Soyano and Mushirobira, 2018). This avoidance strategy, however, incurs high energy demands and requires the physiological capacity to escape the unfavourable conditions (Brodersen et al., 2008; Marchand, 2013). Thus, migration can be risky, and it may be more beneficial to remain in the cold (Soyano and Mushirobira, 2018). If fishes do not or cannot avoid frigid temperatures, they must modify their behaviour and/or physiology to cope with the cold

(Lee et al., 2003; Shuter et al., 2012; Marchand, 2013; Deslauriers et al., 2018). Thus, a second strategy is to remain in the cold and undergo dormancy, where fish conserve their energy by becoming inactive, not feeding, and having a low metabolic rate (Crawshaw et al., 1982; Marchand, 2013; Soyano and Mushirobira, 2018; Reeve et al., 2022). During dormancy, a fish relies on stored energy reserves and having access to a safe refuge, with a trade-off of putting normal life processes ‘on pause’ (Marchand, 2013; Speers-Roesch et al., 2018; Reeve et al., 2022). The third overwintering strategy is compensatory physiological acclimation, where fish acclimate to the cold by adjusting their physiology to compensate for the slowing effect of the cold, which costs energy but allows them to continue to perform critical activities such as feeding and predator avoidance (Marchand, 2013; Soyano and Mushirobira, 2018). Examples of compensatory modification in the cold are an increase in metabolic enzyme activities to compensate for slowed reaction rates, and adjustments of lipid composition of biological membranes so they are more fluid to allow for regular function/activity to occur at low temperatures (Hazel and Williams, 1990; Angilletta, 2009; Marchand, 2013). Note, at a finer scale, dormancy and compensation strategies appear to exist as the extreme opposite ends along a continuum (Reeve et al., 2022); for example, some fish may be better termed winter-lethargic, which is intermediate between dormancy and compensation where fish are still active and feed during the winter but at very low levels (Shuter et al., 2012; Reeve et al., 2022). Each overwintering strategy has its own benefits and trade-offs, thus presenting alternatives that have evolved repeatedly in various fish species, likely depending on factors such as phylogeny and local environmental characteristics.

Quantifying thermal sensitivity of biological rates

A standard method to quantify the response of biological rates (e.g., metabolic rate, activity, growth, or food consumption) to changing temperature is to use the thermal sensitivity quotient (Q_{10}) of Arrhenius and Van't Hoff. Q_{10} is the fold change in a biological rate standardized over a 10°C change in temperature (Peck, 2016). A Q_{10} of ~2-3 is typical of rates that are simply following physicochemical Arrhenius (thermodynamic) effects on underlying chemical reaction rates (Peck, 2016; Clarke, 2017; Speers-Roesch et al., 2018). On the other hand, higher Q_{10} values indicate a change in the rate that is greater than would be expected based on Arrhenius effects alone, suggesting the involvement of active responses by the animal or major thermal perturbation (i.e., a constraint) of the rate (Crawshaw, 1984; Costa et al., 2013; Staples, 2016; Peck, 2016; Speers-Roesch et al., 2018). Q_{10} values can also indicate a thermal compensation resulting from acclimation, specifically if the Q_{10} after acclimation is reduced compared with that after acute temperature change. Such thermal compensation are the result of physiological adjustments that help offset the effect of temperature and make the biological rate less thermally sensitive (Clarke, 2017). Q_{10} is a simple comparative metric of the thermal sensitivity of important biological rates and is broadly applied to model the impacts of our changing climate (Dillon et al., 2010; Clarke, 2017).

The importance of swimming and metabolic demands of fishes

Movement, achieved via swimming in fishes, is a fundamental trait in animals needed for basic activities, such as feeding, escaping predators/unfavourable environments, and migratory behaviours (Reidy et al., 2000; Deslauriers and Kieffer, 2012; Gunderson & Leal, 2016). Activity level is used as a behavioural characteristic

indicating the magnitude of movement; it can be affected by changing environmental conditions such as decreasing temperatures, in part because activity depends on temperature-sensitive physiological processes and also the level of activity can be related to covariation in an animal's demands (e.g., food consumption, which itself is affected by temperature; Beamish, 1978; Karasov, 1992; Nilsson et al., 1993; Sogard and Olla, 1998; Speers-Roesch et al., 2018). Movement is costly because increases in muscular contraction and associated physiological processes increase tissue energy demands and thus the metabolic rate of the animal; thus, modifications of activity levels can save energy or expend it as needed (Reeve et al., 2022).

Fish utilize different types of swimming behaviours, reflecting required activity levels and environmental constraints (Kieffer, 2000). Spontaneous movement/activity is defined as the random or exploratory swimming behaviour that fish perform during their normal daily activities (Brett, 1964; Peterson and Anderson, 1969; Nilsson et al., 1993; Speers-Roesch et al., 2018; Reeve et al., 2022). Sustained swimming is constant movement that is not particularly fast and can last for hours or even days (Jones, 1982; Deslauriers and Kieffer, 2012; Hvas and Oppedal, 2017). Spontaneous activity and sustained swimming rely heavily on aerobic metabolism primarily in red muscle, which can supply energy at a relatively slow but long-term rate (Brett and Grove, 1979; Deslauriers and Kieffer, 2012; Palstra and Planas, 2011). On the other hand, burst swimming occurs when the fish swims at high velocities for short periods of time. Supported by anaerobic metabolism, which generates rapid and large amounts of energy but cannot be sustained due to accumulation of anaerobic by-products, this type of

swimming is typically utilized during predator-prey interactions and/or strenuous periods during migration (Jones, 1982; Kieffer 2000; Deslauriers and Kieffer, 2012).

Swim performance is a survival- and fitness-linked trait for fish and thus a focus of investigations into the impacts of stressful environments on fishes (Kolok, 1999; Kieffer, 2000; Plaut, 2001). Two commonly used methods to measure swim performance include critical swimming speed (U_{crit} , aerobic swimming) and burst swimming (U_{burst} , anaerobic swimming) (Plaut, 2001; Deslauriers and Kieffer, 2012). These can be measured by placing a fish in a swim tunnel/flume and either slowly (U_{crit}) or quickly (U_{burst}) increasing the water velocity until the fish is fatigued and can no longer swim against the current (Deslauriers and Kieffer, 2012). Of the two swimming tests, the burst swimming (U_{burst}) test (seconds to minutes) is quicker to complete experimentally while being particularly relevant to life-or-death situations such as capturing prey or avoiding predation. While assessments of U_{crit} and U_{burst} test the swimming performance of fish, measurement of spontaneous activity has increasingly been recognized as an important metric when assessing the impact of environmental conditions on fishes, given that fish spend most of their time spontaneously moving around and this costs a substantial portion of their daily energy budget, consequently impacting their food consumption and need to forage (Brett, 1964; Peterson and Anderson, 1969; Nilsson et al., 1993; Kolok, 1999; Lee et al., 2003; Speers-Roesch et al., 2018; Reeve et al., 2022). It is commonly noted in many fish species that activity and swimming is positively correlated with temperature (at least up to the T_{opt}) and thus, measuring swim performance and activity is an important metric when investigating a fish's thermal biology (MacCrimmon and Robbins, 1981; Randall and Brauner, 1991; Sogard and Olla, 1998; Wurtsbaugh and

Cech, 1983; Pang et al., 2013; Penghan et al., 2014; Downie et al., 2020; Yin et al., 2021).

Activity can vary within an individual and can have impacts on their energy demands, and higher activity can cause an increase in their energy expenditure and require greater food consumption. Therefore, changes in activity are correlated to changes in metabolic rate (Reeve et al., 2022). Metabolic rate can be used to estimate a fish's energy expenditure, which in turn can relate to growth rate and overall fitness (Johnston and Dunn, 1987; Morash et al., 2021; Norin and Speers-Roesch, 2021). Whole-animal metabolic rate can be measured as oxygen uptake rate (MO_2) using respirometry (Clark et al., 2013; Norin and Speers-Roesch, 2021). Spontaneous activity increases an ectotherm's energy expenditure (routine metabolic rate; RMR) above their standard metabolic rate (SMR), which indicates the basic costs of living (Norin and Speers-Roesch, 2021). There are many factors that can cause changes to a fish's metabolic rate, such as temperature, salinity, and food availability; temperature, in particular, is a master controller of metabolic rate (Richards, 2010; Norin and Speers-Roesch, 2021). Metabolic rate is sustained by food consumption, by which animals gather their energy (Pörtner and Peck, 2010; Rodgers et al., 2019). Thus, metabolic rate sets the demands of an animal for food (Norin and Speers-Roesch, 2021). Because temperature influences metabolic rate, it also impacts food consumption, with appetite normally increasing with warming and decreasing with cooling temperatures in line with increasing and decreasing metabolic rate, respectively (Hathaway, 1927; Brett, 1964; Shrable et al., 1969; Jutfelt et al., 2021). In winter, the ability to acquire energy can become a challenge as food becomes scarce and swimming can become difficult, although the cold also lowers energy requirements

(Shuter et al., 2012; Rodgers et al., 2019). Overall, measurements of activity and feeding can help to determine the influence certain environmental factors, such as temperature, have on a fish's physiology and their capacity to optimally perform basic activities (Johnston and Dunn, 1987; Giberson and Litvak, 2003; Pörtner and Peck, 2010; Deslauriers and Kieffer, 2012).

Life histories of Atlantic sturgeon and shortnose sturgeon

Sturgeons (Order Acipenseriformes) are an ancient group of fishes with a natural distribution restricted to the northern hemisphere, where they can experience a broad range of temperatures (Auer, 2004; Cech Jr. and Doroshov, 2004). All 27 species of sturgeon have been listed as species of special concern or endangered (IUCN, 2004). This is largely due to the anthropogenic impacts of increased damming, pollution and overfishing combined with the slow-growing, long-lived life history of sturgeons (Kynard et al., 2016; Bard and Kieffer, 2019). Sturgeon are culturally and ecologically important species and are very important aquaculture species in North America, as they are used for caviar and meat (Bronzi et al., 2011; Kynard et al., 2016; Tavakoli et al., 2021). There is a general lack of information on their biology, including the functional responses to environmental change that have allowed them to persist across vast geological time scales (Baker et al., 2005; COSEWIC, 2011; Zhang and Kieffer, 2014; Fisheries and Oceans Canada, 2016).

Two species of sturgeon inhabit coastal waters of eastern North America: the Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*; AS) and shortnose sturgeon (*Acipenser brevirostrum*; SN). These co-generic species have overlapping distributions from the southern parts of Florida, United States to Labrador, Canada (Dadswell, 1979;

Smith and Dingley, 1984; Kynard 1997; COSEWIC, 2005; Taylor et al., 2016). They are distributed from the southern parts of Florida, United States to Labrador, Canada (Kynard 1997; Taylor et al., 2016). Northern shortnose sturgeon species are amphidromous; they are known to reside primarily in freshwater or estuaries as juveniles as well as adults (Dadswell, 1979; Kynard, 1997; Kynard et al., 2016), while Atlantic sturgeon are anadromous, meaning they spend most of their time in brackish or coastal marine habitats as adults and return to freshwater to breed and spawn (Fernandes et al., 2010; Stewart et al., 2015; Beardsall et al., 2016; Whitmore and Litvak, 2018). However, both species as juveniles share the same habitat year-round. For example, in New Brunswick Canada, they are known to stay in the Saint John River for the first few years of their lives (Fernandes et al., 2010; Whitmore and Litvak, 2018). In the wild, feeding seems to be restricted during the winter for adult shortnose sturgeon because they move into estuaries in the colder months and reduce food consumption when temperatures drop below ~10°C (Dadswell, 1979; Kynard, 1997; COSEWIC, 2015). On the other hand, they feed voraciously in the warm months, starting in June into September (Dadswell, 1979; Dadswell et al. 1984; Kynard et al. 2016). Less is known about adult Atlantic sturgeon feeding behavior, but they are known to forage in marine coastal regions, such as the Bay of Fundy, close to their natal rivers, where temperatures seem to be more constant year-round (Bain, 1997; Taylor et al., 2016; Beardsall et al., 2016; Whitmore and Litvak, 2018).

Even though these two species can be found in similar habitats, they appear to have evolved divergent overwintering strategies as adults, specifically in New Brunswick, Canada (Krieger et al., 2000; Li et al., 2007; Taylor et al., 2016). For

example, during winter in the Saint John River basin, New Brunswick, adult Atlantic sturgeon are found in the warmer seawater of the Bay of Fundy (~6-8°C), while shortnose sturgeon remain in the colder freshwater (~1°C) (Dadswell, 1979; Smith and Dingley, 1984; Li et al., 2007; Fernandes et al., 2010; Stewart et al., 2015; Taylor et al., 2016; Beardsall et al., 2016; Andrews et al., 2020). The explanation for the differing overwintering strategies between the two species is unknown but could be related to differences in thermal performance of physiological and/or behavioural traits.

Comparing thermal responses of Atlantic sturgeon and shortnose sturgeon

Atlantic sturgeon and shortnose sturgeon have been focal species for investigation into the thermal tolerance and performance of sturgeon. Studies have examined the effects of temperature on a broad range of traits in each of these two species, including their critical thermal maximum (CT_{max}), swim performance, metabolic rate, growth, and hematological stress responses (Kelly and Arnold, 1999; Hardy and Litvak, 2004; Ziegeweid et al., 2008; Kieffer et al., 2011; Deslauriers and Kieffer, 2012; Zhang and Kieffer, 2014; Penny and Kieffer, 2014; Kieffer et al., 2014; Spear and Kieffer, 2016; Zhang and Kieffer, 2017). However, overall, little comparative research has been done on these two species' responses to temperature and even less in response to cold. Spear and Kieffer (2016) showed that 15°C-acclimated juvenile shortnose sturgeon and Atlantic sturgeon have a similar critical thermal maximum (CT_{max}) of approximately 30°C. Hardy and Litvak (2004) found that Atlantic sturgeon larvae were more efficient at utilizing their yolk and developing faster with varying temperatures (13-21°C) compared to shortnose sturgeon. Most work to date has focused on temperatures above 5°C, often at relatively warm temperatures of 10°C or 15°C. Less is known about these species'

physiological and behavioural responses to cold temperatures, as the lowest temperature that any of these papers examined was 5°C, even though juveniles of both species can inhabit waters as low as 1°C for several months in winter (Li et al., 2007; Fernandes et al., 2010; Taylor et al., 2016). Furthermore, most of the literature is focused on these two species' thermal physiology; less is known about their behavioural responses to temperature change (Colchen et al., 2016). Specifically, there is a lack of knowledge on the effect that frigid temperatures in winter have on Atlantic sturgeon and shortnose sturgeon's behavioural responses, such as activity and feeding (Krieger et al., 2000; Baker et al., 2005; COSEWIC, 2011; Zhang and Kieffer, 2014; Taylor et al., 2016; Fisheries and Oceans Canada, 2016).

Outline and goals of my study

For my MSc thesis, I compared the responses of shortnose sturgeon and Atlantic sturgeon to acute cooling and cold acclimation to help assess whether species-specific differences in thermal sensitivity of important behavioural traits are associated with these species' divergent overwintering strategies. Specifically, the objectives of my study were: i) to characterize potential species-specific effects of cooling to winter temperatures on activity and feeding; and ii) to identify whether fish size affects these responses to the cold, because both Atlantic and shortnose sturgeon are known to have ontogenetic shifts in habitat use (Kynard and Horgan, 2002; Kynard et al, 2016). Using video recordings and automated tracking software in the laboratory, I monitored the food consumption and activity of two different size/age classes of Atlantic sturgeon and shortnose sturgeon (~25g and <1 year; ~150g and >1 year) in response to acute cooling (15°C→3-2°C) and cold acclimation (3 weeks at ~2°C). In addition, the effects of acute cooling on swim

performance in shortnose sturgeon was assessed. I used the results to help evaluate if shortnose sturgeon and Atlantic sturgeon, which live in the same areas, have differing overwintering strategies due to potential species-specific thermal responses.

I hypothesized that shortnose sturgeon have evolved a ‘cold-adapted’ phenotype characterized by better cold tolerance and performance (i.e., lower thermal sensitivity), while Atlantic sturgeon will have a ‘warm-adapted’ phenotype in which performance in the cold will be greatly constrained (i.e., greater thermal sensitivity), consistent with their adult behavioural overwintering strategies of moving into the warmer Bay of Fundy. I predict that shortnose sturgeon, in comparison with the Atlantic sturgeon, will have greater activity in the cold (potentially via greater compensation resulting from acclimation), and a better capacity to feed opportunistically in the cold. Testing juveniles will be informative since this is the stage of life when they are both found in the same freshwater environments yet may exhibit distinct responses to cold that will help explain the development in adults of their differing winter coping strategies. As well, looking at different sized juveniles may further provide information on these species’ thermal biology (sensitivity) since they show ontogenetic shifts in winter habitat use. For example, this was observed by Zhang and Kieffer (2014), who determined there was a size-dependent effect on upper thermal tolerance (CT_{max}).

METHODS

Experimental Species

Atlantic sturgeon and shortnose sturgeon juveniles (<1 years old; Figure 2) of mixed sexes were obtained from Acadian Sturgeon and Caviar (Carter’s Point, Kingston,

New Brunswick, Canada; <https://www.acadian-sturgeon.com/en>) in 2019. Sturgeon species were initially kept in separate one meter circular flow-through holding tanks supplied with dechlorinated Saint John city freshwater (12:12 light:dark) for approximately one month before the start of Experiment 1.

The shortnose sturgeon and Atlantic sturgeon used for Experiment 1 (<1 year old; see below) were then transferred to a separate re-circulating freshwater system (12:12 light: dark) consisting of four 80 L glass aquaria [50 cm (L) x 27 cm (W) x 25 cm (H); Figure 3; 20 fish per aquaria] and a sump containing bio-media (Sweetwater SWX Bio-Media, Pentair Aquatic Eco-Systems, Inc. Cary, NC USA) to reduce ammonia. Water temperature was maintained at $15^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$ using a water chiller (Arctica 1/3 horsepower, JBJ Chillers, St. Charles, MO). The fish were held for a minimum of four weeks in the recirculation systems before experimentation. Fish were fed daily with dry 1.5mm sinking pellets (EWOS, Surrey, British Columbia, Canada) to satiation. All experimental fish were weighed, and total length was measured before and after the experiment. These <1 year old fish used for Experiment 1 were of similar weights between species (SN: 24.4 ± 1.0 g, n=17; AS: 24.8 ± 2.0 g, n=15; Table 1).

For Experiment 2 (see below), shortnose sturgeon and Atlantic sturgeon originally obtained from Acadian Sturgeon and Caviar in 2019 were kept and continued to grow for ~ 1 year such that they were larger and >1 year in age by the time they were used for Experiment 2. These fish were kept in one-meter circular flow-through holding tanks and temperature was maintained at $15^{\circ}\text{C} \pm 1^{\circ}\text{C}$ using aquarium heaters (Aquatop 800W titanium aquarium heater, Aquatic ecosystems, Florida, USA). Fish were fed daily with dry 5mm sinking salmonid pellets (EWOS, Surrey, British Columbia, Canada) to

satiation. Fish were held under these conditions for a minimum of four weeks before experimentation. Fish were elastomer tagged (Kozlowski et al., 2016; Northwest Marine Technology Inc., Anacortes, Washington, USA), and were allowed to recover for approximately seven days before the start of Experiment 2. Experiment 2 was conducted with sturgeon of comparable weights between species (SN: 174.5 ± 15.2 g; AS: 131.2 ± 7.8 g; Table 1).

Experiment 1: Routine activity and food consumption during acute cooling in small (~25g) juvenile Atlantic sturgeon and shortnose sturgeon

Experimental set-up

I constructed an experimental set-up consisting of 12 individual plastic arenas [24 cm (L) x 36 cm (W) x 12 cm (H)] placed within a shallow freshwater re-circulating plexiglass wet table [183 cm (L) x 97 cm (W) x 20 cm (H); Figure 4a]. The size of the arenas was selected to allow fish to freely swim within (specifically, able to turn around), while limiting the overall size of the set-up so that we could record all the fish's behaviour simultaneously. Eight continuously operating infrared lights (940 nm; CMvision, Houston, TX, USA) were placed below the wet table, situated off to the side to silhouette the fish from below, while photoperiod was maintained at 12:12 with overhead visible lights. A white translucent acrylic sheet was placed between the infrared illumination area and the wet table to diffuse the infrared light. Sturgeon did not show any indication that they could see the infrared lights (e.g., there was no startle response or other movements in response to on/off of the lights), which matches observations for other fish species under similar conditions (Speers-Roesch et al., 2018; Reeve et al., 2022). Sunrise/sunset lights were used to gradually introduce and remove light from the

system at the beginning and ending of each light period (30 min brightening or dimming periods) to minimize potential changes in behaviour due to rapid on/off lighting (Ryu et al. 2020). Routine activity of the fish was monitored by two near-infrared-sensitive cameras (640 x 480 p, 10-15 fps; IDS Camera, IDS Imaging, Obersulm, Germany) mounted above the arenas and videos were recorded in ISpy (v.7.2.10; Figure 5). The plastic arenas were fitted with an inflow and outflow that allowed water to circulate within the arena before draining out through the water bath back into the sump, where it was filtered, chilled to the required temperature, and then pumped back to the arenas.

Experimental protocol

Each acute cooling trial involved simultaneously cooling shortnose sturgeon and Atlantic sturgeon within the experimental set-up at 1.5°C per day from 15°C to 3°C. At the beginning of each trial six shortnose sturgeon and six Atlantic sturgeon acclimated to 15°C were randomly chosen by numbering the containers and using an online random number generator (<https://www.random.org/>) to determine which fish was placed in each of the 12 arenas (each fish was placed in an individual arena). The fish were allowed to adjust to the arenas for 24 hours prior to starting the experiment. A clear acrylic sheet was placed on top of the arenas to prevent escape. Recording started on the morning of Day 1 when water temperatures were 15°C. Temperature was lowered 1.5°C each subsequent morning (this took ~1hr) down to 3°C (reached on day 8) using an in-line Arctica chiller (1/3 hp Arctica, JBJ Chillers, St. Charles, MO). Thus, a recording was made for approximately 24 hours at each temperature, which was used to measure activity of fish at each temperature (see below). During each morning cooling period, fish were fed 1% of their bodyweight with 1.5 mm commercial pellets (EWOS Canada Ltd.,

Surrey, BC, Canada); the next morning any uneaten pellets were removed from the chamber and counted to obtain the food consumption ((# of pellets eaten/# of pellets given) x 100) over 24 hours at each temperature. Overall, there were three trials each of which included 6 fish per species. Directly after the acute cooling experiment, one of the three trials of shortnose sturgeon (n=5) and Atlantic sturgeon (n=3) were left in the experimental set-up for an additional 5 days at 3°C to examine the stability of the response at 3°C. In this trial, three Atlantic sturgeon did not feed at 15°C (i.e., after being acclimated to the arenas) and one shortnose died part way through the acute cooling experiment, so they were removed from all analyses since these Atlantic sturgeon did not eat even at the initial 15°C temperatures and the shortnose sturgeon did not finish the trial. Thus, sample sizes in Experiment 1 were 17 for shortnose sturgeon and 15 for Atlantic sturgeon (except for the additional 5 days at 3°C as explained above).

Swim performance of acutely cooled shortnose sturgeon

Using a group of different fish taken from the holding tanks, the acute temperature-dependency of swim performance was measured in shortnose sturgeon using the burst swimming (U_{burst}) test. 15°C acclimated sturgeon (n=8-10) were exposed to acute cooling (1.5°C per day) in a small re-circulating system until they reached one of the desired test temperature (either 15°C, 12°C, 9°C, 6°C or 3°C). Fish were only tested at one temperature (i.e., different groups of fish were used for each acute temperature exposure) and were similar in size to the fish used in the activity and feeding portion of Experiment 1 (Table 4). Once at the testing temperature, an individual fish was placed into a 30 L swim flume (Loligo Systems, Denmark; Figure 6A), where it was allowed to adjust for 30 minutes at a minimal velocity of 5cm s⁻¹ (Deslauriers and Kieffer, 2012).

Following the adjustment period, the water velocity was rapidly increased by a set speed of 10 cm s⁻¹, every five seconds, until the fish could not maintain position in the flume and became impinged on the back screen of the flume (Figure 6B). The swimming test for each fish was recorded using a video camera, to confirm the time and speed at which the fish became exhausted. U_{burst} was calculated using the formula: $U_{burst} (\text{cm s}^{-1}) = V_f + [T_1 \times (t \times d_v)^{-1}]$. V_f is the speed (cm s⁻¹) of the last swimming period, T_1 is the final time (s) before the fish becomes fatigued, t is the time interval (s) between each velocity increment and d_v is the velocity (cm s⁻¹) increment. The velocities measured in the swim test were divided by the total length of each fish to report U_{burst} in body lengths per second (BL s⁻¹). Atlantic sturgeon would not swim in the flume (K. Middleton, personal observation; Miller and Kieffer, unpublished); thus, U_{burst} speeds could not be evaluated for this species.

Experiment 2: Routine activity and food consumption during acute cooling and following cold acclimation in larger (~150g) juvenile shortnose sturgeon and Atlantic sturgeon

The experimental system used for Experiment 2 was similar to Experiment 1 except there were only 3 individual fish plastic arenas run at once instead of 12 in Experiment 1, but each arena was larger [94 cm (L) x 37 cm (W) x 11 cm (H)]. Using a similar protocol described above for the acute cooling experiment, larger (~150 g) shortnose sturgeon and Atlantic sturgeon were placed individually into the three experimental plastic arenas (n=11). Species were allocated in a 2:1 ratio, and the dominant ratio was alternated between species between trials; for example, if in the first trial 2 shortnose sturgeon and 1 Atlantic sturgeon were run, then for the subsequent trial 2 Atlantic sturgeon and 1 shortnose sturgeon were run, etc. Temperature was controlled by

two in-line Arctica chillers (Arctica, JBJ Chillers, St. Charles, MO) to reach the lowest temperature of 2°C. Six continuously operating infrared lights (850nm, CMvision Houston, TX, USA) were directed underneath the experimental containers to silhouette the fish from below, while photoperiod was maintained at 12:12 with overhead visible lights. Two near-infrared-sensitive cameras (IDS, Germany) were mounted approximately one meter above the arenas to continuously record the fish activity. Fish were fed 1% of their bodyweight daily using larger 5mm commercial pellets (EWOS Canada Ltd., Surrey, BC).

Activity and daily food consumption were measured during acute cooling by decreasing temperature 1.5°C a day from 15°C to ~2°C over 9 days, following the same methods as Experiment 1. Then, the fish were taken from the arenas and placed in a re-circulating 1 m diameter circular holding tank supplied with dechlorinated Saint John city freshwater at 2°C and held there for 3 weeks (12:12 light:dark). The temperature was controlled by an in-line Arctica chiller (1/3 hp Arctica, JBJ Chillers, St. Charles, MO). After approximately 3-week cold acclimation, fish were returned to the experimental set-up for 72 hours at 2°C to measure their activity and food consumption as described above.

Data Analysis and Statistics

Analysis of routine activity using automated tracking software

The routine activity of the fish was measured over ~2-hour intervals within the ~24 hour video recording at each temperature using an automatic tracking software (ToxTrac, v2.84; Rodriguez et al., 2017, Rodriguez et al., 2018; <https://toxtrac.sourceforge.io/>). The pixel-to-distance calibration required for calculations

of distance moved was carried out using known distances and video pixel measurements in ImageJ (Version 1.52a, National Institutes of Health, USA; Schneider et al., 2012). In the Toxtrac program, the total distance travelled by each fish over the 2-hour interval at each temperature was divided by the total visible time tracked during the same interval, to obtain an average velocity over the 2-hour period. Cooling to the next lower temperature occurred in the morning prior to fish feeding at 10am and any measurements during this 1hr period were not included in the analyses. For the analyses, the period representing the day was 11:00-19:00, and night was represented from 20:00-7:00. The average velocity of each fish over the ~2-hour intervals was then averaged over each of these day and night periods at each temperature to obtain a single day value and a single night value for each fish at each temperature. The routine day and night activity of each fish was standardized to the fish's total body length and reported as body lengths per minute (BL min^{-1}).

The Toxtrac software is sensitive to small movements such as tail twitches or minor movements from water flow, so such background noise must be removed from the tracking analyses of routine activity. The background noise was determined by calculating the average velocity of each fish during a period where we visually confirmed their inactivity (a typical period was ~5-10 minutes of inactivity for any individual fish) and subtracting this value from the same fish's overall velocity (mm/s) measurements. Velocity was then standardized to the fish's body length (see above). For the small fish (~25g) the average background noise was 0.99 mm s^{-1} (1.62% of average activity was explained by background noise) and for the large fish (~150g) was 1.08 mm s^{-1} (2.0% of average activity was explained by background noise).

Calculating Q₁₀'s

Thermal sensitivity (Q_{10}) of routine activity and food consumption, as well as U_{burst} (in shortnose sturgeon), was calculated across relevant temperature intervals (see Table 2) using the equation: $Q_{10} = (R_2/R_1)^{(10/(T_2-T_1))}$. R_1 is the initial rate for any biological process at temperature T_1 , and R_2 is the new rate at the new temperature T_2 .

Calculating specific growth rate (SGR) of sturgeon

Specific growth rate (SGR) of the fish were calculated using the equation: $SGR = (\ln W_f - \ln W_i \times 100)/t$. W_f is the final weight of fish after being acclimated to 2°C, W_i is the initial weight of fish at 15°C before start of experiment, and t is the time (days) between the initial and final weights (Lemke, 1977; Fonds et al., 1992; Crane et al., 2019).

Statistical analysis

All statistical analyses were done in R (Version 1.2.5033, Rstudio Inc., Boston, MA, USA; <http://www.rstudio.com>). Significance was accepted at $p \leq 0.05$ and values are stated as means \pm standard error of the means (S.E.M.), unless otherwise noted.

For Experiment 1 and 2, routine activity data was analyzed using a generalized linear mixed effects model (GLMM: lme4 package; family: Gamma, link= “log”). The Gamma distribution was chosen because the data for activity level was positive continuous and not normally distributed. For the initial analysis for each species, the dependent variable was routine activity, with fixed factors of temperature, and day/night, with the individual fish being a random factor. The factor, day/night were subsequently removed from the model as there was only a small difference between day and night within either species at each temperature.

After removing the variable day/night from the analyses, Experiment 1 and 2 were analyzed separately using generalized linear mixed effects models (GLMM: lme4 package; family: Gamma, link= “log”). This statistical approach was used to ascertain the effect of species and temperature on activity. Using the function glmer (lme4 package), a GLMM was run with activity as the dependent variable, with the fixed factors species and temperature, and the random factor being the individual fish.

The effect of acute cooling on food consumption was analyzed using a GLMM Template Model Builder (glmmTMB package; family=beta; link=logit). This distribution was chosen because the data for percent food consumption was not normally distributed and contained zeroes. The food consumption data were converted to proportions and transformed to remove zeroes ($y'=(y*(n-1)+0.5)/n$); where y is food consumption (proportion) and n is the sample size) to satisfy the assumptions of glmmTMB (family: beta) procedure. Food consumption (% food consumption converted to a proportion) was the dependent variable, species and temperature were the fixed factors and the individual fish were considered a random variable.

To compare the acute and acclimated 2°C activity and feeding (Experiment 2) or to compare the acute 3°C activity and feeding to the additional days at 3°C (Experiment 1), a GLMM was used. I was only interested in differences between the levels after acute exposure to 2°C versus acclimation to 2°C or acute 3°C versus the 5 additional days at 3°C. Therefore, the data was filtered to examine the 2°C or 3°C data alone, with activity and feeding being the dependent variables, but all the factors in the analyses remained the same. For food consumption (glmmTMB package; family=beta; link= logit) and routine activity (GLMM: lme4 package; family: Gamma, link= “log”) the fixed factors for

Experiment 1 were days at 3°C (acute 3°C, days 1, 2, 3, 4, 5 at 3°C) and species, the random factor was the individual fish. For Experiment 2, the fixed factors were days at 2°C (acute 2°C, days 1 and 2 at 2°C after a 3 week acclimation period) and species, with the random factor being the individual fish.

Once GLMMs were fitted to the data, all significant effects were determined by using the Anova function in R, where p-values were calculated with a Type II Wald chi-square test. Bonferroni post-hoc multiple comparisons tests were done to determine the significant differences between the levels of the significant factors using the estimated marginal means (emmeans package).

Swim performance was analyzed using a one-way factorial analysis of variance (ANOVA) with Tukey post-hoc test, where U_{burst} was the dependent variable and temperature was the fixed factor. This was used to determine differences in swim performance between temperatures. A linear regression was also performed to determine the predictability of swim performance using temperature, with U_{burst} as the dependent variable and temperature as the independent variable.

Specific Growth Rate (SGR) was analyzed using a Welch two sample t-test, where species was the fixed factor and SGR was the dependent variable.

RESULTS

Experiment 1: Routine activity and food consumption during acute cooling in small (~25g) juvenile shortnose sturgeon and Atlantic sturgeon

Food consumption

Food consumption was high (>70% of daily ration) at the warmer temperatures (15-9°C) but decreased with cooling below 6°C in both species (Figure 7; glmmTMB:

$\text{Chisq}=118$; $\text{df}=1$; $p<2e^{-16}$); food consumption was similar between species during cooling (Figure 7; glmmTMB: $\text{Chisq}=2.61$; $\text{df}=1$; $p=0.11$) and there was no interaction between temperature and species (Figure 7; glmmTMB: $\text{Chisq}=0.101$; $\text{df}=1$; $p=0.75$). Food consumption was low at 3°C for both species, where feeding decreased by 60% from the level at 15°C in shortnose sturgeon and 44% in Atlantic sturgeon. Even at the coldest temperatures, both Atlantic sturgeon and shortnose sturgeon continued to feed at a low level. The overall thermal sensitivity of food consumption was similar between the two species (from 15-3°C: Atlantic sturgeon $Q_{10}=1.61$; shortnose sturgeon $Q_{10}=2.13$).

The fish measured for the additional five days at 3°C (AS: $n=3$; SN: $n=5$) showed no difference in food consumption between the acute 3°C value (i.e., first day at 3°C) and the values of the additional 5 days at 3°C (Figure 7; glmmTMB: $\text{Chisq}=0.55$; $\text{df}=1$; $p=0.46$). However, Atlantic sturgeon showed higher levels of food consumption compared with shortnose sturgeon during the additional five days at 3°C (Figure 7; glmmTMB; $\text{Chisq}=7.8$ $\text{df}=1$; $p=0.0052$). There was also a weak interaction between species and days at 3°C (Figure 7; glmmTMB: $\text{Chisq}=3.91$; $\text{df}=1$; $p=0.048$).

On average, SGR showed positive growth for both shortnose sturgeon (0.107) and Atlantic sturgeon (0.0548) (Table 3). There was no significant difference between species SGR ($t=-0.437$; $\text{df}=28.61$; $p=0.665$).

Routine activity

Routine activity was similar between the day and night during the acute test for shortnose sturgeon (Figure 8; GLMM: $\text{Chisq}=0.441$; $\text{df}=1$; $p=0.51$). Nighttime activity was significantly higher than daytime in Atlantic sturgeon (Figure 8; GLMM: $\text{Chisq}=5.15$; $\text{df}=1$; $p=0.023$), with no significant interaction between temperature and

day/night (Figure 8; GLMM: Chisq=1.81; df=8; p=0.986) During the additional 5 days at 3°C, both Atlantic sturgeon (Figure 8; GLMM: Chisq=10.17; df=1; p=0.0014) and shortnose sturgeon (Figure 8; GLMM: Chisq=12.07; df=1; p=0.0005) showed a significant difference between day/night, where night had relatively higher activity than day. However, since there was only a small difference in diel pattern in either species, day/night activity were combined at each temperature (24 hours) within a species for the rest of the analyses.

Activity decreased with acute cooling in both species (Figure 9; GLMM: Chisq=144.1; df=8; p<0.0001), however Atlantic sturgeon had a higher activity than shortnose sturgeon from 15°C to 10.5°C (Figure 9; GLMM: Chisq=6.16; df=1; p=0.0131). At the cooler temperatures (6-3°C), the activity levels of the two species became similar and even at the coldest temperatures both species showed a low level of activity (Figure 9). However, the thermal sensitivities of activity from 15-3°C differed substantially between species. Activity of shortnose sturgeon was much less thermally sensitive ($Q_{10}=2.68$) compared with activity of Atlantic sturgeon ($Q_{10}=5.99$); shortnose sturgeon and Atlantic sturgeon activities at 3°C were 31% and 12% of the starting value at 15°C, respectively. Supporting a divergent response between species for activity, there was an interaction between species and temperature (Figure 9; GLMM: Chisq=19.22; df=8; p=0.0137).

The fish measured for an additional five days at 3°C (AS: n=3; SN: n=5) showed no difference in activity level between the acute 3°C response (i.e., first day 3°C) and the response with the additional 5 days at 3°C (Figure 9; GLMM: Chisq=0.518; df=1; p=0.47). However, shortnose sturgeon showed higher activity levels compared with

Atlantic sturgeon (Figure 9; GLMM; Chisq=7.701 df=1; p=0.0055). There was no interaction between species and days at 3°C (Figure 9; GLMM: Chisq= 0.435; df=1; p=0.51).

Swim performance in shortnose sturgeon during acute cooling

Swim performance (U_{burst}) in shortnose sturgeon decreased with acute cooling (Figure 10; ANOVA: $F_4=3.24$; $p=0.023$), but only the 15°C vs. 3°C comparison was significantly different (Tukey post-hoc; $p=0.009$). At the coldest temperature (3°C) U_{burst} was 57% of the value at 15°C, but the fish were still able to swim (2.1 BL/sec), and Q_{10} was 1.60 between 15-3°C. The regression analysis showed a significant effect of cooling on swim performance ($y=1.91+0.11x$; $F_{1,38}= 12.2$; $R^2=0.24$; $p=0.0012$), where y = swimming performance and x represents water temperature.

Experiment 2: Routine activity and food consumption during acute cooling and following cold acclimation in large (~150g) juvenile shortnose sturgeon and Atlantic sturgeon

Food consumption

Food consumption decreased with acute cooling from 15-2°C in both species (Figure 11; glmmTMB: Chisq=217; df=1; $p<2e^{-16}$). Atlantic sturgeon and shortnose sturgeon food consumption was initially above 88% of daily ration at 15°C, but at 2°C had decreased to 18.2% for shortnose sturgeon and 7.5% for Atlantic sturgeon at 2°C. There was no difference in food consumption between species (Figure 11; glmmTMB: Chisq=0.32; df=1; p=0.57) and feeding persisted at a low level at the coldest temperatures (3 and 2°C). However, shortnose sturgeon had a lower thermal sensitivity for food consumption ($Q_{10}=3.38$) from 15°C to 2°C compared with Atlantic sturgeon

($Q_{10}=6.89$). No interaction existed between temperature and species (Figure 11; glmmTMB: Chisq=0.095; df=1; p=0.76).

There was no difference in food consumption between fish exposed to 2°C acutely or acclimated to 2°C (Figure 11; glmmTMB: Chisq=0.024; df=1; p=0.88). There was no interaction between species and 2°C acute/acclimated food consumption (Figure 11; glmmTMB: Chisq=0.52; df=1; p=0.47). There was weak statistical evidence for a difference in food consumption between the species (Figure 11; glmmTMB: Chisq=3.71; df=1; p=0.054). In fact, following acclimation to 2°C, there remained a difference between species in thermal sensitivity of food consumption with shortnose sturgeon having a lower thermal sensitivity ($Q_{10}=3.01$) from 15°C to 2°C (average on the second day) than Atlantic sturgeon ($Q_{10}=7.28$).

On average, SGR showed negative growth for both shortnose sturgeon (-0.124) and Atlantic sturgeon (-0.157) (Table 3). There was no significant difference between species SGR ($t=-0.436$; df=18.63; p=0.668).

Routine activity

Activity levels were significantly different between day and night for Atlantic sturgeon (Figure 12; GLMM: Chisq=7.19; df=1; p=0.0073), but no interaction between day/night and temperature (Figure 12; GLMM: Chisq=7.99; df=9; p=0.535). Shortnose sturgeon showed differences in activity between day and night (Figure 12; GLMM: Chisq=5.33; df=1; p=0.021), however, no interaction between day/night and temperature (Figure 12; GLMM: Chisq=5.32; df=9; p=0.806). There was no difference in activity between day and night following cold acclimation in both Atlantic sturgeon (GLMM: Chisq=1.205; df=1; p=0.27) and shortnose sturgeon (GLMM: Chisq=0.0635; df=1;

$p=0.80$) (Figure 12). Therefore, since there was only a small difference of diel pattern in my experiment, day and night activity were combined at each temperature (24 hours) within a species for further analyses.

Activity decreased with acute cooling in both species (Figure 13; GLMM: Chisq=463.71; df= 9; $p<0.0001$). Overall, activities of the species were similar throughout the acute cooling experiment (Figure 13; GLMM: Chisq=0.618; df=1; $p=0.43$). There was a weak, non-significant interaction between species and temperature (Figure 13; GLMM: Chisq=16.29; df=9; $p=0.061$). Shortnose sturgeon activity decreased by 79%, while Atlantic sturgeon decreased by 93%. Thus, the average thermal sensitivities ($15-2^{\circ}\text{C}$) differed substantially between species, with shortnose sturgeon having a Q_{10} of 3.28 and Atlantic sturgeon having a Q_{10} of 7.47, indicating a much greater thermal sensitivity in the latter species.

There was an interaction between acute/acclimated 2°C and species (Figure 13; GLMM: Chisq=8.09; df=2; $p=0.018$), and a difference between species' activity once acclimated to 2°C , where shortnose sturgeon increased activity by 46% from their acute 2°C activity, whereas Atlantic sturgeon increased activity by 24% after acclimation (Figure 13; GLMM: Chisq=9.52; df=1; $p=0.0020$). As a result, shortnose sturgeon activity showed a lower Q_{10} following cold acclimation ($Q_{10}=2.03$ between 15°C and the second day 2°C acclimated data) compared with their acute thermal sensitivity ($Q_{10}=3.28$ between 15°C and acute 2°C). In contrast, activity of Atlantic sturgeon remained highly thermally sensitive, although less sensitive compared to compared with the acute thermal sensitivity mentioned above ($Q_{10}=6.08$ following 2°C acclimation vs. $Q_{10}=7.47$ following acute 2°C exposure).

DISCUSSION

My study provides evidence of species-specific thermal sensitivity of behavioural responses to cooling to winter cold temperatures in juvenile shortnose sturgeon and Atlantic sturgeon. In general, feeding and activity decreased markedly with acute cooling in both species and remained reduced after 3 weeks at 2°C. Even at the coldest temperatures (e.g., 2-3°C), there was a low level of feeding and activity observed, suggesting that juvenile Atlantic and shortnose sturgeon do not enter dormancy when exposed to cold temperatures. This comparable level of activity and feeding in both species in the cold corresponds with the existing literature on juveniles, which are both found in their same natal river systems for the first few years of their life, overwintering at similar low temperatures (Krieger et al., 2000; Li et al., 2007; Fernandes et al., 2010; Taylor et al., 2016; Whitmore and Litvak, 2018). However, while levels of food consumption and activity were statistically similar between the species in most cases during the acute cooling experiments, the thermal sensitivities of the species differed. Specifically, at the warmer temperatures (13.5°C -10.5°C) activity was similar or higher for Atlantic sturgeon compared to shortnose sturgeon, while activity levels of Atlantic sturgeon were lower at the coldest (3°C) temperatures compared to shortnose sturgeon. Thus, activity in Atlantic sturgeon was more thermally sensitive (i.e., greater Q₁₀ values) than in shortnose sturgeon. A similar finding of higher thermal sensitivity in Atlantic sturgeon was seen for food consumption in the larger sturgeon. Furthermore, following the three-week acclimation to 2°C in Experiment 2, the Atlantic sturgeon still had a great

cold sensitivity for both activity and food consumption ($Q_{10} > 6$), whereas the Q_{10} in shortnose sturgeon remained low and decreased (e.g., Q_{10} from 3.28 to 2.03 for activity). Overall, shortnose sturgeon and Atlantic sturgeon both appear to be winter-lethargic species, defined as a species that reduces its activity and feeding to low but sustained levels at cold temperatures (Shuter et al., 2012; Reeve et al., 2022). Nonetheless, shortnose sturgeon appear to be better able to compensate for low temperatures based on their lower thermal sensitivities. The responses to cooling were similar regardless of the size of the juveniles (the only exception was that feeding was higher in the cold for the small Atlantic sturgeon), supporting the idea that the interspecific differences in thermal sensitivity in the juvenile fish from my study could persist into adulthood to help explain the differences in the overwintering behaviour of adult sturgeon. Wild, larger Atlantic sturgeon, which may have a higher thermal sensitivity, overwinter in the warmer waters of the Bay of Fundy, while the potentially less thermally sensitive adult shortnose sturgeon reside within the cooler river systems (Dadswell, 1979; Smith and Dingley, 1984; Li et al., 2007; Fernandes et al., 2010; Stewart et al., 2015; Taylor et al., 2016; Beardsall et al., 2016). The overwintering behaviour of sturgeon has not been well studied, but my study provides the first thorough behavioural characterization of responses to cold among two dominant eastern North American species that can provide a useful complement to existing ecological knowledge from less-controlled field studies (Dadswell, 1979; Smith and Dingley, 1984; Li et al., 2007; Fernandes et al., 2010; Stewart et al., 2015; Taylor et al., 2016; Beardsall et al., 2016).

Comparison of the response of food consumption to cooling in Atlantic sturgeon and shortnose sturgeon

Food consumption fell below 50% of daily ration for small shortnose sturgeon and small Atlantic sturgeon after acute exposure to 3°C, eventually becoming as low as 10-20% in small shortnose sturgeon following several days at 3°C (Fig. 7). Food consumption dropped below 20% for the larger fish of both species at 2°C after acute exposure and remained at this low level following a 3-week acclimation period (Fig. 11). Many fish species reduce feeding (to varying degrees depending on daily ration) in cold water (Shrable et al., 1969; Crawshaw et al., 1982; Lemons and Crawshaw, 1985; Fracalossi and Lovell, 1994; Marchand, 2013; Soyano and Mushirobira, 2018), which is likely the result of a lower metabolism at cold temperatures and potentially a shift to an energy savings strategy or the relatively higher costs of digesting a meal in the cold (i.e., higher temperature-corrected specific dynamic action) (Peck, 2016). Like other fish, shortnose sturgeon and Atlantic sturgeon have lowered metabolic rates at colder temperatures (Secor and Gunderson, 1998; Kieffer et al., 2014; Zhang and Kieffer, 2017) and therefore, food requirements are less. The decreases in food consumption were substantial in both sturgeon species (which corresponded with negative growth in SGR for Experiment 1; Table 3), in particular in the large Atlantic sturgeon and especially given the daily ration was already relatively low. However, the sturgeons did not cease feeding, nor did they show enhanced feeding following acclimation, which is altogether consistent with an overwintering strategy of winter lethargy (Reeve et al., 2022). In contrast, winter-dormant species cease feeding in the cold (e.g., cunner, Reeve et al.,

2022) while winter active species continue to feed at a relatively high level (e.g., brook char; E. Middleton, unpublished data, 2020; Cunjak et al., 1987).

Although my findings showed that both shortnose sturgeon and Atlantic sturgeon have the capacity to continue to feed at the coldest temperatures, albeit at a low level, their thermal sensitivities differed from one another, especially in the larger (~150 g) fish. A Q_{10} of ~2-3 is typically associated with the effect of temperature on biological rates, including feeding rate (Fonds et al., 1992; Ojanguren et al., 2001; Schram et al., 2013; Peck, 2016; Remen et al., 2016; Clarke, 2017; Speers-Roesch et al., 2018; Jutfelt et al., 2021). In the larger sturgeon, shortnose sturgeon were less cold-sensitive with higher rates of feeding and lower Q_{10} 's (Q_{10} 's=3.38) compared to Atlantic sturgeon (Q_{10} =6.89). In comparison, Mayfield and Cech (2004) found that green sturgeon (age-0; ~150g) in the lab had a Q_{10} value for food consumption rate of 3.33 (between 11°C and 15°C) and 1.16 (between 15°C and 19°C), which is comparable to the Q_{10} 's I found for shortnose sturgeon (Table 2). Juvenile pallid sturgeon (~21-59g) in the lab had a Q_{10} of 5.89 (between 13°C and 28°C) for food consumption (Chipps et al., 2010), with the preferred water temperature for feeding around 28°C (Wildhaber et al., 2017). These results are comparable to the higher Q_{10} values for food consumption in Atlantic sturgeon found in my study (Table 2). Until my study, there has been little lab-based data on thermal sensitivity (Q_{10}) of food consumption for Atlantic sturgeon and shortnose sturgeon, even at their preferred temperatures for feeding. However, a modelling study found food consumption for Atlantic sturgeon to be the greatest around 20-26°C (Niklitschek and Secor, 2009), while shortnose sturgeon in the wild have been found to still feed heavily at temperatures between 7-12°C (Dadswell et al., 1979; Usvyatsov et al., 2012). This

interspecific difference is consistent with my finding of lower cold sensitivity of feeding in shortnose sturgeon compared with Atlantic sturgeon that may be more limited by frigid temperatures. Overall, my and previous findings for North American East coast sturgeon suggest that there is variability among species' feeding responses to temperature change, which could relate to species-specific thermal niche although further study is needed. The cold-induced reductions in feeding in my laboratory experiments on sturgeon are consistent with studies on shortnose sturgeon showing reduced feeding in the winter (Dadswell, 1979; Bain, 1997; COSEWIC, 2015). For example, Usvyatsov et al. (2012) examined the gut contents of wild shortnose sturgeon in the Saint John River and found that there was a reduction in food consumption starting in November when temperatures decreased to 8°C. To the best of my knowledge little if nothing is known about winter feeding in wild Atlantic sturgeon. However, in a lab-based study King (2004) examined the growth of juvenile Atlantic sturgeon with differing diets at various seasonal temperatures (~6-17°C). Across all diets, mean weight remained similar at the coldest winter temperatures (~6-10°C) and increased at the warmer temperatures (~11-17°C). Based on these findings it could be inferred that there was reduced feeding in the cold as there was no growth during the cold exposure. These results from King (2004) are comparable with those from my study, where the weight of larger Atlantic sturgeon and shortnose sturgeon over 3 weeks of acclimation at 2°C showed small negative growth (Appendix 1; Table 3). Overall, there is broad agreement between my laboratory studies and existing studies that feeding is greatly reduced in winter in shortnose and Atlantic sturgeon, further reflecting winter lethargy.

However, it is important to consider that winter can be associated with prey scarcity, meaning fish will need to exert additional energy to find food (Huusko et al., 2007; Shuter et al., 2012; Marchand, 2013). Food availability is known to influence feeding patterns (and motivation) in many fish (Colgan, 1973); further study is required to determine if food was not easily accessible to the experimental animals, whether feeding rate may have been adjusted in a different way, such as a further decrease in appetite (and possibly shift their strategy towards even lower activity).

Comparison of the response of routine activity to cooling in Atlantic sturgeon and shortnose sturgeon

In both shortnose sturgeon and Atlantic sturgeon across all temperatures, I found only a small difference in diel pattern of activity (Fig. 8; Fig. 12). The available literature suggests diel activity cycles in sturgeons are variable, and broadly agree with our finding of a flexible and free-running diel activity pattern in shortnose and Atlantic sturgeons. McCleave et al. (1977) tagged adult shortnose sturgeon (~102 cm in length) during the summer months (12-22°C) and found no differences in patterns of daily movements between day and night. Similarly, Kynard and Horgan (2002) found that there was no difference in activity between day and night for larval Atlantic sturgeon. In contrast, Kynard and Horgan (2002) observed under laboratory conditions (21-24°C) that larval shortnose sturgeon were more active during the day, and Richmond and Kynard (1995) and Parker et al. (2012) determined that Connecticut River larval shortnose sturgeon (13-15°C) and adult shortnose sturgeon (3-11°C) were more active at night. McLean et al. (2014) determined that adult Atlantic sturgeon had an increase in activity at dawn and dusk (i.e., crepuscular diel pattern). Richmond and Kynard (1995) found that larval

shortnose sturgeon were more active at night at 13–15°C. Why patterns of diel activity vary so much between studies is difficult to ascertain, but it may be related to predator avoidance, changes in the food supply, or flow conditions under natural conditions, where higher activity at night might be necessary to avoid predation in the wild (Orpwood et al., 2006; Huusko et al., 2007; McLean et al., 2014; Whitmore and Livak, 2018), particularly at early life stages. Additional data, across various ontogenetic stages, is required to fully understand diel activity patterns in sturgeon.

Activity in both small and large sturgeon decreased with acute cooling (Fig. 9; Fig. 13); reduction of activity in the cold is common among fishes (Brett and Higgs, 1970; Metcalfe et al., 1986; Metcalfe and Thorpe, 1992; Huusko et al., 2007; Colchen et al., 2016; Reeve et al., 2022). At the lowest temperatures, shortnose sturgeon had higher activity levels on average compared to Atlantic sturgeon. In both small and large fish, activity of Atlantic sturgeon was found to be more thermally sensitive overall (i.e., had higher Q₁₀'s) than for shortnose sturgeon following acute cooling. Furthermore, activity was higher on average in shortnose sturgeon following cold acclimation whereas it remained low or even decreased on average in Atlantic sturgeon. This greater cold sensitivity of activity in Atlantic sturgeon compared with shortnose sturgeon largely parallels the results found for food consumption (see above), and is consistent with their species-specific behavioural response in the winter as adults, where shortnose sturgeon migrate downstream in the river systems towards estuaries but remain in cold water, while Atlantic sturgeon move into the warmer ocean (Dadswell, 1979; Bain, 1997; Kynard, 1997; Fernandes et al., 2010; Beardsall et al., 2016; Taylor et al., 2016; Whitmore and Litvak, 2018; Andrews et al., 2020). Decreases in activity in the cold also

has been seen in lake and Gulf sturgeons under field conditions (Rusak and Mosindy, 1997; Thayer et al., 2017; Kynard et al., 2018; Moore et al., 2021). Regardless of the variation in cold sensitivity, both species were able to continue to move about in the cold; the ability to continue to swim at low levels at cold temperatures is seen in many sturgeon species (Parsley et al., 2007; Quist et al., 1999; Huff et al., 2012; Kessel et al., 2017). In particular, and in agreement with my findings, a study done on juvenile shortnose sturgeon found that decreasing temperatures lowered activity (mean number of bottom gridlines crossed by 20 fish), however, they were still slightly active (0-5 gridlines crossed daily) at the coldest temperature of 3°C (Kynard et al., 2018).

The decrease in activity with cooling seen in the present study and previous literature suggests sturgeon can continue to stay active at a low level in the cold and further supports the idea of these sturgeon species being winter-lethargic (Shuter et al. 2012; Reeve et al., 2022). Atlantic sturgeon and shortnose sturgeon of both sizes had a large decrease (70-90%) in activity from 15-2°C but did not become fully inactive in the cold. In comparison, the mummichog is a winter lethargic species that shows a comparable decrease in activity from 14-2°C, including maintenance of a low level of activity at 2°C (Reeve et al. 2022). In contrast, dormant species such as cunner and American eel have zero/near zero activity in the cold (Reeve et al., 2022) and winter-active species like brook char remain highly active (E. Middleton, unpublished results). Based on their activity (and feeding) response to cooling, Atlantic and shortnose sturgeon (and possibly other sturgeons based on literature reviewed above) may be best classified as winter lethargic species.

It has been shown that associated decreases in activity, feeding, and metabolism occur in certain fish species as part of an energy savings strategy during the cold, food poor winter (Nilsson et al., 1993; Speers-Roesch et al., 2018; Reeve et al., 2022). Therefore, it is important to understand the relationships between temperature and metabolism in ectotherms- in particular, to determine any limitations that exist that may influence other physiological or behavioural processes (Schulte, 2015). Shortnose sturgeon and Atlantic sturgeon have been shown to reduce their metabolic rate with decreasing temperatures (Secor and Gunderson, 1998; Kieffer et al., 2014; Zhang and Kieffer, 2017). Shortnose sturgeon (~11g) were found to be less thermally sensitive to temperature once acclimated, where the Q_{10} value remained around 1.0 from 25°C to 15°C, but with a higher Q_{10} value of 4.3 from 15°C to 10°C (Kieffer et al., 2014). Secor and Gunderson (1998) found that Atlantic sturgeon (10-70g) respiration rate had a Q_{10} of 1.11 and 1.76 from 26°C to 19°C, showing a typical change of rate that would be seen with changing temperatures (Secor and Gunderson, 1998). Comparison of these metabolic rate Q_{10} to my results for feeding and activity are complicated by the fact that the previous studies examined warmer temperatures than in my study. It is possible that at lower temperatures of 15°C to 2°C the metabolic rate Q_{10} could be higher. Additional research on the effects of cold temperatures on metabolic rates in Atlantic and shortnose sturgeon are required to fully understand thermal sensitivities in their physiology and any potential differences in activity and overwintering strategies.

At the coldest temperatures, shortnose sturgeon and Atlantic sturgeon were still active at low levels, showing that their range of temperature tolerance can be as low as 2°C and that they can persist in these challenging conditions. The results from the U_{burst}

experiments showed that small shortnose sturgeon swim performance decreased by 55% from 15°C to 2-3°C during the acute temperature experiments (Fig. 10), but that the thermal sensitivity from 15°C to 3°C was low (Q_{10} of 1.60). Thus, even at the coldest temperatures, shortnose sturgeon are still capable of reaching high burst swimming speeds which demonstrates their ability to engage in important exercise-related performance at low, winter-like temperatures. The U_{burst} swimming protocol tests anaerobic, burst type swimming, which is known to be relatively temperature insensitive with a typical Q_{10} value of 1-2 so the shortnose sturgeon is typical in this regard (Bennett, 1990; Kieffer, 2000, Guderley et al., 2001; Zeng et al., 2009; Deslauriers and Kieffer, 2012). My findings for U_{burst} are similar to studies that have tested the aerobic swimming capacity in sturgeons (using the critical swimming performance protocols) where Q_{10} was also between 1-2 (Kieffer et al., 1998; Peake et al., 1999; Zeng et al., 2009; Deslauriers and Kieffer, 2012; Yuan et al., 2017). Specifically, Deslauriers and Kieffer (2012) examined the critical swimming speeds (U_{crit}) across various acclimation temperatures (5-25°C) and found that the thermal sensitivity of this metric was 1.14 from 25°C to 5°C, which is comparable to the U_{burst} Q_{10} of 1.60 from 15°C to 3°C in my study. As well, in my study, small Atlantic sturgeon would not swim in the swim flume, even at 15°C; rather, they hit the back wall of the flume during the adjustment period with a low water velocity of 5cm/s. Interestingly, I observed when handling both species acclimated to 2°C that shortnose sturgeon would quickly swim away as was seen at 15°C for both species, while Atlantic sturgeon were less responsive and could easily be picked up compared to their 15°C response. This latter observation was also noted by Miller and Kieffer (unpublished data) who examined the physiological responses to exhaustive exercise in

Atlantic sturgeon at 3°C. These observations further support the idea that Atlantic sturgeon are more cold sensitive compared with shortnose sturgeon.

Comparison of the response of routine activity and food consumption to cooling in different sized Atlantic sturgeon and shortnose sturgeon

Overall, large and small sturgeon showed similar responses in activity and food consumption to cooling. Food consumption at the lowest temperatures, however, was overall greater in the smaller Atlantic sturgeon compared with the larger Atlantic sturgeon. This could be due to the risk of smaller fish depleting their energy stores faster if food became unavailable, as smaller fish will have higher mass-specific metabolic demand and smaller lipid reserves (Miranda and Hubbard, 1994; Sogard and Olla, 2000), while the larger fish may be more tolerant of periods of food scarcity. In any case, my results show species-specific differences occur in juvenile Atlantic sturgeon and shortnose sturgeon. My findings for the larger sturgeon may help explain the differing overwintering strategies of adults, if we assume the differences persist into adulthood.

Caveats and future directions

I experimentally characterized how the feeding and activity of Atlantic sturgeon and shortnose sturgeon respond to cold temperatures, providing insight into their overwintering strategies. However, my study raises other questions that still need to be addressed.

Although I was able to examine both the acute and acclimation effects of cooling temperature, time and space constraints prevented investigation of the influence of another important seasonal factor: photoperiod. Future studies should examine whether

using a shorter winter photoperiod than the 12L:12D used here causes a difference in the behavioural effects of cooling. It is known that photoperiod can impact behavioural responses in fishes (Guthrie, 1986; Oppedal et al., 2001; Almazán-Rueda et al., 2005; Kitagawa et al., 2015). For example, Atlantic salmon continue to be active and feed when exposed to constant light, while during a natural photoperiod they reduced swimming and feed intake during the dark phase of the day (Oppedal et al., 2001). Photoperiod has also been shown to interact with some temperature responses in fish, such as growth rate and critical swimming speeds (Kolok, 1991; Jonassen et al., 2001; Guderley et al., 2001). For example, juvenile Atlantic halibut have higher growth rates when reared under continuous light regardless of temperature at 6°C or 12°C compared to the natural photoperiod (Jonassen et al., 2001). Since winter photoperiod can be as low as 9:15 (light: dark) in Atlantic Canada, if I had used a shorter photoperiod with fewer hours of light, the fish's behaviour may have differed. However, the influence of using 12:12 rather than 9:15 is likely less than the aforementioned studies where continuous light was investigated. This may be particularly true for sturgeon, given I found the study species' activity did not indicate a consistent difference between day and night suggesting that their response to changes in photoperiod are probably less than for species with a strong diurnal or nocturnal cycle. Additionally, a 12:12 photoperiod at Saint John's latitude occurs in March, so it is still winter-relevant to my experimental species (<https://nrc.canada.ca/en/research-development/products-services/software-applications/sun-calculator/>). Still, it would be worth investigating whether variation in photoperiod could alter feeding and activity of sturgeons at low temperatures as days can be shorter during the winter months.

During the present study, fish were provided with a 3-week period to acclimate to 2°C. While this is a commonly used thermal acclimation duration, studies have shown that there can be species-specific variability in the time it takes for acclimation to reach a new steady state. Sandblom et al. (2014) studied the duration needed for shorthorn sculpin (*Myoxocephalus scorpius*) to acclimate to a new temperature of 16°C and found that 8 weeks were required for routine metabolic rate to return to normal levels. On the other hand, Peterson and Anderson (1969) found that this process only took 2 weeks in Atlantic salmon. Thus, species-specific acclimation periods appear to exist, and it is possible that Atlantic sturgeon and shortnose sturgeon require additional time to show thermal compensation or, conversely, a further decrease in activity or feeding at 2°C. Fish can experience these winter cold temperatures for months (Shuter et al., 1989; Clarke, 2017), so it would be relevant to see if there were longer-term effects of cold on food consumption and activity in Atlantic sturgeon and shortnose sturgeon. Nevertheless, in both Atlantic sturgeon and shortnose sturgeon there was little change in these responses between the acute exposure vs. 3-week acclimation to the coldest temperature, so it is more likely that I captured the cold acclimated behavioural phenotype of my study species. Thus, the response to cooling in Atlantic and shortnose sturgeons may be similar whether acutely exposed or acclimated.

In the wild, fish at northern latitudes can experience rapidly fluctuating temperatures (Morash et al., 2021). However, it is less likely for them to encounter an acute decrease in temperature as rapid as done in this study (decrease of 1.5°C per day); it is more likely for streams and lakes in the winter to stay between 0°C to 4°C with some variation in river temperature up to 9°C (Rodgers, 1987; Cunjak, 1988; Morash et al.,

2021). Thus, acclimating the fish to each of the cooling temperature (15, 12, 9, 6, 3 and 2°C) would simulate a more realistic characterization of their response to cooling winter temperature that would be seen in the wild, compared with acutely cooling the fish stepwise to each temperature. Using this acclimation experimental design was the initial intention of the thesis; however, space and available fish prevented the necessary number of replications and temperature conditions to address this. In any case, given the consistency I observed in behavioural responses to acute 2°C or acclimated 2°C exposures, it is probable that the acute responses I measured at other temperatures reflect the acclimated response that would be observed.

The sample sizes, logistical feasibility, and experimental approaches I used necessitated the use of juvenile hatchery fish. When examining behavioural responses, hatchery fish may differ from wild fish (McDonald et al., 1998; Álvarez and Nicieza, 2003). This can be due to differences in ‘experience’, for example as seen in a study with brown trout, where wild trout had a better capacity to avoid predators and had differing diel patterns compared to hatchery trout (Álvarez and Nicieza, 2003). However, due to constraints such as the difficulty of catching wild juvenile sturgeon and difficulty of studying large adults in the lab, use of hatchery-raised juvenile fish was more feasible and still represents a good first step to understanding these species’ response to temperature especially since the fish I used were born of wild parents. I found that juvenile shortnose sturgeon are less sensitive to cold than Atlantic sturgeon, which is consistent with their adult overwintering behaviour where shortnose sturgeon stay in the colder freshwater and Atlantic sturgeon move to the warmer ocean. Nonetheless, in future studies, comparison of overwintering strategies for adults of Atlantic sturgeon and

shortnose sturgeon would be informative to confirm that the results I see for juveniles do indeed apply to adults.

Temperature profoundly affects ectotherms (Fry, 1947), but other environmental factors may co-vary with temperature to cause distinct biological responses. For example, salinity is another abiotic factor that can influence behaviour and physiology of fishes (Pörtner and Peck, 2010). In general, the overwintering behaviour of adult shortnose sturgeon and Atlantic sturgeon in Atlantic Canada includes changes in salinity, such that shortnose sturgeon have been seen to move to estuaries, while Atlantic sturgeon move to the seawater of the Bay of Fundy. Thus, there could be a different response observed in these two species if variation in salinity was introduced. For example, Niklitschek and Secor (2010) looked at the habitat preference of juvenile Atlantic sturgeon and shortnose sturgeon, finding that Atlantic sturgeon overall chose higher salinities than shortnose sturgeon. However, selection of temperature was similar as both species preferred 20°C. There were some species-specific responses, for example Atlantic sturgeon avoided the highest temperature of 28°C while shortnose sturgeon avoided the coldest temperature of 12°C (Niklitschek and Secor, 2010). Furthermore, a study done by Penny and Kieffer (2014) determined that juvenile shortnose sturgeon are challenged with regulating ions and water levels when exposed to seawater (32ppt) such that there is a significant decrease in oxygen consumption and increased levels of plasma ions but can seem to tolerate short periods (24 hours) of high salinity exposure with no mortalities. These results indicate that shortnose sturgeon may prefer not to be in frigid winter cold but are essentially forced to because of a physiological constraint of poor salinity tolerance.

Conclusions and perspectives

Overall, I found that shortnose sturgeon and Atlantic sturgeon have broadly similar behavioural responses to the cold, where they can both remain active and feed at low levels even at 2°C, identifying them both as having a lethargic overwintering strategy. Their general responses to cooling are consistent with existing knowledge that both species as juveniles remain in the colder freshwater over winter (Bain, 1997; Fernandes et al., 2010; Whitmore and Litvak, 2018). However, my Q₁₀ analysis indicates that the feeding and activity of Atlantic sturgeon are more thermally sensitive to acute cooling and cold acclimation compared with shortnose sturgeon. Thus, cold may impose a greater constraint on Atlantic sturgeon that may correlate with their overwintering strategy of outmigration to the warmer ocean as adults. However, given I found that both species have the capacity to tolerate the cold, other abiotic factors such as salinity could be influencing the overwintering behavioural response for these two sturgeon species (Jarvis et al., 2001). It is known that juvenile Atlantic sturgeon and shortnose sturgeon have low salt tolerance and therefore, stay in freshwater during the first few years of their life (Bain, 1997; Fernandes et al., 2010; Allen et al., 2014; Penny and Kieffer, 2014; Downie and Kieffer, 2016; Whitmore and Litvak, 2018). While adult Atlantic sturgeon have the osmoregulatory ability and seawater tolerance to move out to the ocean during the winter, adult shortnose sturgeon stay in freshwater and/or brackish water (Kynard, 1997; Jarvis et al., 2001). It is generally thought that fish species can select physiologically optimal environmental conditions (Jobling, 1981; Cardona, 2000; Edeline and Elie, 2004; Webster and Dill, 2006; Niklitschek and Secor, 2010). Therefore, although Atlantic sturgeon can cope with the cold, at least as juveniles, their ability to

tolerate seawater as adults and greater sensitivity to the cold may make the warmer ocean a preferred overwintering habitat for them compared with shortnose sturgeon (Dadswell, 1979; Smith and Dingley, 1984; Li et al., 2007; Fernandes et al., 2010; Stewart et al., 2015; Taylor et al., 2016; Beardsall et al., 2016; Andrews et al., 2020). Shortnose sturgeon, on the other hand, cannot tolerate high levels of salinity and are less sensitive to the cold, which matches their strategy of remaining in cold freshwater during winter. In both species, the behavioral responses to cooling that I have characterized may be the precursor to the adult stage where the variation in overwintering strategy is realized. Future research should examine the effects of the interaction between cold and salinity on these two species' biological responses at multiple life stages.

Table 1. Body weight, and total length of shortnose sturgeon and Atlantic sturgeon used for all experiments.

Experiment	Species	Number of fish used	Body Weight (g)	Total Length (cm)
1	Shortnose	17	24.4±1.0	18.5±0.3
1	Atlantic	15	24.8±2.0	19.3±0.5
2	Shortnose	11	174.5±15.2	34.8±0.6
2	Atlantic	11	131.2±7.8	35.9±0.9
U _{burst}	Shortnose	43	25.9±1.7	19.0±0.4

Data are means +/-S.E.M.

Table 2. Thermal sensitivities (Q_{10}) of activity, food consumption and swim performance in small (~25 g, <1 y) or large (~150 g, >1 y) shortnose sturgeon and Atlantic sturgeon. Q_{10} were calculated using the mean values at each temperature.

Experiment	Temperature Interval	Species	Activity	Food consumption	Swim performance
1 (Small)	15°C- acclimated to 3°C acutely cooled	Shortnose	2.68	2.13	1.60
1 (Small)		Atlantic	5.99	1.61	NM
2 (Large)	15°C- acclimated to 2°C acutely cooled	Shortnose	3.28	3.38	
2 (Large)		Atlantic	7.47	6.89	
2 (Large)	15°C acclimated to 2°C- acclimated	Shortnose	2.03	3.01	
2 (Large)		Atlantic	6.08	7.28	

NM, not measured.

Table 3. Average specific growth rate (SGR) of the small and large Atlantic sturgeon and shortnose sturgeon (Experiment 1 and 2). Experiment 1 was over a 10 day period of acute cooling from 15°C to 3°C and Experiment 2 over a 9 day period of acute cooling starting at 15°C and ~3 weeks of acclimation to 2°C. There was no significant difference in SGR between the two species in either Experiment (t-test, p=0.67).

Experiment	Species	Size	SGR
1	Shortnose Sturgeon	Small	0.107 +/-0.096
1	Atlantic Sturgeon	Small	0.055 +/-0.072
2	Shortnose Sturgeon	Large	-0.124 +/-0.061
2	Atlantic Sturgeon	Large	-0.157 +/-0.046

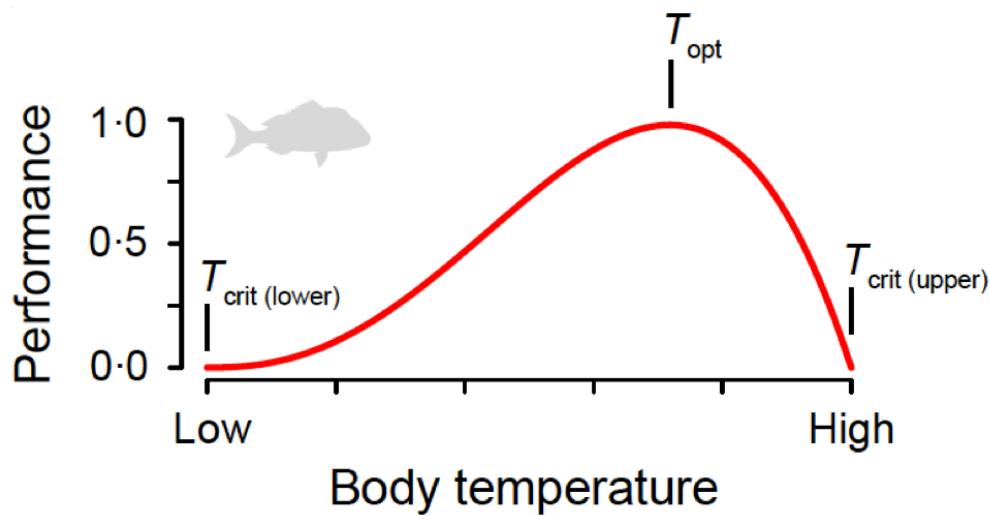


Figure 1. A thermal performance curve, which models the effects of temperature on animal performance (modified from Speers-Roesch and Norin, 2016). The lower and upper T_{crit} indicate an individual's critical thermal range; warming or cooling beyond these temperatures causes mortality. The thermal optimum (T_{opt}) is the temperature that the individual performs at its maximum.



Figure 2. Representative individuals of small (~25g) juvenile shortnose (A) and Atlantic (B) sturgeon.



Figure 3. The recirculating aquarium system used to acclimate small shortnose sturgeon and Atlantic sturgeon to 15°C



Figure 4. Experimental set-up for monitoring activity and feeding during acute cooling experiment for small sturgeon (Experiment 1). For the larger sturgeon (Experiment 2), a similar set-up was used but with bigger arenas, and only 3 arenas with 3 sturgeon per trial.

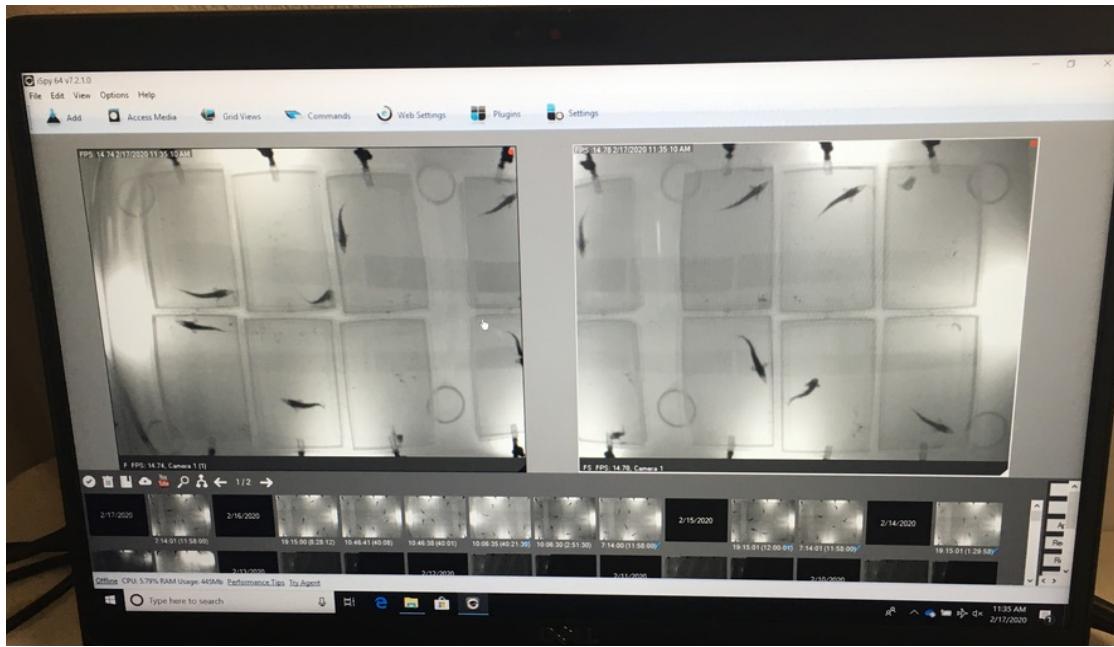


Figure 5. Photograph showing representative live video recordings of sturgeon in the iSpy software during Experiment 1.

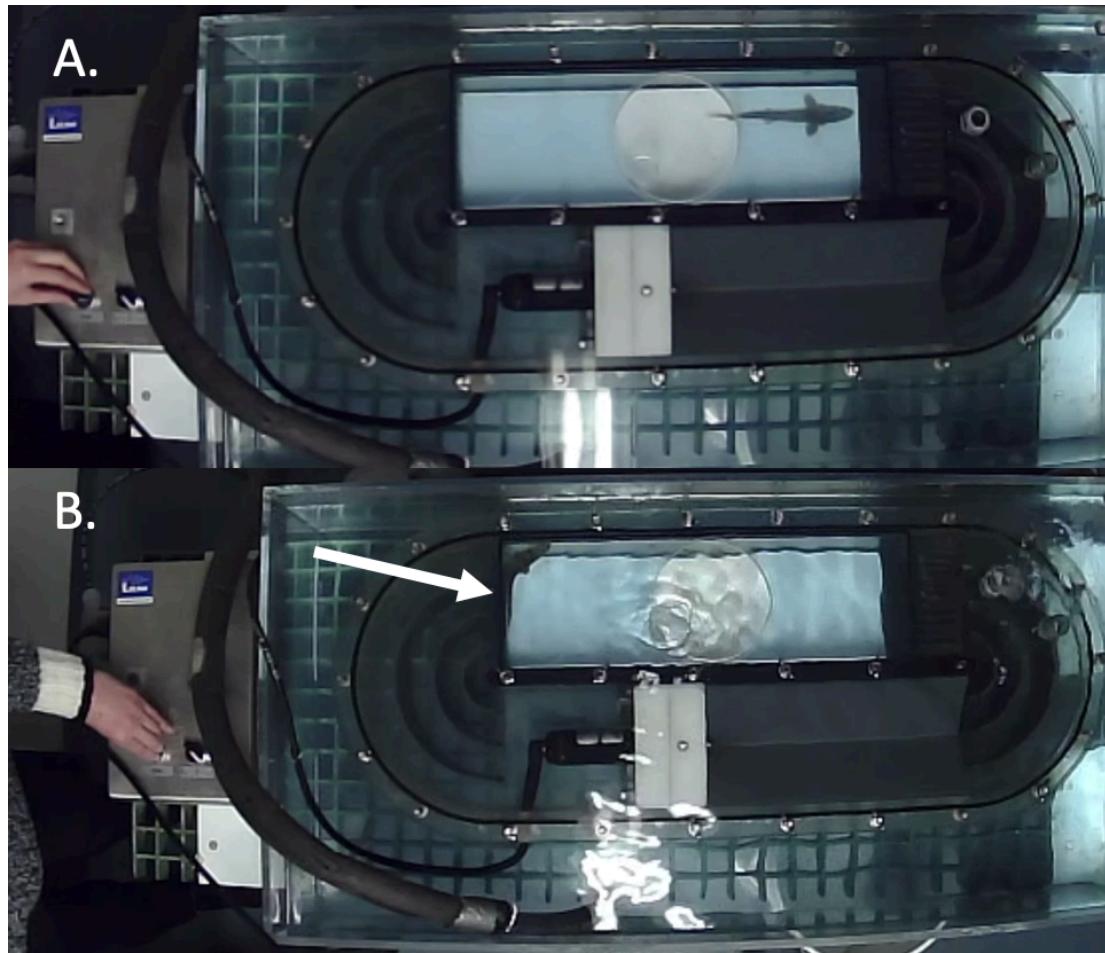


Figure 6. Assessment of swim performance using a swim tunnel flume. (A) Sturgeon is within the 30L flume at one of the test temperatures during the adjustment period (maintaining 5cm/s velocity). (B) A U_{burst} test where the fish was considered to not be maintaining position in the flume (the fish is impinged on the back screen of the flume, indicated by white arrow); at this point, the test stops and the water velocity was used to calculate U_{burst} .

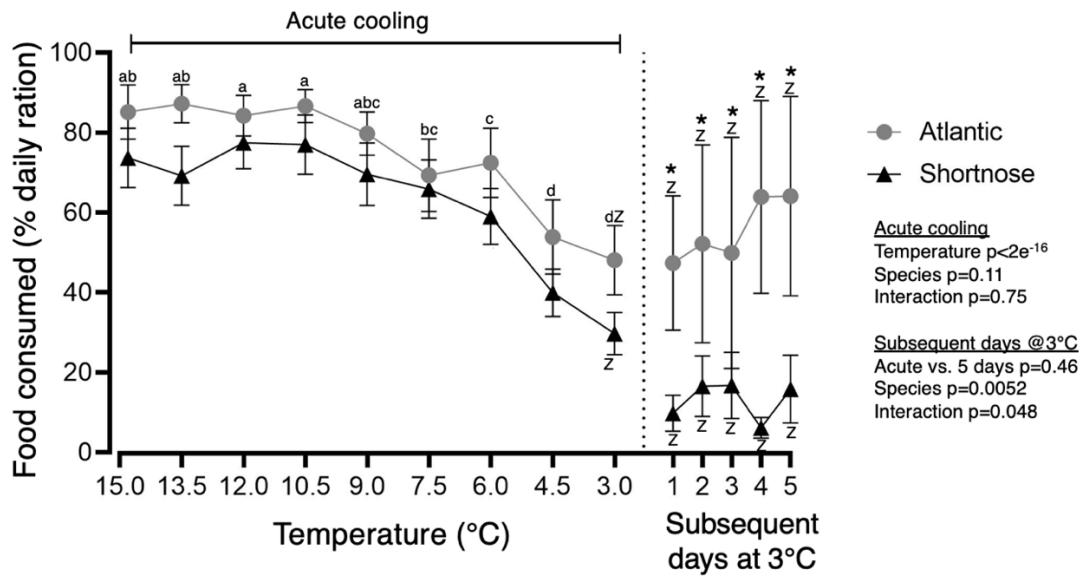


Figure 7. Food consumption (% of daily ration) of juvenile small (~25g) Atlantic sturgeon (AS; grey closed circles) and shortnose sturgeon (SN; black closed triangles) during acute cooling (1.5°C/day) followed by a further 5 days at 3°C (Experiment 1). Data are means ± S.E.M (SN: n=17; AS: n=15 except for the 5 days at 3°C where SN: n=5, AS: n=3). Food consumption was measured as the percentage of the daily ration (% of body weight) consumed over 24 hours at a given temperature. A generalized linear mixed model using Template Model Building (glmmTMB) was used to examine the effect of species and acute 3°C vs subsequent days at 3°C on food consumption. When statistical significant in the glmmTMB model was observed, a Bonferroni post-hoc multiple comparisons test was used to determine the level of significance at each temperature. Values with different lower case letters (i.e., a-c) are significantly different between acute temperature exposures. Values with different upper case letters (i.e., Z) are significantly different between the acute 3°C and the subsequent days at 3°C; there was

no difference between the acute 3°C value and the values on subsequent days at 3°C, which is indicated by the same letter on all values (Z).

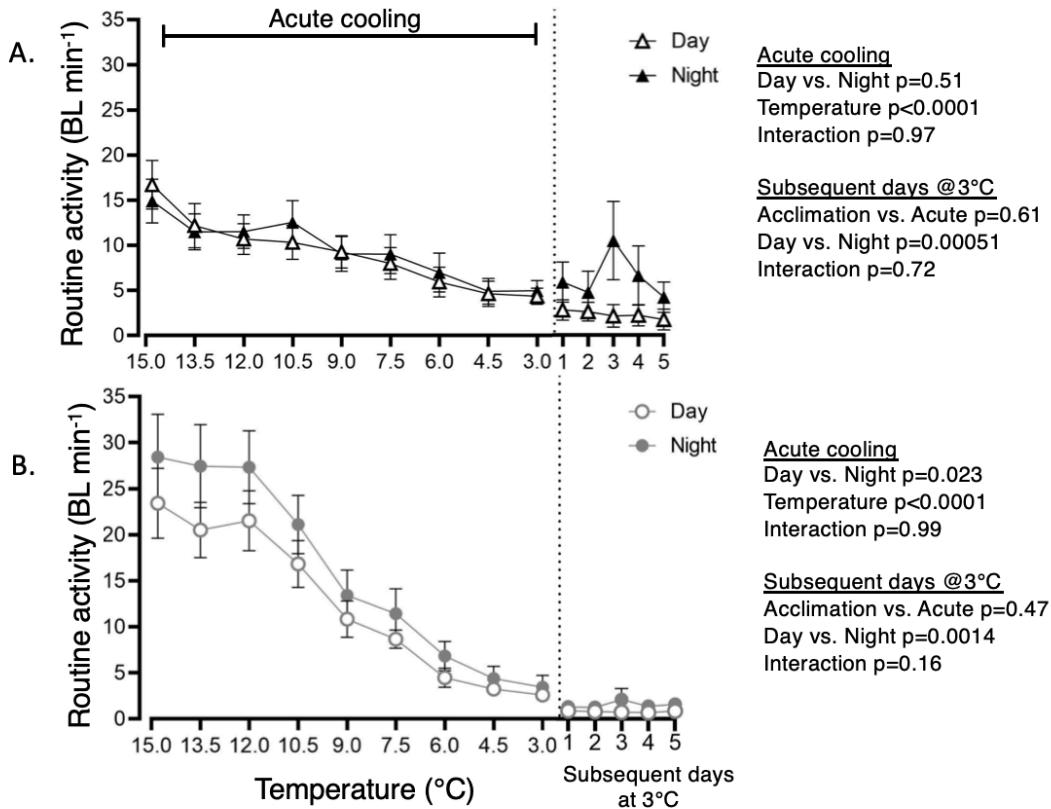


Figure 8. Daytime and nighttime routine activity of juvenile small (~25g) shortnose sturgeon (SN) (A) and Atlantic sturgeon (AS) (B) during acute cooling (1.5°C/day) followed by a further 5 days in the cold (3°C) (Experimental 1). Daytime is indicated by open symbols and nighttime is indicated by closed symbols. Data are means ± S.E.M. (SN: n=17, AS: n=15; except for the 5 days at 3°C where SN: n=5, AS: n=3). A generalized linear mixed model (GLMM) was used to determine statistical significance between day and night within a species at each step-wise temperature. When statistical significant in the GLMM model was observed, a Bonferroni post-hoc multiple comparisons test was used to determine the level of significance at each temperature. For

clarity, pairwise comparisons between temperature treatments are not shown (but see Figure 9).

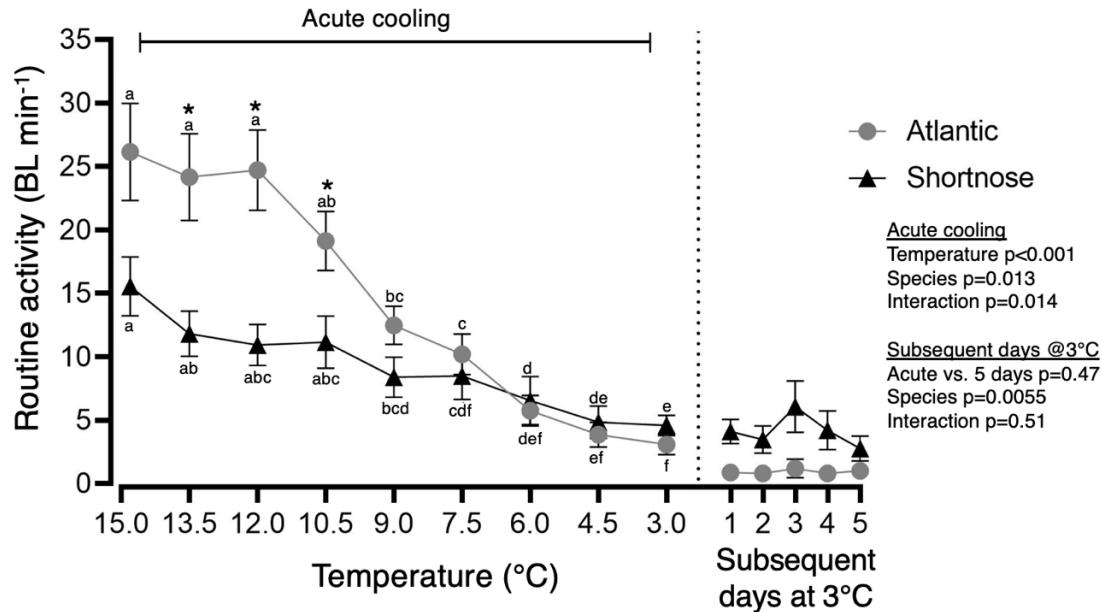


Figure 9. Routine activity of juvenile small (~25g) Atlantic (AS; grey closed circles) and shortnose (SN; black closed triangles) sturgeon during acute cooling followed by a further 5 days in the cold (3°C) (Experiment 1). Data are means ± S.E.M. (SN: n=17, AS: n=15, except for the 5 days at 3°C where SN: n=5, AS: n=3). The activity is for the combined daytime and nighttime periods (full diel cycle, ~24 h) at each temperature. A generalized linear mixed model (GLMM) was used to examine the effect of temperature, species, and acute 3°C vs subsequent days at 3°C on activity. When statistical significant in the GLMM model was observed, a Bonferroni post-hoc multiple comparisons test was used to determine the level of significance at each temperature. Values with different lower case letters (i.e., a-d) are significantly different between acute temperature exposures within a species. Asterisks indicate significant differences between species within a single temperature.

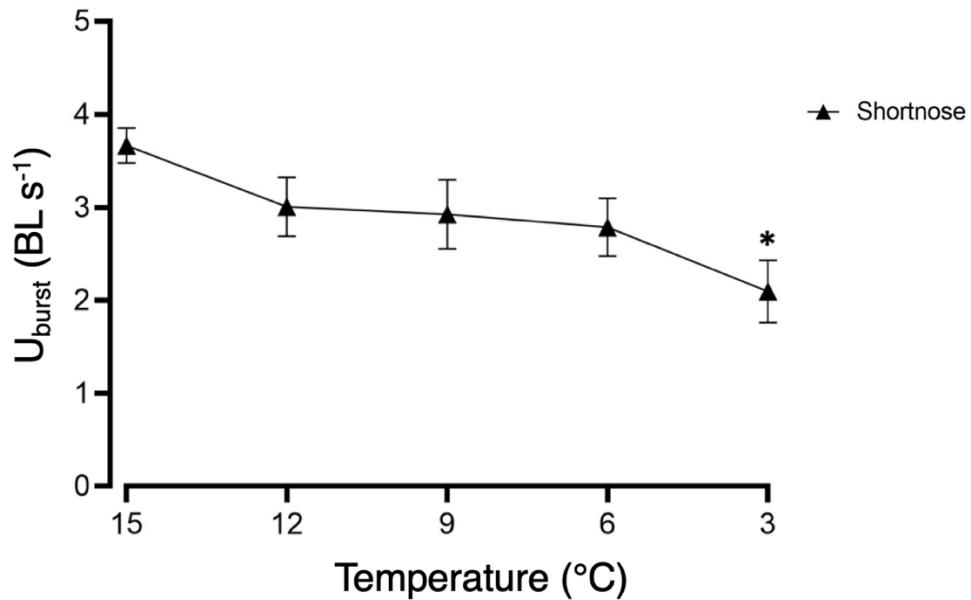


Figure 10. Burst swimming test (U_{burst}) indicating swim performance of juvenile small (~25g) shortnose sturgeon following acute cooling (1.5°C/day) to one of the desired temperatures (15, 12, 9, 6 or 3°C). Data are means \pm S.E.M. ($n=8$ at each temperature except 3°C where $n=10$, with different groups of fish at each temperature). A one-way factorial analysis of variance (ANOVA) with Tukey post-hoc test was used to determine statistical significance. Asterix indicates significant difference in swim performance compared with the 15°C value (Figure 10; ANOVA: $f_4=3.24$; $p=0.023$).

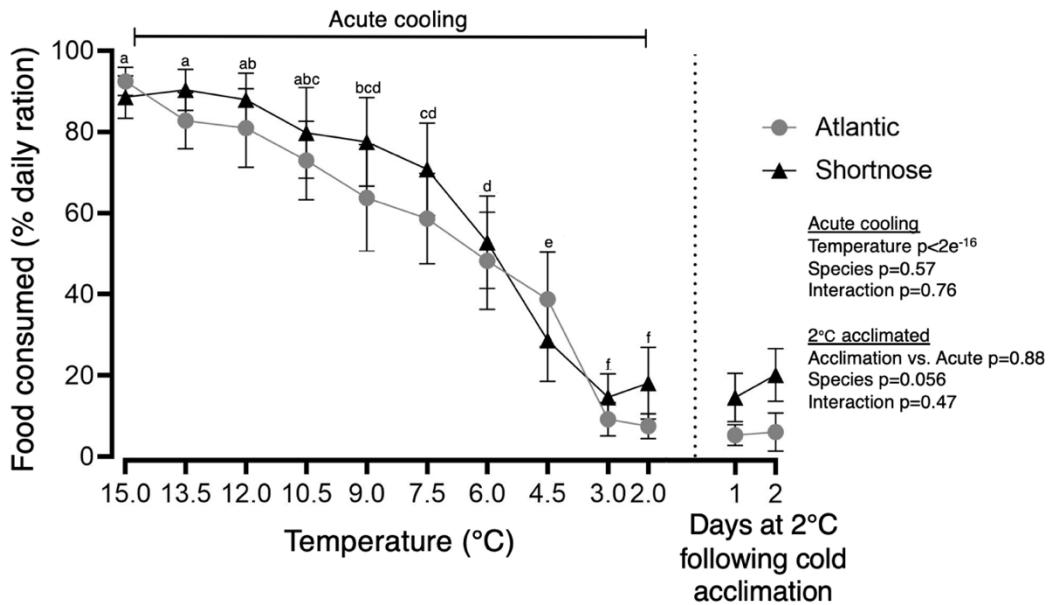


Figure 11. Food consumption (% daily ration) of juvenile large (~150g) Atlantic sturgeon (AS; grey closed circles) and shortnose sturgeon (SN; black closed triangles) during acute cooling (1.5°C/day) and for two days at 2°C following 3 weeks of 2°C acclimation. Data are means \pm S.E.M (SN: n=11, AS: n=11). Food consumption was measured as the percentage of the daily ration (1% of body weight) consumed over the ~24 hours at each temperature. A generalized linear mixed model using Template Model Building (glmmTMB) was used to examine the effects of temperature and species. Another GLMM was used to examine the effect of 2°C acute vs 2°C acclimated days on food consumption. When statistical significant in the glmmTMB model was observed, a Bonferroni post-hoc multiple comparisons test was used to determine the level of significance at each temperature. Values with different lower case letters (i.e., a-d) are significantly different between acute temperatures.

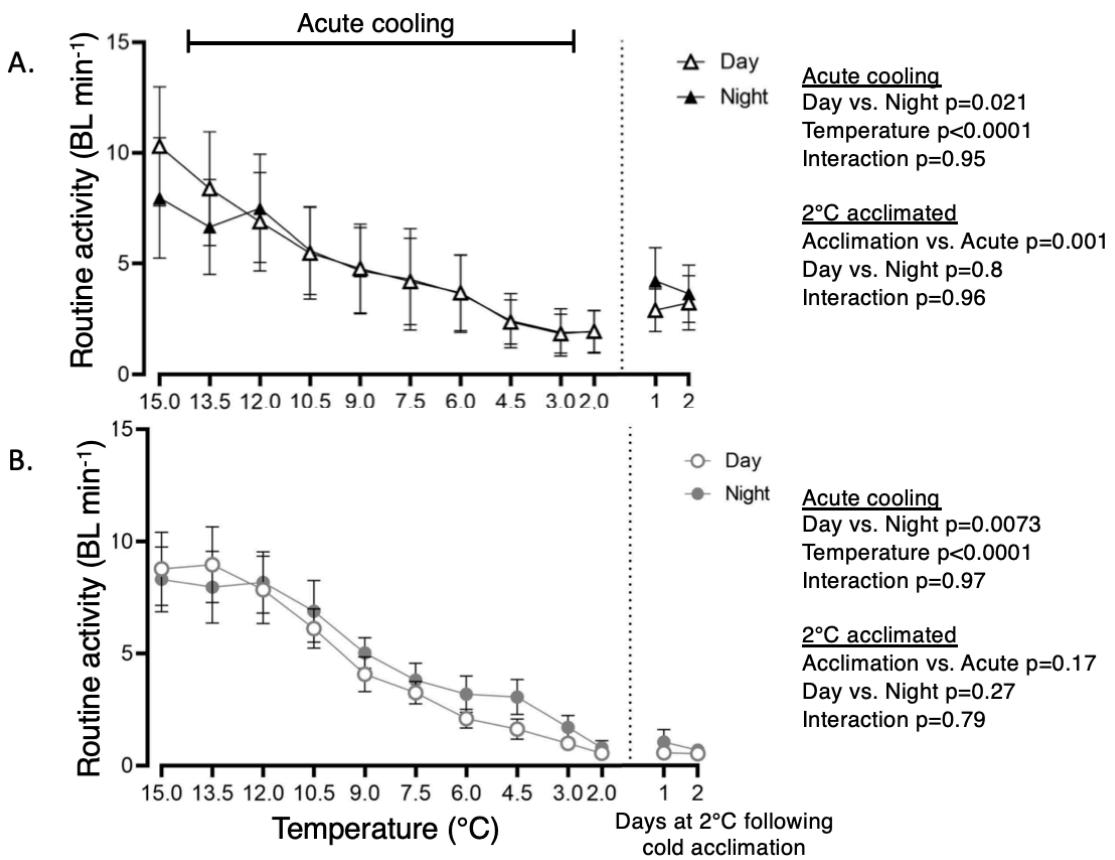


Figure 12. Daytime and nighttime routine activity of juvenile large (~150g) shortnose sturgeon (SN) (A) and Atlantic sturgeon (AS) (B) during acute cooling (1.5°C/day) and for two days at 2°C following 3 weeks of 2°C acclimation (Experiment 2). Daytime is indicated by open symbols and nighttime is indicated by closed symbols. Data are means ± S.E.M (SN: n=11, AS: n=11). A generalized linear mixed model (GLMM) was used to determine statistical significance between daytime and nighttime within a species at each step-wise temperature. When statistical significant in the GLMM model was observed, a Bonferroni post-hoc multiple comparisons test was used to determine the level of significance at each temperature. For clarity, pairwise comparisons between temperature treatments are not shown (but see Figure 13).

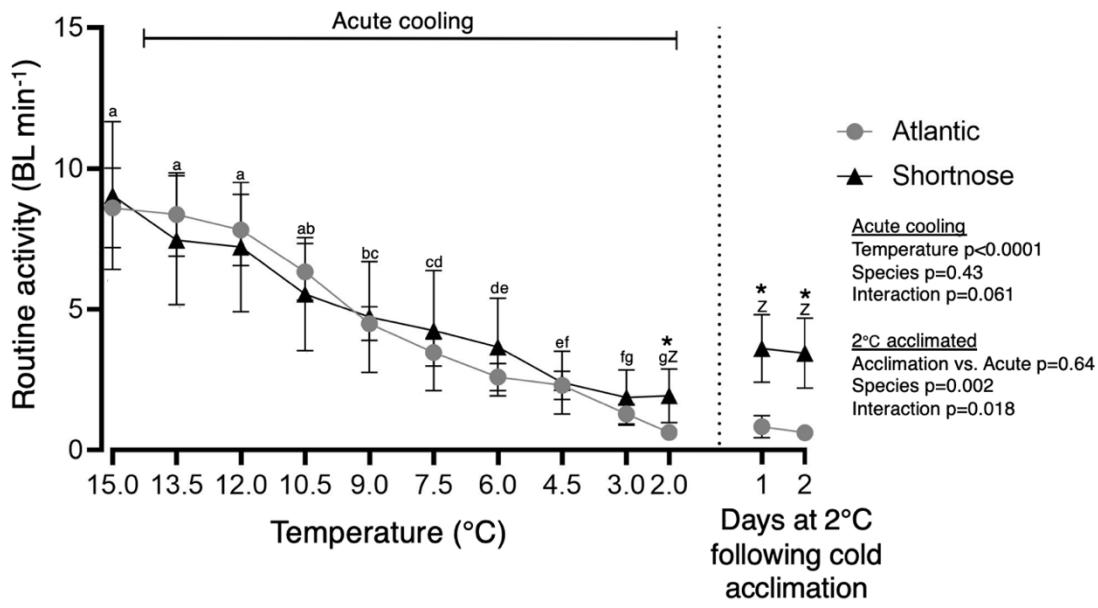


Figure 13. Routine activity of juvenile large (~150g) Atlantic (AS; grey closed circles) and shortnose (SN; black closed triangles) sturgeon during acute cooling (1.5°C/day) and for 2 days at 2°C following 3 weeks of 2°C acclimation. Data are means ± S.E.M. (SN: n=11, AS: n=11). The activity is for the combined daytime and nighttime periods (full diel cycle, ~24 h) at each temperature. A generalized linear mixed model (GLMM) was used to examine the effects of temperature and species. Another GLMM was used to examine the effect of 2°C acute vs 2°C acclimated days on activity. When statistical significant in the GLMM model was observed, a Bonferroni post-hoc multiple comparisons test was used to determine the level of significance at each temperature. Values with different lower case letters (i.e., a-f) are significantly different between acute temperatures. Values with different upper case letters (i.e., Z) are significantly different between the acute 2°C and the days at 2°C following cold acclimation; there was no effect of acclimation in either species. Asterisks indicate significant differences between species within a single temperature.

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APPENDIX

Appendix 1. Length (cm) and weight (g) of large (~150g; >1 year) Atlantic and shortnose sturgeon before and after acclimation to 2°C.

Before acclimation to 2°C			After acclimation at 2°C	
Shortnose	Length (cm)	Weight (g)	Length (cm)	Weight (g)
SN1	32	112.35	33	118.4
SN2	36.1	208.86	36.1	213.2
SN3	33.4	132.51	35.5	140.4
SN4	34.1	145.7	34.1	134.99
SN5	34.1	135.32	34.1	126.04
SN6	33.1	137.76	33.2	135.85
SN7	38.6	199.82	39	197.05
SN8	34.1	231.47	34.1	249.36
SN9	38.6	273.51	38.6	242.98
SN10	35.3	196.3	35.8	194.04
SN11	33.9	145.48	33.9	140.92
Atlantic	Length (cm)	Weight (g)	Length (cm)	Weight (g)
AS1	38.1	146.85	37.8	145.9
AS2	36.5	132.45	36.5	126
AS5	39.6	164.9	39.6	160.13
AS6	32.6	87.86	32.6	89.16
AS7	38.1	138.03	38.1	132.01

AS8	34.1	115.63	34.1	116.91
AS9	31.7	109.6	32	109.35
AS10	35.8	145.54	35.8	143.43
AS11	40.5	174.12	40.5	179.52
AS12	34.1	113.42	34.6	106.64
AS13	33.9	114.82	33.9	116.78

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