Effects of Temperature and Diet on the Metabolic Phenotypes of Lake Sturgeon, *Acipenser fulvescens*: Implications for Conservation Aquaculture

By

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Preface

This thesis was written as manuscript style under the guidelines of University of Manitoba Faculty of Graduate Studies. The care and use of experimental animals within the thesis complied with guidelines established by the Canadian Council on Animal Care under animal use protocol F15-007 as approved by the Animal Care Committee of the University of Manitoba Fort Garry campus.

This thesis is an original work by Gwangseok Yoon. I, Gwangseok Yoon, declare that I performed the majority of the work presented in this original Ph.D. thesis. However, I will use pronoun "we" and "our" throughout the thesis because collaborative efforts have been made. All experiment chapters have been published in the peer reviewed literature. The roles and contribution of all authors for each chapter are described below.

Chapter 2: Effects of dietary shifts on ontogenetic development of metabolic rates in age-0 lake sturgeon *Acipenser fulvescens*.

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Chapter 4: Long-term effects of temperature on growth, energy density, whole-body composition and aerobic scope of age-0 Lake sturgeon (*Acipenser fulvescens*)

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Chapter 6: Long-term effects of EPA and DHA enriched diets on digestive enzyme activity, aerobic scope, growth and survival in age-0 Lake Sturgeon (*Acipenser fulvescens*)

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Abstract

The environment during early life history strongly impacts phenotypic development in all organisms, which further influences developmental trajectory and ecological fitness later in life. Depending on the developmental stage and magnitude of change in the environment, phenotypes may become irreversible and thus have a long-lasting effect later in life.

This thesis was designed to better understand how changes in the environment may influence plasticity and variation of metabolic phenotypes of Lake Sturgeon (*Acipenser fulvescens*) within the first year of life. Broadly speaking, the thesis tested two hypotheses that 1) all measured phenotypes would be plastic; and 2) durations of environmental effects on phenotypic development would be correlated with distinct developmental windows. Studies were developed to examine 1) short-term effects of temperature or diet on metabolic phenotypes such as metabolic rate, energy density, fatty acid profiles, and growth (Chapters 2 and 3) and 2) longer-term effects of temperature or diet during early life on these metabolic phenotypes (Chapters 4, 5 and 6).

The first experimental chapter (Chapter 2) examined ontogenetic development of metabolic rate and demonstrated that dietary shifts between *Artemia* to bloodworm resulted in cessation of growth with elevated routine metabolic rate. Chapter 3 examined how fatty acid profiles and plasma cortisol concentration were influenced by environmental temperature and showed that decreasing temperature led to increases in mono- and polyunsaturated fatty acids in both phospholipids and triglycerides, and food deprivation resulted in lack of difference between baseline and peak cortisol concentrations. Chapter 4 examined how temperature during early life influenced plasticity of growth and showed that temperature post-dietary transition resulted in a transient effect on growth and energy metabolism without long-term effects post-winter. Chapter 5 examined how temperature during early life could influence growth and fatty acid metabolism when fish were exposed to colder temperatures later in life and demonstrated that elevated temperatures resulted in a longer-term effect on growth but lack of transcriptional responses of desaturating fatty acids when exposed to a cold temperature (3.5°C) later in life. The final experimental chapter, Chapter 6 examined longer-term effects of diet at the onset of exogenous feeding on metabolism and growth and demonstrated that an enriched diet resulted in prolonged effects on growth, digestive enzyme activity and survival prior to a simulated overwintering.

This doctoral thesis research revealed that all measured metabolic phenotypes were plastic, but subtle changes in temperature and diet during early life history resulted in transient or prolonged effects on growth and metabolism in age-0 lake sturgeon. Results will aid our understanding of cohort and population dynamics as well as contribute to the development of conservation strategies for lake sturgeon, a species at risk or endangered across its natural range.

Dedication

This thesis is dedicated to my son Jason Jinsung Yoon, who inspired me with a lot of research ideas and encouraged me to complete my PhD program.

Quote

Row, row, row your boat,

Gently down the stream.

Merrily, merrily, merrily, merrily,

Life is but a dream.

Acknowledgement

I would like to start off thanking Dr. Gary Anderson for guiding me throughout my PhD program. I cannot find a better word to describe how fortunate I have been to have Dr. Anderson as my advisor and mentor in my life. I still remember him generously having offered his backyard for my wedding party for friends in Winnipeg followed by the first one in Korea, and all our friends from our lab gathered to make food together before the party. That event remains me of the unforgettable moments during my graduate degree programs. It was so much fun with never ending shenanigans. Dr. Anderson is the best advisor and mentor in my life! Also, I am very happy that I have had Dr. Suh in my PhD program, and I whole-heartedly thank you for mentoring and supporting me through my PhD program. You have shown me how to become a passionate scientist with warm-heart.

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1 Introduction

1.1 Phenotypic and developmental plasticity

Phenotypes are measurable biological traits that are the result of environment and genotype interactions. Measurement of phenotypes in growing organisms helps us to understand how organisms can adjust their physiology and/or behaviour in response to changing environments. Phenotypic plasticity is the ability to express a range of phenotypes within the same genotype within a cohort or population, which confers flexibility in response to changing environments (Agrawal 2016). Phenotypic plasticity may vary depending on the biological function of traits (e.g. blood glucose vs metabolic rate) and the magnitude and duration of environmental changes (Lindström 1999; Monaghan 2008; Burggren 2020). Developmental plasticity is a similar concept to phenotypic plasticity, but is more focused on irreversible changes, which typically occur during sensitive developmental windows (i.e. critical window) (Piersma and Drent 2003). In fish, a critical window for growth is hypothesized to be from the embyrogenesis to the onset of exogenous feeding (Jonsson and Jonsson 2019). While it remains unknown if developmental plasticity is whether an adaptive response or passive effects of biochemical reaction on developmental processes, it can strongly influence developmental trajectory, growth and thus variation in life history traits (Johnston 2006; Burggren 2015; Jonsson and Jonsson 2019). In this thesis, phenotypic plasticity will refer to transient changes in a phenotype in response to changes in the environment whereas developmental plasticity will refer to persistent changes in phenotype due to environmental effects during early life history.

1.2 Effects of environment on phenotypic development

In ectotherms, temperature is the most pervasive abiotic factor as it strongly influences physicochemical reactions and thus all biological processes. It is well known that environmental temperature influences metabolism, development rate, growth and survival (Schulte et al. 2011; McKenzie et al. 2020) and that changes in temperature typically result in highly plastic responses in fish with metabolic rate being a well studied trait (Clarke and Johnston 1999). For example, metabolic rate is often described as an unimodal curve where metabolic rate increases with temperature until an optimum is reached then sharply decreases as temperature keeps increasing (Schulte et al. 2011). Although metabolic rate is recognized to be a plastic trait that is strongly influenced by environmental temperature (Brown et al. 2004), temperature changes during early

life may have irreversible effects on metabolic phenotypes (e.g. enzyme activity and growth rate) and thus plasticity of metabolic rate later in life (Ryu et al. 2018).

The exact mechanism in prolonged effects of environmental temperature during early life on metabolic phenotypes remains unknown, but it is hypothesized to be due to increased sensitivity in physiological processes and transcriptional regulation in response to temperature during early life (Mueller 2018). In fish, a critical window for growth is hypothesized to be during embryogenesis and/or the larval life history period (Melendez and Mueller 2021). Studies have demonstrated that temperature changes during embryonic development could result in long-term effects on oxidative phosphorylation enzymes and/or muscle cellularity (Johnston 2006; Martell and Kieffer 2007; Schnurr et al. 2014; Donelson et al. 2018; Ryu et al. 2020), which would influence metabolic rate and growth. For example, muscle development in fish is comprised of various physiological processes such as cellular differentiation, enzyme activity and gene expression, all of which are sensitive to temperature change (Johnston et al. 2011). As fish rapidly develop during embryogenesis, temperature may have prolonged effects on muscle plasticity and thus growth in fish (Johnston 2006). Furthermore, temperature during early life could influence transcriptional regulators (i.e. epigenetics) such as DNA methylation and histone modification that can lead to persistent effects on phenotypic development (Jonsson and Jonsson 2019).

In fishes, another critical window for growth is hypothesized to be the larval life history period, including onset of exogenous feeding (Hou and Fuiman 2020). In the majority of fishes, development of biological systems remain incomplete at hatch (Post and Lee 1996; Gisbert 1999), and ontogenesis continues relying on the endogenous yolk during which both morphological and physiological development enable exogenous feeding (Yúfera and Darias 2007; Conceição et al. 2010). Afterwards, fish must obtain nutrition and energy from their diet, thus the importance of nutritional condition during early life has received a great deal of attention due to its prolonged effects on phenotypic development (Metcalfe and Monaghan 2001; Monaghan 2008).

For example, in humans it has been shown that malnutrition *in utero* can cause hypoplasia of the pancreas and thus hyposecretion of insulin by pancreatic β-cells, leading to increased sensitivity to glucose and insulin resistance (Hales and Barker 1992). Although this phenotype may be beneficial under malnutrition due to enhanced capabilities of storing fats and maintaining

blood glucose levels, such a phenotype becomes malicious in the adult if there is high-calorie food intake, which in turn gives rise to development of impaired glucose tolerance, obesity and potentially coronary heart disease (Barker 2002). Studies have also highlighted that dietary intake of different types of fatty acids during early life may have prolonged effects on development of metabolic phenotypes (Innis 2011), which may influence growth and life history traits later in life (Criscuolo et al. 2008).

Fatty acids are carboxylic acids with aliphatic chains that are either saturated or unsaturated. Fatty acids are a core molecule of lipid classes, such as phospholipids, triglycerides, cholesterol esters and wax esters as well as non-esterified forms such as free fatty acids found in the circulation and tissues. Fatty acids are known to play an important role in functional biology, such as the structure of phospholipids in the cell membrane (Hazel 1997); immune responses through oxylipin metabolism (Tocher 2003); ontogenesis (Tocher 2010); energy reserves in various lipid molecules (triglyceride and wax esters); gene regulation (Jump and Clarke 1999) and metabolic fuel for ATP production through β-oxidation in mitochondria (Lind 1992). Saturated fatty acids have straight carbon chains with an even number of carbon atoms whereas unsaturated fatty acids have double bonds that occur between carbon positions. In biological molecules, a double bond in an acyl chain typically has a *cis* configuration that is thermodynamically less stable than *trans* forms, which gives the characteristic of a lower melting point and greater flexibility. Monounsaturated fatty acids (MUFAs) have a single double bond in the carbon chain whereas polyunsaturated fatty acids (PUFAs) have two or more. When the first double bond occurs between the third and fourth carbon atom from the methyl end in the fatty acid, it is called omega-3 fatty acid whereas omega-6 fatty acids have the first double bond between the sixth and seventh carbons from the methyl end (Figure 1.1). PUFAs are known to play important roles in ontogenesis, growth and homeoviscous adaptation (Tocher 2010; Ernst et al. 2016; Park 2018). The majority of PUFAs are produced in plants, but many animals, including fish, are known to have limited desaturation and elongation capacity synthesizing PUFAs.

Fatty acid metabolism in vertebrates has been well characterized, and is hypothesized to be almost identical across species (Sprecher 2000; Miyazaki and Ntambi 2003). Synthesis of MUFAs is comprised of desaturation and elongation. Stearoyl-CoA desaturase (*SCD*) is a fatty acid desaturating enzyme that inserts a double bond in the 9th carbon from the carboxyl end in

the fatty acid chain for the synthesis of C16:1n7 and C18:1n9, and further conversion to C18:1n7 and C22:1n9 by elongation (Miyazaki and Ntambi 2003). The synthesis pathway of LC-PUFAs in fish is grouped by omega-3 and 6 fatty acids, which is not interchangable (Park 2018). For example, eicosapentaenoic acid (C20:5n3; EPA) and docosahexaenoic acid (C22:6n3; DHA) could be metabolized from C18:3n3 (alpha-linoleic acid; ALA) whereas arachidonic acid (C20:4n6; AA) could be from C18:2n6 (linoleic acid; LA) *in vivo*. Like other vertebrates, it is hypothesized that fish cannot *de novo* synthesize LA nor ALA due to the lack of Δ 12 and 15 desaturases (Monroig et al. 2018). Although some fish may synthesize AA and DHA from dietary LA and ALA (Tocher et al. 2009), the process of conversion may not be efficient becauses of the limited physiological and molecular capacity of fatty acid desaturation and elongation (Park et al. 2018). Thus, these fatty acids must be obtained from the diet to meet the physiological needs of the animal (i.e., essential fatty acids) (Tocher 2015), and sufficient dietary intake of essential fatty acids is particularly important during early life for growth and survival in fish (Hamre et al. 2013).

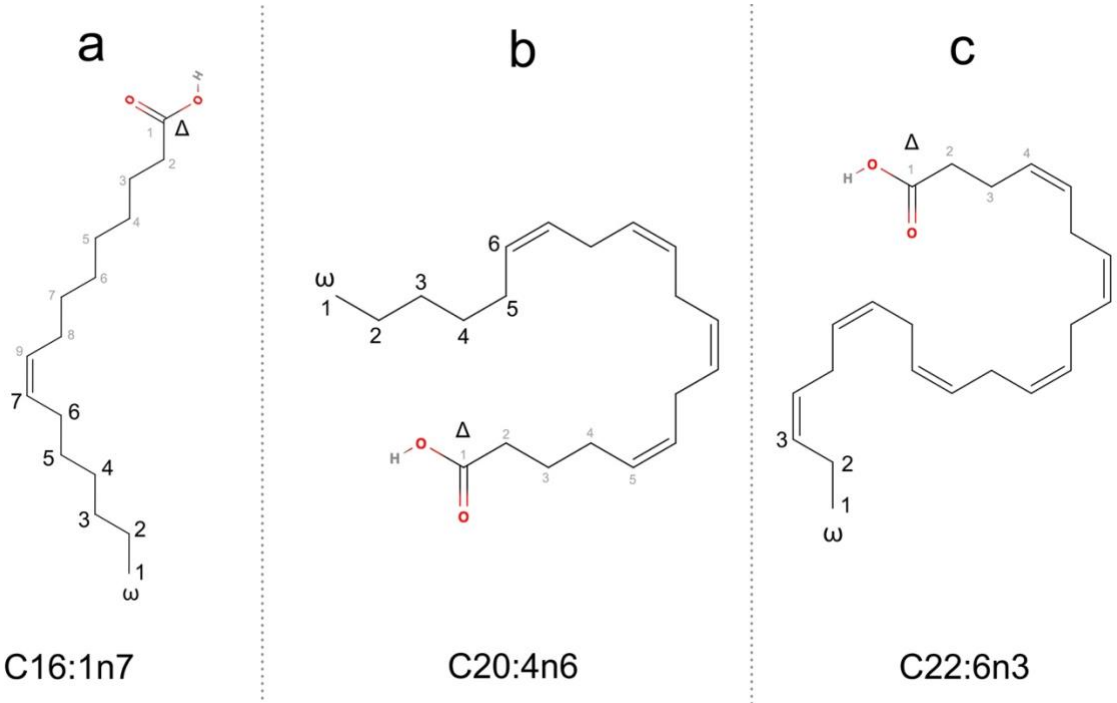

Figure 1.1. Chemical structure of palmitoleic acid (a), arachidonic acid (b) and docosahexaenoic acid (c) to illustrate how carbon atoms are numbered from carboxyl (Δ) and methyl ends (ω). Chemical structure was drawn using a public program PubChem Sketcher V2.4 (https://pubchem.ncbi.nlm.nih.gov//edit3/index.html).

For example, in Atlantic sturgeon (*Acipenser oxyrinchus*), feeding dietary omega-3 fatty acids at the onset of exogenous feeding significantly increased growth performance during early life (Kolman et al. 2018). In Adriatic sturgeon (*Acipenser nacarii*) and European eel (*Anguilla anguilla*), diet enriched with omega-3 fatty acids (i.e. EPA and DHA) significantly decreased both routine metabolic rate and standard metabolic rate (McKenzie 2001), which may indicate reduced basal metabolic costs and thus increased metabolic capacity for growth. Furthermore, dietary supplementation of DHA increased survival rate in milkfish (*Chanos chanos*) and red porgy (*Pagrus pagrus*) during early life (Gapasin and Duray 2001; Watanabe et al. 2016). Also, provision of omega-3 fatty acids (i.e. EPA and DHA) during early life could result in longer-term effects of increased swimming performance, growth and survival rates later in life as seen in red drum (*Sciaenops ocellatus*) (Fuiman and Perez 2015).

1.3 Implications for conservation aquaculture

Understanding how temperature and diet could shape metabolic phenotypes during early life may have implications for interpreting cohort and population dynamics as well as conservation biology where sustainable populations are the key to success of conservation programs. For example, in conservation aquaculture, hatchery-reared fish are released to wild systems to improve the status of the species of interest. However, the success of these programs is highly variable (Brannon et al. 2004). A major limitation of hatchery practices is low post-release survival rates, which may be partly due to prolonged effects of early rearing environments on metabolic phenotypes in hatchery fish (Jonsson and Jonsson 2019). In conservation aquaculture, unnatural environments such as fixed temperature regimes and artificial diets are employed to facilitate management of the hatchery (Araki et al. 2008). However, absence of natural cues in such environments result in phenotypes that are significantly different from wild fish (Chittenden et al. 2008; Gessner et al. 2009; Baker et al. 2014), leading to an inability to acclimate to the wild environment and thus lower post-release survival rates (Crossman et al. 2014). Furthermore, when these hatchery-reared individuals are released and mate with existing individuals, maladaptive phenotypic changes may occur in wild cohorts (Sundstrom et al. 2005; Hindar et al. 2006; Hoekstra et al. 2007).

Lake sturgeon (*Acipenser fulvescens*) is a freshwater, cartilaginous and benthic species that is native to North America, and is currently endangered or at risk across its natural range (Bruch et al. 2016). Conservation aquaculture programs have been ongoing to improve the status of

populations for decades. In Manitoba, Canada, typical hatchery practice involves fixed rearing temperatures (i.e. 16°C) and *Artemia* and bloodworm as the main diet prior to release into the natural environment. However, success of conservation efforts is limited often as a result of low survival rates during dietary shifts at early life stages and also low post-release survival of stocked fish particularly for age-0 fall fingerlings compared to age-1 spring yearlings (McDougall et al. 2020). As mortality is naturally high during early development in fish (Osse et al. 1997), it was suggested that release of larger spring yearlings may be more desirable for the conservation of lake sturgeon. However, release of smaller age-0 fall fingerlings is often required due to logistical and financial constraints at the fish hatchery. Thus, it is important to understand how current hatchery practices could influence development of phenotypes during early life and how these phenotypes might impact survival during the first winter of life.

Although fixed temperatures may facilitate the management, optimum temperature for growth could vary with developmental stages and growth trajectory (Handeland et al. 2008; Khan et al. 2014; Killen 2014). *Artemia* has been used as a popular start diet for rearing larval fish for decades as it is cost-effective for mass production (Conceição et al. 2010); however, *Artemia* is known to have a low content of EPA and DHA (Watanabe et al. 1983), both of which are essential fatty acids that play a crucial role in ontogenesis and neural development in vertebrates (Monroig et al. 2018; Hou and Fuiman 2020). To better guide conservation aquaculture programs, studies have suggested inclusion of more natural factors such as a natural temperature regime and enriched diet that would enhance growth performance as well as recognition of prey that may enhance post-release survival rate (Reid et al. 2010; Yoon et al. 2019, 2020). For example, *Artemia* is frequently enriched with essential fatty acids in industrial aquaculture, but the protocol of enrichment has not been employed in conservation aquaculture of lake sturgeon. However, these suggestions should be sustainable with financial and/or logistical constraints at fish hatchery operations (Froehlich et al. 2017). Thus, recent studies have focused on identifying a key developmental window as well as timing and duration of environmental manipulation for growth in age-0 lake sturgeon (Earhart et al. 2020; Brandt et al. 2022), which could guide more practical rearing protocols at conservation fish hatcheries in Manitoba. Therefore, understanding 1) how metabolism changes in response to dietary shifts during early life in age-0 lake sturgeon, 2) how age-0 lake sturgeon cope with overwintering conditions and 3) how environment during early life could shape development of metabolic

phenotypes associated with overwintering performance (i.e. growth and homeoviscous adaptation) will significantly enhance the current conservation stocking program in Manitoba.

1.4 Factors that influence overwintering survival

Overwintering survival is driven by interactions between energetic status of animals and severity of wintering conditions (Hurst 2007). Thus, success of overwintering is often largely dependent up on body size prior to the winter. Growth performance of fish can be estimated using traditional methods by simply measuring length and body mass of the fish (Breck 2014), but this method does not demonstrate how much energy has been allocated toward somatic growth or storage. Energy density (ED) represents the standardized amount of energy per unit of body mass ($J \cdot g^{-1}$) and measurement of ED can help us understand changes in energetic assimilation at different developmental stages (Wuenschel et al. 2006) or under different environmental conditions (Tirelli et al. 2006; Canale 2014). Previously, it was shown that ED could be used to predict the survivability of age-0 lake sturgeon during a simulated overwintering event (Deslauriers et al. 2018). Furthermore, metabolism during early life could influence energy allocation toward somatic growth (i.e. protein) and energy reserves (i.e. lipids), which would reflect proximate composition in fish (Sloat et al. 2014). Thus, measurement of these metrics in addition to length and body mass in fish would provide a holistic understanding of growth and general nutritional status that may promote overwintering survival.

Metabolic costs for growth can be estimated through measurement of metabolic rate. Metabolic rate in organisms can be estimated through measuring oxygen consumption, carbon dioxide production, heat production or food consumption/egestion, but in fish it is commonly indirectly assessed by measuring oxygen consumption where ~ 90% oxygen is used in aerobic respiration for ATP regeneration (Rolfe and Brown 1997). Standard metabolic rate (SMR) is defined as the minimum energy expenditure to support vital processes such as substrate cycling, protein turnover and organismal integrity (Fry 1957; Beamish 1964; Nelson 2016). Thus, SMR is measured when a subject is post-absorptive, unstressed and resting (Hochachka and Somero 2001). SMR has been shown to be correlated with traits such as growth, energy assimilation and behaviour in some fish; therefore, changes in SMR can have ultimate consequences on ecological fitness (Burton et al. 2011; Killen 2014). Routine metabolic rate (RMR) is a similar concept to SMR, but includes potential energetic costs for routine activities such as body movements and digestion. In fish, RMR has been measured where reliable measurement of SMR

may not be possible due to logistical constraints or behaviour of the species of interest, especially at early life stages (Killen et al. 2012; Jung et al. 2019; Yoon et al. 2021). Maximum metabolic rate (MMR) is the maximum oxygen consumption rate that can be achieved to support maximal activity following exhaustive exercise (Norin and Clark 2016). Thus, MMR could be informative to understand metabolic responses to exercise or compare aerobic performance of individuals (McKenzie 2001; Kieffer et al. 2014), which may influence prey capture, predator avoidance or migration (Kieffer 2010). Lastly, aerobic scope (AS) is the difference between SMR and MMR, and AS is the metabolic capacity that can be allocated toward routine activities above basal such as locomotion, foraging, digestion and growth (Clark et al. 2013; Jutfelt et al. 2021).

Although body mass plays a major role in overwintering survival, it is critical to maintain homeostasis of membrane fluidity in cold temperatures to keep cellular functionality. Homeoviscous adaptation (HVA) is a biochemical process of reconstructing membrane lipid molecules in response to temperature changes where adaptation to cold temperatures typically leads to increased MUFAs and PUFAs with a concomitant decrease in saturated fatty acids in phospholipids (Hazel 1997). AA, EPA and DHA are long-chain PUFAs (LC-PUFA) that are commonly found in phospholipids and known to play a key role in HVA in fish (Monroig et al. 2018). During HVA, these fatty acids could be supplied from dietary intake or LC-PUFA could be synthesized *in vivo* via a series of desaturation, elongation and peroxisomal β-oxidation steps although the synthetic capacity in fish is hypothesized to be limited (Tocher and Ghioni 1999; Park 2018). Where these LC-PUFAs may not be available due to seasonal changes in dietary fatty acid composition of prey (Næsje et al. 2006; Hixson and Arts 2016), the physiological and/or molecular capacity of fatty acid synthesis and incorporation to phospholipids may strongly influence successful HVA at colder temperatures (Ernst et al. 2016).

It is hypothesized that fatty acid metabolism is strongly influenced by temperature and diet. For example, in carp (*Cyprinus carpio*) and milkfish (*Chanos chanos*), cold exposure increased gene expression of SCD (Trueman et al. 2000; Hsieh et al. 2003) to increase MUFAs from saturated fatty acids (SATs). Also, in low temperature acclimated fish, enzyme activity of Δ 6 desaturase, a rate limiting enzyme for desaturating fatty acids, was shown to increase suggesting a mechanism of synthesizing LC-PUFAs during HVA (Schünke and Wodtke 1983; Hagar and Hazel 1985; Tocher et al. 2004). In addition, dietary fatty acids could strongly influence downstream production of PUFAs. For example, Δ 6 desaturase is promiscuous with both

omega-3 and 6 fatty acids, and it can bind one of four potential substrates—C18:3n3, C18:2n6, C24:5n3 or C24:4n6 although it was suggested that binding affinity may be higher for omega-3 fatty acids in vertebrates (Gerster 1998). Studies have suggested that dietary availability of these fatty acids can compete for Δ 6 desaturase, which can significantly influence downstream production of LC-PUFA, AA, EPA and DHA in mammals (Emken et al. 1994; De Antueno et al. 2001). Importantly, dietary fatty acids during early life history could have prolonged effects on survival and growth (Fuiman and Perez 2015).

1.5 Hypotheses and Predictions

In this thesis, I examined how temperature and diet could result in short-term (phenotypic plasticity) and long-term (developmental plasticity) changes in phenotypes of developing lake sturgeon (Figure 1.2.).

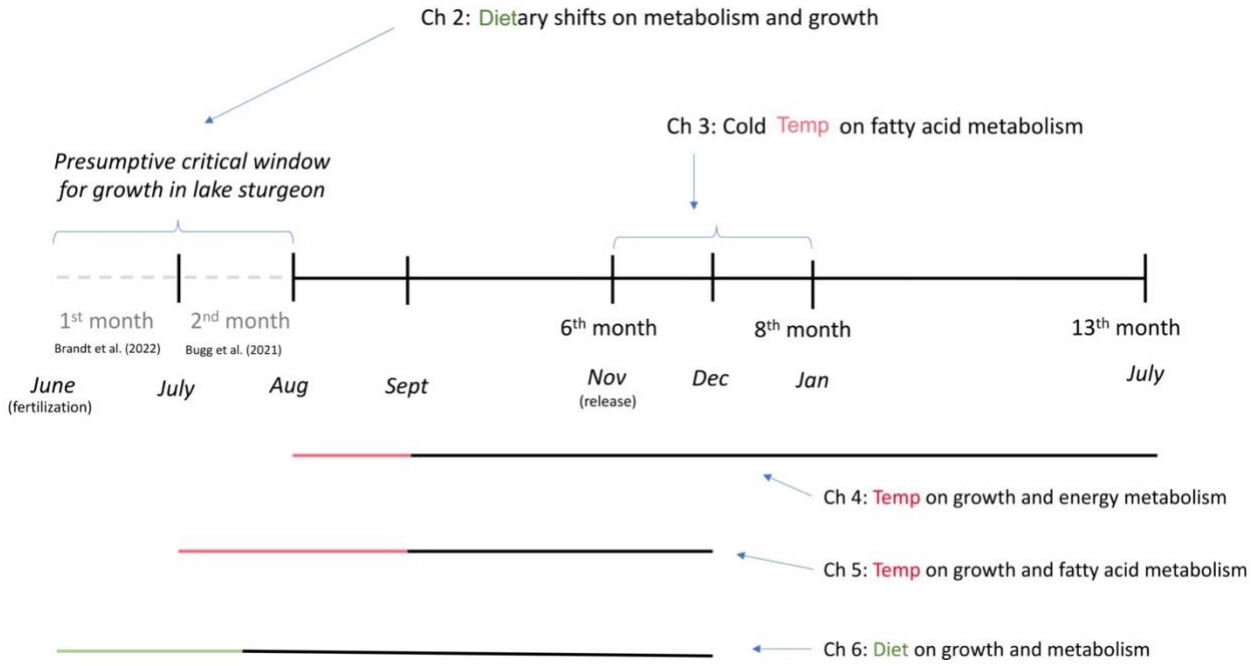

Figure 1.2. Schematic figure of experimental flow in each chapter. Month represents approximate developmental time of lake sturgeon in Manitoba, Canada. In Chapters 4, 5 and 6, fish were exposed to temperature or diet manipulation during early life (i.e. colour red or green) and subsequently raised at the same environment of temperature or diet to examine plasticity of metabolic phenotypes within the first year of life.

Broadly speaking my PhD research tested two hypotheses and specific predictions in each experimental chapter are described below:

1) all measured metabolic phenotypes will be plastic (Chapters 2 and 3) and;
2) duration of environmental effects on phenotypic development will be correlated with distinct developmental windows (Chapters 4, 5 and 6).

In the first experimental chapter (Chapter 2), I examined how dietary shifts during early life history could influence development of metabolic phenotypes. I predicted that a change in diet from yolk to *Artemia* and from *Artemia* to bloodworm during early life would result in a significant increase in RMR but not MMR. In Chapter 3, I examined how decreases in temperature and food availability as the fish approach the first winter of life could influence fatty acid composition and plasma cortisol concentration. I predicted that under an overwintering condition of reduced temperature and food availability, MUFAs and PUFAs would significantly increase in the liver tissue, but there would be no difference between baseline and peak plasma cortisol concentrations.

In Chapter 4, based on the assumption that the critical window for growth phenotypes in lake sturgeon is the first month of life (Brandt et al. 2022), I examined how temperature manipulation during early life may influence plasticity of growth. I predicted that temperature manipulation past the first month of life would result in a transient effect on growth. In Chapter 5, I examined how temperature manipulation during early life could influence growth and fatty acid metabolism when fish were later exposed to cold temperatures. I predicted that temperature manipulation at the first month of life would result in a persistent effect on growth and fatty acid metabolism even when fish encountered cold temperatures later in life. Finally in Chapter 6, based on the assumption that the dietary critical window for growth phenotypes in Siberian sturgeon (*Acipenser baerii*) is at the onset of exogenous feeding (Luo et al. 2019), I examined how provision of EPA and DHA at the onset of exogenous feeding might influence growth and fatty acid metabolism. I predicted that dietary EPA and DHA at the onset of exogenous feeding would have a prolonged effect on growth and fatty acid metabolism.

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2 Chapter 2: Effects of dietary shifts on ontogenetic development of metabolic rates in age-0 lake sturgeon *Acipenser fulvescens*

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Keywords: ontogenetic dietary shift, diet transition, oxygen consumption rate, larval fish, mortality

2.1 Abstract

In many fish species, ontogenetic dietary shifts cause changes in both quantitative and qualitative intake of energy, and these transitions can act as significant bottlenecks in survival within young of the year class. In the present study, we estimated routine (RMR) and forced maximum metabolic rate (FMR) in age-0 lake sturgeon *Acipenser fulvescens* on a weekly basis from 6 to 76 days post hatch (dph) within the same cohort of fish. We were particularly interested in the period of dietary transition from yolk to exogenous feeding between 6 and 17 dph and as the fish transitioned from an *Artemia* based diet to a predominantly bloodworm diet between 49 and 67 dph. Measurement of growth rate and energy density throughout the study indicated there was a transient growth arrest during the transition from artemia to bloodworm. The highest mass-specific ($mgO_2Kg^{-1}Hr^{-1}$) RMR recorded throughout the first 76 days of development occurred during the yolk-sac phase and during transition from *Artemia* to bloodworm. Similarly, diet transition from *Artemia* to bloodworm – when growth arrest was observed – increased scaled RMR (i.e., $mgO_2Kg^{-0.89}Hr^{-1}$), and it did not significantly differ from scaled FMR. Log-log relationships between non mass-specific (i.e., mgO_2Hr^{-1}) RMR or FMR and body mass significantly changed as the growing fish adapted to the nutritional differences of their primary diet. We demonstrate that dietary change during early ontogeny has consequences for growth that may reflect altered metabolic performance. Results have implications for understanding cohort and population dynamics during early life and effective management for conservation fish hatcheries.

2.2 Introduction

In the majority of fish, development of biological systems typically remains incomplete at hatch (Post and Lee 1996; Gisbert et al. 1999; Gisbert and Doroshov 2006; Kamler 2008). Ontogenesis continues post-hatch relying on endogenous yolk to fuel both morphological and physiological development prior to exogenous feeding (Osse and van den Boogaart 1995; Yúfera and Darias 2007; Conceição et al. 2010). For example, gastrointestinal development prior to exogenous feeding is known to be stage specific during early development and genetically predetermined to facilitate uptake of nutrients at the onset of exogenous feeding (Zambonino Infante and Cahu 2001).

In fish, following the onset of exogenous feeding, changes in diet frequently occur during development, a process termed ontogenetic dietary shift. Ontogenetic dietary shift is key to understanding how changing diets, with associated changes in nutritional quantity and/or quality, influence the subsequent developmental trajectory and survival (Nunn et al. 2007, 2010; Sánchez-Hernández et al. 2019). It is hypothesized that ontogenetic dietary shift is driven by morphological and physiological constraints in fish. Body size is related to gape size of fish, which limits the size and type of prey that can be ingested (John 1999; Murchie et al. 2019). Further, physiology of the digestive tract may influence efficiency of nutrient uptake (Zambonino Infante and Cahu 2001, 2007; Yúfera and Darias 2007; Davis et al. 2013). Importantly changes in growth of the gastrointestinal tract during early development may lead to a dramatic change in metabolic rates (Weatherley and Gill 1983; Post and Lee 1996; Wang et al. 2001; Yagi et al. 2010; Rombough 2011; Rosenfeld et al. 2015). Furthermore, changes in quantity or quality of diet could influence duration of digestive processes and thus may influence metabolic rates (Secor 2009; Chabot et al. 2016a, 2016b). For example, in juvenile European seabass (*Dicentrarchus labrax*), dietary supplement of seaweed (*Gracilaria* spp., *Ulva* spp., and *Fucus* spp.) for 49 days significantly increased routine metabolic rate (Peixoto et al. 2016). In Adriatic sturgeon (*Acipenser nacarii*) and European eel (*Anguilla anguilla*), diet enriched with omega-3 polyunsaturated fatty acids significantly decreased both routine metabolic rate (RMR) and standard metabolic rate (SMR) (McKenzie 2001). Conversely, in juvenile rainbow trout (*Oncorhynchus mykiss*), isoenergetic diets with varying levels of protein and lipid did not affect RMR (Eliason et al. 2007).

Understanding changes in metabolic rate with respect to ontogeny and diet could provide a physiological underpinning of how much metabolism is relegated to support routine activities and growth. SMR is the minimum metabolic cost to support vital processes such as ion regulation, protein turnover and organism integrity (Rolfe and Brown 1997; Chabot et al. 2016b; Treberg et al. 2016), and SMR is known to be correlated with behavioural traits and growth performance in some fish species (Álvarez and Nicieza 2005; Killen et al. 2013; Metcalfe et al. 2016; Zeng et al. 2017). RMR is similar to SMR, but includes additional metabolic costs for body movements, digestion (i.e., specific dynamic action; SDA) or growth, and measurement of RMR has frequently been used where reliable measurement of SMR may not be possible due to logistical constraints or behaviour of the species of interest (Lee et al. 2003; Killen et al. 2012; Zhang and Kieffer 2017; Jung et al. 2019; Yoon et al. 2021). Maximum metabolic rate (MMR) is the maximum oxygen consumption rate that can be achieved by the organism (Norin and Clark 2016). Thus, MMR is typically estimated by forcing fish to perform intense exercise that functions to elevate oxygen consumption rates ($\dot{M}O_2$), and is often described as forced maximum metabolic rate (FMR; relative to spontaneous maximum metabolic rate) (Svendsen et al. 2014; Kissinger et al. 2017; Murray et al. 2017). FMR could be a useful approximation of MMR for larval fish due to the small size and fragility at this life stage (Kissinger et al. 2017). Therefore, because of technical limitations but more importantly the biology of larval fish, measurement of RMR and FMR may be more practical at this life stage (Yoon et al. 2021).

RMR and FMR vary depending on developmental stages (Wieser 1995; Post and Lee 1996; Glazier 2005; Killen et al. 2007; Boucher et al. 2018). For example, relative ontogenetic patterns of organogenesis may influence predictable changes in RMR where organs have differential metabolic activities (Weatherley and Gill 1983; Rolfe and Brown 1997; Hochachka and Somero 2001; Norin and Malte 2012; Boldsen et al. 2013). Also, developmental status of oxygen transport systems such as the cardiorespiratory systems can strongly influence oxygen uptake and utilization (Farrell et al. 1990; Farrell 2009; Hillman et al. 2013; Eliason and Farrell 2016). Thus, understanding ontogenetic changes in RMR and FMR of larval fish will inform how regulation of energy metabolism may influence growth at a critical life history period (Rombough 1994, 2011; Cunha et al. 2007; Burggren and Blank 2009).

It is well documented that metabolic rate increases with body mass and mass-specific metabolic rate decreases with body mass (Kleiber 1932; West et al. 1997; Glazier 2005; Killen et

al. 2007). Thus, metabolic rate can be explained by a power function of body mass: $\dot{M}O_2 = a \times body\ mass^b$. Metabolic scaling explains how metabolic rate is associated with body mass of animals; a concept that has been central to understanding how biophysics, developmental physiology and evolutionary pressure can overlap to shape $\dot{M}O_2$ (Oikawa et al. 1991; Post and Lee 1996; Agutter and Wheatley 2004; Brown et al. 2004; Hulbert 2007; Pernet et al. 2007; White and Kearney 2013; Yagi and Oikawa 2014; Sibly et al. 2015; Gillooly et al. 2016). Over the last century, numerous studies have been conducted to suggest a unified mass scaling exponent of metabolic rate across taxa ranging from 2/3 to 1 (Kleiber 1932; West et al. 1997; Clarke and Johnston 1999; Wang et al. 2001; Glazier 2005). West et al. (1997) stated that metabolic scaling exponent of 0.75 may show precision over a great range of body mass; however, it may deviate from 0.75 due to early ontogeny or taxonomic characteristics. Specifically, during early development fish may show a higher scaling exponent (Oikawa et al. 1991; Wang et al. 2001; Kamler 2008), and mass scaling exponent could greatly change during dietary shifts (Gisbert et al. 2001; Boucher et al. 2018). For example, in larval green sturgeon (*Acipenser medirostris*), mass scaling exponent for RMR significantly decreased from 1.64 ± 0.2 during endogenous yolk (0 – 14 days post hatch; dph) to 1.04 ± 0.1 during exogenous feeding (15 – 30 dph) (Gisbert et al. 2001). As dietary shifts often cause a temporal limitation in food uptake, it is hypothesized that dietary shifts will significantly influence metabolic scaling during these key early life history phases in fishes (Gisbert et al. 2001).

In this study we set out to examine how metabolism changed during early life history in lake sturgeon *Acipenser fulvescens*. In particular we focussed on how diet transitions from yolk to *Artmeia* and from *Artemia* to bloodworm influenced mass-specific RMR and FMR. Previously, it was reported that mortality is particularly high during the diet transition from *Artemia* to bloodworm in age-0 lake sturgeon at a conservation fish hatchery setting (Klassen et al. 2007). Thus, we chose to use these diets to mimic the feeding protocol and investigate how increased mortality during the diet transition may be corrleated with changes in growth and metabolism. Furthermore, we calculated metabolic scaling exponents in lake sturgeon during dietary shifts to compare developmental patterns of metabolism and predict that mass-specific RMR and FMR would increase as diet changed.

2.3 Materials and Methods

All experiments and described procedures were approved by the University of Manitoba Animal Care Committee under the requirements established by the Canadian Council for Animal Care under protocol number F015–007.

2.3.1 Animal husbandry

Gamete collection, fertilization and animal rearing were performed as previously described (Bugg et al. 2020). In brief, spawning lake sturgeon were caught by gill net on the Winnipeg River (50°17’52N, 95°32’51W) and gametes were collected from 1 female and 2 males. Sperm was activated by adding de-chlorinated freshwater, and subsequently eggs were added to fertilize. Following the fertilization, embryos were de-adhered by submerging embryos in a clay solution (i.e., Fullers earth) and gently stirred by hand for 1 h. Embryos were then rinsed with de-chlorinated freshwater and incubated in tumbling jars until hatch. Hatching completed within 2 days, and hatched larvae were transferred to one of two duplicate 12L flow-through aquaria at 16°C on a 12:12h day:night cycle. Following the yolk-absorption, at 14 dph, a small amount of freshly hatched *Artemia* (Brine Shrimp Direct, Ogden, Utah) was introduced to promote recognition of prey. At 17 dph fish were fed *Artemia* three times a day *ad libitum*. Any mortality was removed at each feeding and recorded. At 49 dph, when gape size would allow, ground bloodworm (Hikari Bloodworm, Hikari, California) was introduced to fish at a ratio of 1:9 (10% of total mixture) and transition to 100% bloodworm was completed by 67 dph (AB Transition) as the ratio of bloodworm increased by 10% every two days. Fish were fed solely on bloodworm from 76 dph.

2.3.2 Whole body metabolic rate

$\dot{M}O_2$ was assessed approximately weekly for 70 days from 6 to 76 dph through intermittent flow respirometry as outlined previously (Yoon et al., 2021) with some modifications. We used two sizes of metabolic chambers (chamber volume of 3.36 ± 0.15 mL; tubing volume of 1.54 ± 0.01 mL; mean ± S.D and chamber volume of 42.32 ± 1.06 mL; tubing volume of 3.07 ± 0.11 mL; mean ± S.D). The smaller chambers were used for fish from 6 to 48 dph and the larger chambers for fish from 55 to 76 dph. The average ratio between chamber

volume to body mass was 72.58 ± 60.53 for the smaller chambers and 72.62 ± 29.67 for the bigger chambers.

Each week throughout development individual fish (n=7-8 per week) were haphazardly captured by dip net and placed in individual metabolic chambers. The respirometry system was comprised of a Witrox 4 Oxygen meter (Loligo Systems, Viborg, Denmark) with fiber optic cables with oxygen sensor spots (PreSens, Regensburg, Germany) attached on the surface of borosilicate glass chambers. Oxygen readings were corrected to water temperature measured to the nearest 0.01°C, salinity of 0 ppt and barometric pressure of 1013 hPa. All eight chambers were situated in the same water bath (L400 x W225 x H140 mm) and supplied with recirculation and flush pumps with oxygen impermeable tubing (Tygon™) provided by the manufacturer (Loligo Systems, Viborg, Denmark). Flow rate was set to replenish oxygen but not cause physical disturbance, and the entire setup was surrounded by a black curtain to avoid visual disturbance but not eliminate the influence of artificial photoperiod. It is important to note that lights were on and off at 07:00 and 19:00 respectively. Throughout the entire experiment, the last meal was provided at 18:00 and on the following day, experiments started at 10:30 and were completed by 18:30. Fish were left in the chambers for 6 hours to measure $\dot{M}O_2$ and the duration of measurement cycles (flush for 360s, wait for 60-180s and measure for 300-900s) was adjusted to ensure that declines in oxygen saturation were linear and greater than 2%, but oxygen saturation did not drop below 80% as metabolic suppression in FMR has been reported at this level of oxygen in age1+ lake sturgeon (Svendsen et al. 2014). Of the 6 hours fish spent in respirometry chambers, RMR was assessed during the final 2 hours. Then, a standardized forced exercise protocol was performed to induce FMR as previously described (Yoon et al. 2021). The fragility of younger larvae made it challenging to move them in and out of chambers without physical damage, so fish from 6 to 48 dph were forced to exercise by increasing flow rates (0.19 ml s^{-1} to 0.86 ml s^{-1}) through adjusting voltages of pumps to induce swimming within chambers for 15 minutes. In contrast, fish from 55 to 76 dph were removed from respirometry chambers and individually chased for 15 minutes and were then returned to respirometry chambers for two measurement cycles (Yoon et al. 2021).

FMR was assessed as the highest $\dot{M}O_2$ among the two consecutive measurements following the standardized exercise protocol. During FMR measurement, waiting and measurement time were adjusted to ensure appropriate mixing prior to measurement and oxygen

decline greater than at least 2%, which was suggested by the manufacturer of the respirometry system (see above). At 6 and 14 dph, the first measurement consisted of 180 or 120 s of waiting, respectively, to ensure proper mixing inside the chamber followed by 300 s of measurement; the second measurement consisted of 360 s of flushing, 180 or 120 s of waiting, and 300 s of measurement. Also, at 55 dph the first measurement consisted of 60 s waiting and 900 s of measurement time, which was necessary to ensure an oxygen decline of 2%; the second measurement consisted of 360 s flush, 60 s of waiting and 900 s of measurement. For the rest of sampling timepoints, the first measurement consisted of 60 s waiting followed by 300 s of measurement and the second measurement consisted of 360 s of flush, 60 s of waiting, and 300 s of measurement.

Only $\dot{M}O_2$ slopes with $r^2 \geq 0.9$ (89.75% of all slopes) were used for data analysis (Chabot et al. 2021). Before and after each trial, oxygen consumption was measured for 15 minutes without fish to estimate background respiration, which was then used to interpolate and correct all corresponding $\dot{M}O_2$ values assuming that background respiration would increase linearly over time (Rodgers et al. 2016). Lastly, in order to examine effects of diet on RMR and FMR, we scaled all RMR and FMR data to 1g of body mass using the previously published intra-specific mass scaling exponent of 0.89 in teleosts with the following equation (Jerde et al. 2019; Norin et al. 2019):

$$\dot{M}O_{2\,scaled} = \dot{M}O_{2\,measured} \cdot (M \cdot Ms^{-1})^{1-b}$$

where $\dot{M}O_{2\,\text{scaled}}$ is the scaled metabolic rate either RMR_s or FMR_s, $\dot{M}O_{2\,\text{measured}}$ is measured $\dot{M}O_2$ either RMR and FMR, M is the measured body mass (g), Ms is the body mass to be scaled (i.e., 1g) and b is the mass scaling exponent (0.89; Jerde et al. 2019).

2.3.3 Growth and energy density

After each $\dot{M}O_2$ experiment, fish were sacrificed by immersion in an overdose of MS-222 (250mg·L; Syndel Laboratory, Vancouver, BC), and total length and body mass were measured to the nearest mm and 0.001g respectively. Carcasses were then dried at 60°C for 48 hours or until constant mass was recorded, and used to estimate whole body energy density as previously described (Yoon et al. 2019).

2.3.4 Statistical analysis

One-way ANOVA was performed to examine significant changes in mass, length, energy density, RMR, FMR and RMR_s and FMR_s across developmental time and dietary shifts. A *post hoc* Tukey's test was used to examine individual comparison of data between life stages. One-tailed hypothesis testing is appropriate to assess a specific and directional hypothesis (Ruxton and Neuhäuser 2010). Thus, we employed a paired t-test with one-tail distribution to test a prediction that FMR and FMR_s are higher than RMR and RMR_s at each life stage (week) and within the same diet. Log-log linear regression was performed to extract a metabolic scaling exponent to explain the relationship between RMR (mgO_2Hr^{-1}) or FMR (mgO_2Hr^{-1}) and body mass across the entire study and within each diet type:

$$\log (RMR \text{ or } FMR) = \log a + \log b \cdot M$$

where a is constant, M is body mass and b is a coefficient scaling factor. Statistical significance was examined at $\alpha < 0.05$. Assumptions of normality and homoskedasticity were visually assessed following the guideline published as previously (Zuur et al. 2010), and data were transformed by square root or log where these assumptions were not met.

2.4 Results

2.4.1 Growth and energy density

Our results showed fish did not significantly grow during the second week during diet transition from *Artemia* to bloodworm (55 to 62 dph) and the final week of study from 70 to 76 dph when fish were fully transitioned to bloodworm ($p>0.05$; Figure 2.1). Energy density significantly decreased during the yolk-sac stage, and there was no significant change during the rest of the study period (Figure 2.2).

2.4.2 Cumulative mortality

We observed a substantial increase in cumulative mortality during the yolk-sac stage (229 mortalities), 12 mortalities within the first two weeks of the onset of exogenous feeding, and 1 mortality during the *Artemia* to bloodworm transition (Figure 2.3).

2.4.3 Whole body metabolic rates

Mass-specific RMR and FMR ($mgO_2Kg^{-1}Hr^{-1}$) were significantly influenced by time throughout development ($p<0.01$; Table 1, Figure 4). Specifically, there was a significant decline in RMR between 6 to 20 dph as the fish shifted from a reliance on endogenous yolk to exogenous *Artemia* as their primary food source. There was a subsequent significant increase in RMR at 55 to 62 dph as fish transitioned from an *Artemia* based diet to a bloodworm based diet. Similarly FMR significantly declined as fish moved from yolk to *Artemia* as a primary food source, however, there was no difference in FMR beyond this initial developmental period. RMR was significantly lower than FMR during periods when fish were reliant on yolk, *Artemia* and bloodworm as their sole diet. Interestingly, however, RMR and FMR were not statistically different during the entire transitionary period from *Artemia* to bloodworm (55-62 dph; $p>0.1$; Figure 2.4, Table 2.4), a period coincidental with growth arrest.

Our results indicated that both non-mass specific RMR (mgO_2Hr^{-1}) and FMR (mgO_2Hr^{-1}) increased with body mass (Figure 2.6). Our log-log linear regression analysis indicated that a significant relationship was only found when fish were on the artemia and bloodworm based diets (20-48 and 70-76 dph; $p<0.01$, Table 2), but not when on a diet of yolk nor when transitioning from *Artemia* to bloodworm (6-14 and 55-62 dph; $p>0.05$, Table 2.2).

While the overall pattern of RMR_s and FMR_s were similar with RMR and FMR (Figure 2.4 and 2.5), results indicated that RMR_s and FMR_s were significantly influenced by dietary shifts ($p<0.001$; Table 2.3, Figure 2.5). Specifically, during transition from *Artemia* to bloodworm RMR_s was higher than when fish were feeding solely on yolk or *Artemia* ($p<0.01$; Figure 2.5). FMR_s indicated a similar trend, although FMR_s in fish fed solely *Artemia* was lower than FMR_s in fish feeding on yolk or bloodworm ($p<0.01$; Figure 2.5). Lastly, RMR_s and FMR_s were not different during the diet transition from *Artemia* to bloodworm ($p>0.1$; Figure 2.5, Table 2.5).

2.5 Discussion

The present study was designed to understand how RMR and FMR changed as larval lake sturgeon grew with a focus on periods of dietary shifts. Specifically, we tested the prediction that RMR and FMR would increase during diet transition from yolk to *Artemia* and then from *Artemia* to bloodworm. Our results partially supported this prediction as there was a significant increase in RMR and a cessation of growth when fish transitioned from *Artemia* to bloodworm.

We found that mortality increased during dietary shifts from consumption of endogenous yolk stores to exogenous feeding on *Artemia*, which agrees with previous findings (Gisbert and Williot 2002; Yúfera and Darias 2007; Hamre et al. 2013; Earhart et al. 2020b). It is not uncommon that mortality rate in fishes typically increases especially during diet transitions (Gisbert and Williot 2002; Yúfera and Darias 2007; Gessner et al. 2009; Conceição et al. 2010). For instance, in lake sturgeon raised in a conservation fish hatchery setting, high mortality has been reported during the dietary transition from *Artemia* to bloodworm (Klassen and Peake 2007; Earhart et al. 2020b; Yoon et al. 2020). In the present study we observed only one mortality during the transition from *Artemia* to bloodworm. Although we did not quantify mortality after 76 dph, a two-week delay in increasing mortality rate after the completion of diet transition was noted in a parallel study (Yoon et al., 2022). Collectively, these results may indicate that fish were struggling to eat enough, digest the food or absorb nutrients during the diet transition from artemia to bloodworm. Diet transition during the early life history may be limited by the ability to forage effectively and/or absorb nutrients from new prey items efficiently (Eggold and Motta 1992; Wilson and Castro 2010; German et al. 2014; Sánchez-Hernández et al. 2019). Body size may determine gape size, which would influence the size of prey that can be ingested and the nutritional profitability of those prey items (Nilsson and Brönmark 2000). Also, developmental status of the gastrointestinal tract is linked to dietary shifts (Buddington, 1985; Gonçalves et al., 2019; Wilson and Castro, 2010). Specifically, metabolic plasticity of the gastrointestinal tract such as regulation of gut morphology and digestive enzymes in response to dietary shifts may increase the metabolic cost for basal maintenance and thus metabolic rates (Penttinen and Holopainen 1992; Secor 2009; Pérez-Sirkin et al. 2020).

We found that RMR appeared to decrease by the onset of exogenous feeding (Figure 4), which agrees with previous studies conducted in steelhead trout, *Salmo gairdneri* (Rombough 1988) and white sturgeon, *Acipenser transmontanus* (Boucher et al. 2018). Changes in RMR during embryonic development may be reflective of distinct development patterns (Finn et al. 1995a; Green 2004). For example, in birds altricial species (i.e. underdeveloped at hatch) show an exponential increase in RMR during embryonic development whereas precocial species (i.e. relatively matured at hatch) show a sigmoidal increase with a transient peak just before hatch (Vleck and Vleck 1987; Vleck and Hoyt 1991). In oviparous fish such as white sturgeon, RMR

increased in embryos just before hatch then showed a significant decrease (Boucher et al. 2018) whereas in oviparous sharks such as white-spotted bamboo shark, *Chiloscyllium plagiosum* (Tullis et al. 2000) and epaulette sharks (*Hemiscyllium ocellatum*) (Wheeler et al. 2021) there was a steady increase followed by a decline in RMR during embryogenesis.

In the present study, three hypotheses may explain the observed decrease in RMR during yolk absorption. Firstly, more rapid growth in body size compared to the circulatory system may have resulted in a decrease in RMR. Post and Lee (1996) demonstrated a biphasic relationship between RMR and body mass in early development, suggesting an allometric decrease in gill surface area may result in a decrease in RMR. Secondly, the relative growth in metabolically active organs such as the heart, gastrointestinal tract and kidneys may show a differential pattern during ontogenesis (Green 2004; Kamler 2008; Yagi et al. 2010; Rosenfeld et al. 2015), which may give rise to changes in RMR during early development (Boldsen et al. 2013). Lastly, as larvae utilize the endogenous yolk for growth as well as basal metabolism, catabolic substrates for oxidative phosphorylation become limited (Finn et al. 1995a, 1995b), which may decrease metabolic rates.

In the present study we observed a growth arrest during the diet transition from artemia to bloodworm, which agrees with previous studies (Shields et al. 2003; Bódis et al. 2007; Walsh et al. 2015) and indicates that diet transition is a particularly challenging event in age-0 fish (Gisbert and Williot 2002; Yúfera and Darias 2007; Hamre et al. 2013; Sánchez-Hernández et al. 2019; Kandathil Radhakrishnan et al. 2020) that might demand an increase in metabolic rate as observed in the increased RMR during this period. Interestingly, we observed the growth arrest during the second week of diet transition and not the first. It is possible that larger individuals with bigger gape size may have taken advantage of feeding bloodworm earlier than smaller individuals leading to an initial unequal distribution of food within the treatment. Increased RMR may indicate changes in fish activity and/or metabolic regulation during diet transition. It is well documented that metabolic rate may be correlated with behaviours of fish (Steinhausen et al. 2005; Millidine et al. 2008; Svendsen et al. 2014). Although we did not quantify behaviour of fish alongside $\dot{M}O_2$, elevation in RMR during diet transition suggests that body movement of larval fish may have increased in search for food (i.e., *Artemia*) to which they were habituated. Further, increased RMR during diet transition may be a result of metabolic responses of the gastrointestinal tract in response to a change in diet as it has been reported that changes in

quantity and quality of dietary intake are known to influence the rate of digestive processes and gut evacuation (McKenzie et al. 1999; Venou et al. 2003; Secor 2009; Lund et al. 2012; Chabot et al. 2016a), both of which could potentially influence measurement of RMR. Conversely, elevated RMR may reflect an increased energetic expenditure for metabolic adjustments in the gastrointestinal tract ()during the transition (Shan et al. 2009; Ostaszewska et al. 2018; Wei et al. 2018; Lipscomb et al. 2020; Pérez-Sirkin et al. 2020). Our observed change in RMR may also be reflected in endocrine regulation of gut development. Previously it was shown that whole body cortisol significantly increased in lake sturgeon during transition from *Artemia* to bloodworm (Earhart et al. 2020a). Not only is cortisol well-recognised as the primary endocrine stress hormone in sturgeons and therefore involved in mobilisation of energy reserves, but also in combination with thyroid hormones, it plays a role in growth and development (Brown and Kim 1995; Mommsen et al. 1999) through facilitation of nutrient uptake across the intestine (Tanaka et al. 1995), intestinal integrity (Rosengren et al. 2018) and metamorphosis (Veillette et al. 1995; Earhart et al. 2020a). Thus, elevated cortisol during this transitionary period may in part be responsible for the observed increased RMR as fish transition from a predominantly *Artemia* based diet to a predominantly bloodworm based diet.

Increased RMR during the diet transition may leave little room for other metabolic processes. Specifically, the lack of difference between RMR and FMR during the diet transition may indicate constrained scope for routine activity such as digestion and growth (aerobic scope; AS). AS is considered as the metabolic capacity for growth in the young of the year fish (Hochachka and Somero 2001), but high energetic demands during the early larval development could result in smaller AS (Wieser 1995; Post and Lee 1996; Killen et al. 2007; Nilsson et al. 2007; Yoon et al. 2021). However, recent studies have suggested that the relationship between AS and growth is equivocal (Grans et al. 2014; Norin et al. 2014; Messmer et al. 2017; Jutfelt et al. 2018). In our study, the growth arrest may be a result of increased body activity and metabolic adjustment and anatomical remodelling of gastrointestinal tract during diet transition. Alternatively, the lack of difference between RMR and FMR between 55 to 76 dph could be due to our method to estimate metabolic rate. Logistical constraints, such as 1) duration of measurement being fixed for 6 hours across all sampling dates as extended hours of measurement could result in mortalities for younger larvae ($\leqq$ 20 dph); 2) frequency of feeding meant larvae may not have been in a post-prandial state during $\dot{M}O_2$ measurement; and 3)

changing animal mass to chamber volume ratios as larvae grew meant we could not confidently assess metabolic costs of potential spontaneous activity particularly in the smaller fish (Bjornson et al. 2020; Yoon et al. 2021), may have lead to RMR values higher than FMR values from some individuals (Figure 2.7). Also, differences in FMR between methods could be due to swimming behaviours of fish (Rummer et al. 2016). For example, FMR measured by a standardized forced exercise protocol of chasing the fish was 36% lower than FMR measured in a swimming respirometer in the bridled monocle bream *Scolopsis bilineata* (Roche et al., 2013). In contrast, a recent meta-analysis demonstrated no difference in FMR between chasing and swimming methods in 121 fishes (Killen et al. 2017). In the present study, it may be possible that the lack of difference between RMR and FMR could be a potential underestimation of FMR due to the chase protocol.

Our results of mass scaling exponents for RMR and FMR were within the range of previously reported values (Clarke and Johnston 1999), but higher than theoretical suggestions (West et al. 1997; Brown et al. 2004; Jerde et al. 2019). Indeed, our trend agrees with a previous study in the similar range of age from 6 to 24 dph white sturgeon (Boucher et al. 2018) and 0 to 30 dph green sturgeon (*Acipenser medirostris*) (Gisbert et al. 2001). Historically, numerous studies have suggested a universal metabolic scaling exponent across taxa ranging from 2/3 to 3/4 (Kleiber 1932; West et al. 1997; Wang et al. 2001). More recently, a study suggested that intra-specific metabolic scaling exponents in teleosts could be close to 0.89 (Jerde et al. 2019). However, intraspecific metabolic scaling exponents have been poorly described in most fish species. Specifically, during early development fish may show a higher scaling exponent, which may reflect the remarkable changes in morphology and physiology associated with digestion (Oikawa et al. 1991; Wang et al. 2001; Kamler 2008). For example, in yolk-sac larvae, it is hypothesized that the relative changes in amount of metabolic substrate (lipid/protein/carbohydrate) in the yolk to growth of inert body mass may influence oxygen consumption and thus potentially result in an overestimate of metabolic scaling exponents (Treberg, *Personal Comm.*). Furthermore, digestibility of prey could significantly influence oxygen consumption rate (i.e. specific dynamic action) (Secor 2009), which may lead to overestimation of metabolic scaling exponents. Importantly, many theoretical frameworks of metabolic scaling in fish have been suggested based on juvenile or adult fishes (Clarke and Johnston 1999; Killen et al. 2010; Jerde et al. 2019); thus, future studies are suggested to

understand the physiological mechanisms that drive elevated metabolic scaling exponents during early life history in fish.

We also saw that log-log relationships between RMR or FMR and body mass changed with dietary shifts. Specifically, we saw the lack of a significant log-log relationship between RMR and body mass during the endogenous yolk (6-14 dph) and diet transition from *Artemia* to bloodworm (55-62 dph) while FMR and body mass showed significant relationships across all diets. Previous studies demonstrated that developmental stages and dietary shifts may influence metabolic scaling in larval sturgeon (Gisbert et al. 2001; Boucher et al. 2018). In green sturgeon non-mass specific RMR (mgO_2Hr^{-1}) did not significantly increase with body mass during the transition from cutaneous to branchial respiration (9-10 dph) and that the mass scaling exponent of RMR in the green sturgeon decreased from 1.64 ± 0.2 during endogenous yolk to 1.04 ± 0.1 during exogenous feeding (Gisbert et al. 2001). In white sturgeon Boucher et al. (2018) reported decreased mass scaling exponents of RMR and MMR after the onset of exogenous feeding compared to endogenous feeding phase (1.34 ± 0.21 to 1.13 ± 0.07 and 0.74 ± 0.22 to 0.60 ± 0.30, mean ± S.E. respectively). Although a significant log-log relationship between FMR and body mass during the entire study period may indicate that development of the cardiorespiratory system was not impacted by dietary shifts (Hillman et al. 2013), it remains unknown why we saw a different trend in RMR compared to two other studies. The result may be due to lack of data or narrow size range prior to 6 dph and/or different diets used in the present study. In both white and green sturgeon, artificial diets were used at the onset of exogenous feeding whereas in our study we used *Artemia* and bloodworm. It is thought that digestibility between artificial diet and diet used in our study may differ, which can influence oxygen consumption during digestion and assimilation (Secor 2009; Chabot et al. 2016a), which may have resulted in different scaling exponents in lake sturgeon than previously reported for other sturgeon species. Also, it is possible that the heterogeneity of metabolic scaling exponents could be attributable to different species, developmental stages, lifestyle and environmental temperature (Oikawa et al. 1991; Post and Lee 1996; Finn et al. 2002; Moran and Wells 2007; Killen et al. 2010; White et al. 2012; Yagi and Oikawa 2014).

2.6 Conclusion

Our results showed growth arrest and elevated routine metabolic rate during the diet transition from *Artemia* to bloodworm, which indicates a physiological response to metabolic

reorganization as diet changes. During the diet transition, there was no statistical difference between RMR and FMR while increased mortality rate was observed albeit at a very low level. Our results demonstrated that larval lake sturgeon showed a higher metabolic scaling exponent than those reported for teleosts, which may suggest a distinct developmental pattern of morphology and physiology in Acipenceriforms that could be life-stage specific. Elevated RMR during dietary shifts in early life history may suggest further constraints on aerobic scope in larval fish, which would in part explain increased elevated mortality rates during these sensitive periods. The current study has implications for understanding cohort and population dynamics as well as development of rearing protocols in conservation hatcheries for lake sturgeon, an endangered species across its natural range.

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Table 2.1. Summary of one-way ANOVA to test significant changes in total length, body mass, energy density, routine (RMR; $mgO_2Kg^{-1}Hr^{-1}$), forced maximum metabolic rate (FMR; $mgO_2Kg^{-1}Hr^{-1}$) by days post hatch (dph) in age-0 lake sturgeon, *Acipenser fulvescens*. df denotes degrees of freedom.

Measurement	Variables	df	Sum Sq	Mean Sq	F-value	P
Total Length	dph	10	170.23	17.02	462.90	<0.001
	Residuals	70	2.57	0.04		
Body mass	dph	10	262.95	26.30	555.80	<0.001
	Residuals	70	3.31	0.05		
Energy density	dph	10	7.299	0.73	10.62	<0.001
	Residuals	69	4.744	0.07		
RMR	dph	10	330	33.00	8.90	<0.001
	Residuals	68	252.2	3.71		
FMR	dph	10	231	23.10	3.21	0.002
	Residuals	69	495.9	7.19		

Table 2.2. Log-log regression analysis for the scaling relationships between routine (RMR; mgO_2Hr^{-1}) or forced maximum metabolic rate (FMR; mgO_2Hr^{-1}) and body mass (g) in age-0 lake sturgeon (*Acipenser fulvescens*). Data were collected on a weekly basis. r^2 : adjusted r^2.

	dph	Diet	Coefficient	Std Error	t-value	r^2	p
RMR	6 - 14	Yolk	0.224	0.394	0.568	-0.055	0.581
	20 - 48	Artemia	1.169	0.079	14.740	0.871	<0.001
	55 - 62	AB Transition	0.860	0.4392	1.958	0.159	0.071
	70 - 76	Bloodworm	1.209	0.330	3.669	0.454	0.003
FMR	6 - 14	Yolk	1.554	0.433	3.588	0.477	0.004
	20 - 48	Artemia	0.906	0.106	8.570	0.681	<0.001
	55 - 62	AB Transition	0.845	0.277	3.050	0.372	0.009
	70 - 76	Bloodworm	1.277	0.301	4.237	0.541	<0.001

Table 2.3. Summary of one-way ANOVA of scaled routine (RMR_s; $mgO_2Kg^{-0.89}Hr^{-1}$) and forced maximum metabolic rate (FMR_s; $mgO_2Kg^{-0.89}Hr^{-1}$) in age-0 lake sturgeon (*Acipenser fulvescens*) by different diets. Square root transformation was applied for the data to meet the assumptions of normality and homoskedasticity.

	Parameters	df	Sum Sq	Mean Sq	F value	p
RMR_s	Diet	3	414.2	138.1	39.3	<0.001
	Residuals	75	263.4	3.51		
FMR_s	Diet	3	129.2	43.1	7.5	<0.001
	Residuals	76	438.5	5.8		

Table 2.4. Results of paired t-test in difference between routine metabolic rate and maximum metabolic rate in age-0 lake sturgeon, *Acipenser fulvescens*. Metabolic rates were assessed on a weekly basis. See the text for details.

Time (dph)	t	df	p
6	-3.9848	6	0.004
14	-3.7589	6	0.005
20	-4.5782	6	0.002
27	-1.1814	4	0.151
34	-3.1046	6	0.011
41	-2.0153	6	0.045
48	-1.9131	6	0.052
55	0.6751	6	0.738
62	-1.3493	7	0.110
70	-1.3691	7	0.107
76	-3.6238	7	0.004

Table 2.5. Results of paired t-test in difference between scaled routine metabolic rate and maximum metabolic rate in age-0 lake sturgeon, *Acipenser fulvescens*. Metabolic rates were scaled to 1g using a mass scaling exponent of 0.89. See the text for details.

Diet	t	df	p
Yolk	-4.643	13	<0.001
Artemia	-4.964	32	<0.001
AB Transition	-0.443	14	0.332
Bloodworms	-2.946	15	0.005

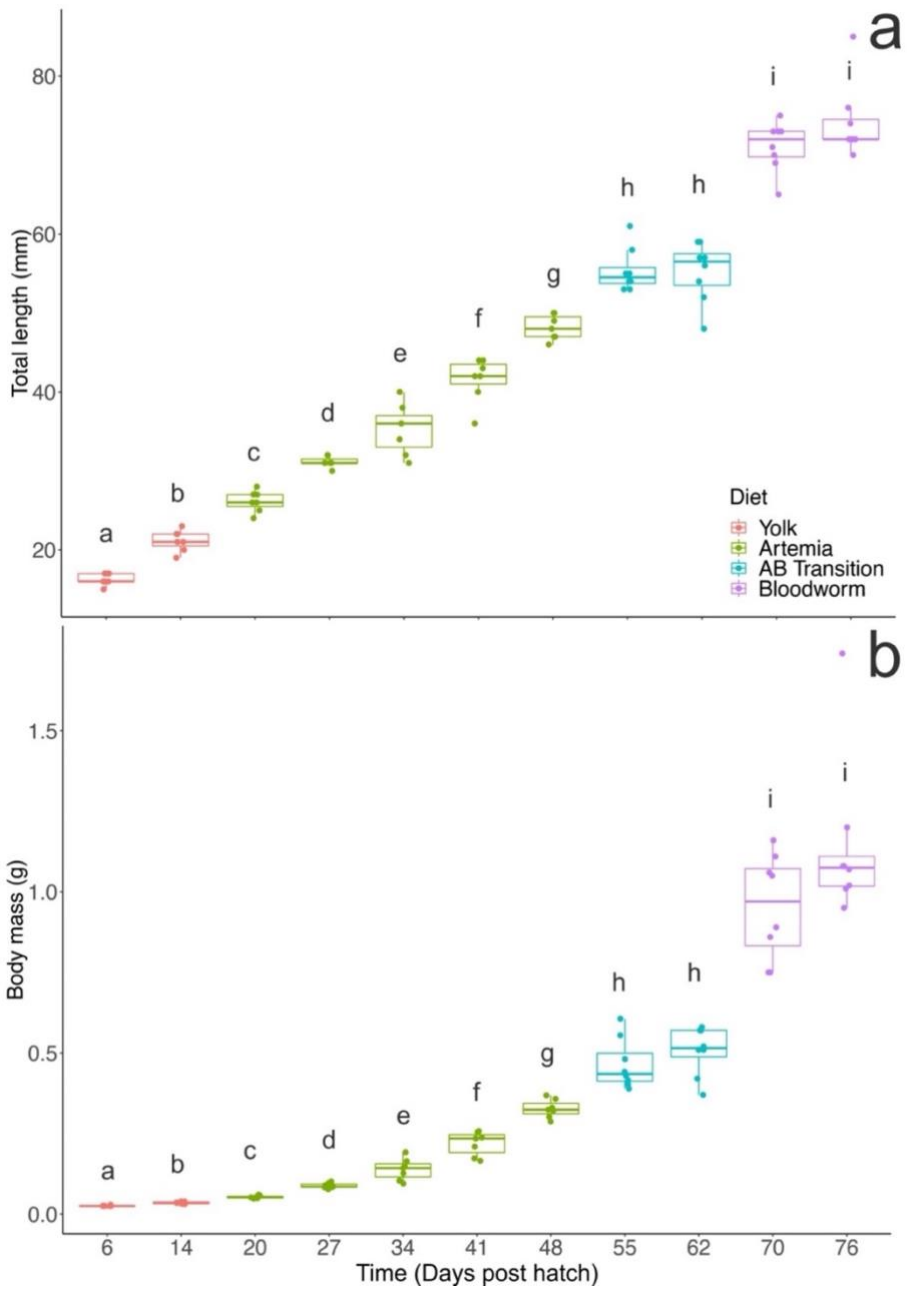

Figure 2.1. Changes in total length (a) and body mass (b) of age-0 lake sturgeon (*Acipenser fulvescens*) reared from 0 to 76 days post hatch (dph) fed *Artemia* from 17 dph, transitioned from *Artemia* to bloodworm (AB Transition) from 49 - 67 dph and then fed completely on bloodworm from 67-76 dph. Different lowercase letters denote statistically significant differences in morphometric traits between dph. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represents data distribution. Dots represent individual data points.

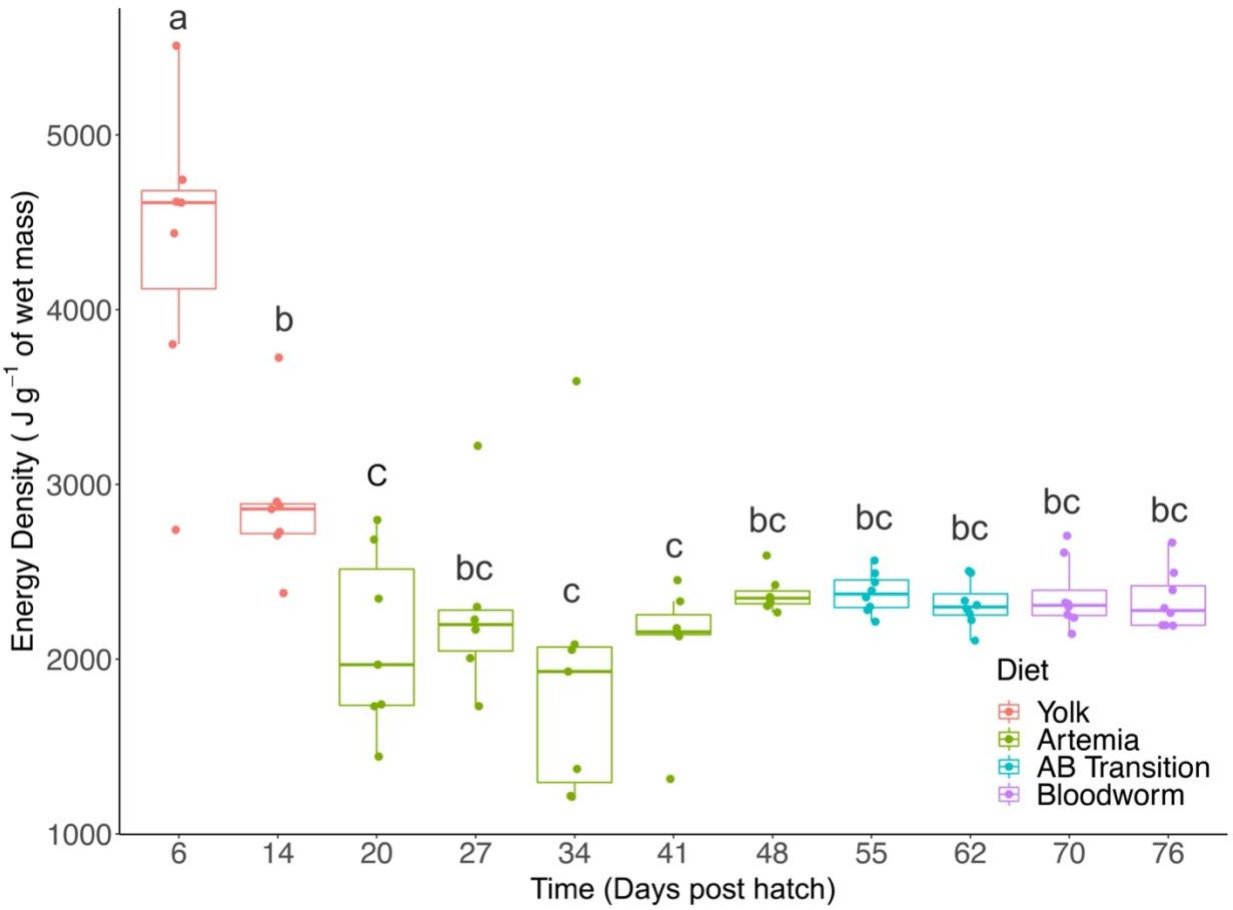

Figure 2.2. Changes in energy density of age-0 lake sturgeon (*Acipenser fulvescens*) in the present study reared from 0 to 76 days post hatch (dph) fed artemia from 17 dph, transitioned from *Artemia* to bloodworm (AB Transition denote *Artemia* to bloodworm transition) from 49-67 dph and then fed completely on bloodworm transition from 67-76 dph. Different lowercase letters denote statistically significant differences in morphometric traits between days post hatch. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots represent individual data points. Data are not transformed for graphical purpose.

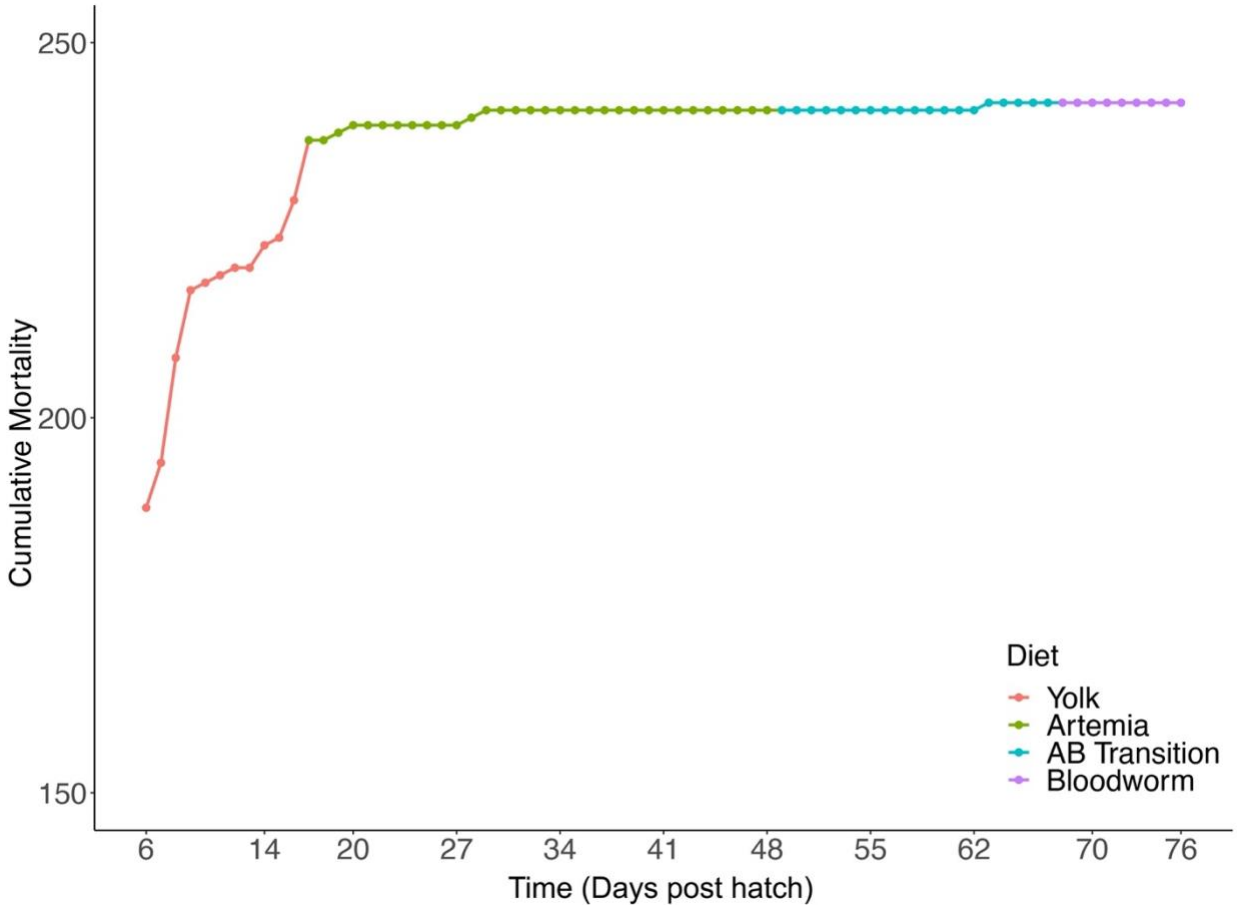

Figure 2.3. Changes in cumulative mortality of age-0 lake sturgeon (*Acipenser fulvescens*) in the present study. Fish were reared from 0 to 76 days post hatch (dph) fed *Artemia* from 17 dph, transitioned from *Artemia* to bloodworm (AB Transition) from 49 - 67 dph and then fed completely on bloodworm from 67-76 dph.

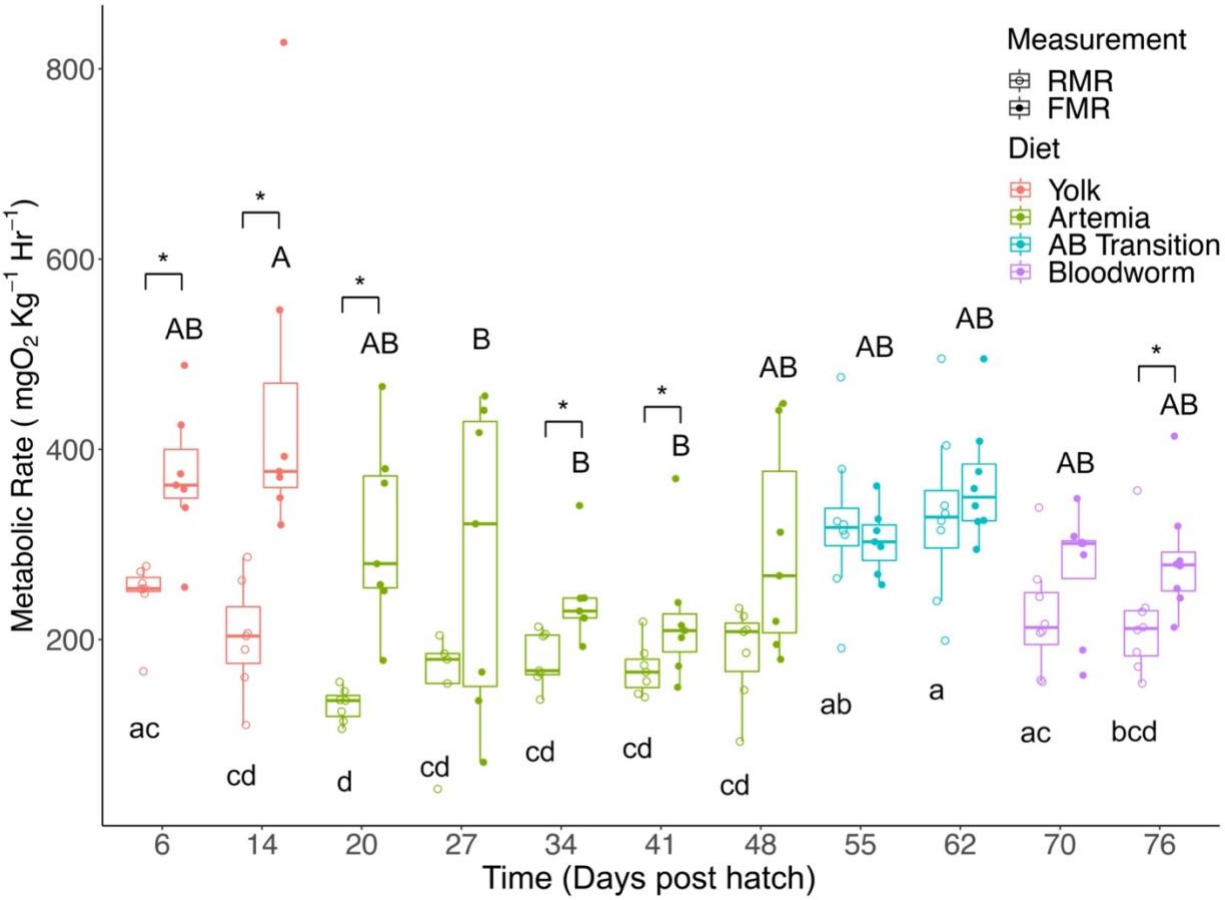

Figure 2.4. Changes in routine (RMR; open dots) and forced maximum (FMR; filled dots) metabolic rates in age-0 lake sturgeon (*Acipenser fulvescens*) used in the present study. Fish were reared from 0 to 76 days post hatch (dph) fed *Artemia* from 17 dph, transitioned from *Artemia* to bloodworm (AB Transition) from 49 - 67 dph and then fed completely on bloodworm from 67-76 dph. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots represent individual data points. Different letters denote significant difference between time points. Letters below or over the boxplot denote for significant difference in RMR (small) or FMR (capital) between time points respectively. * represents significant difference between RMR and maximum FMR at each time point.

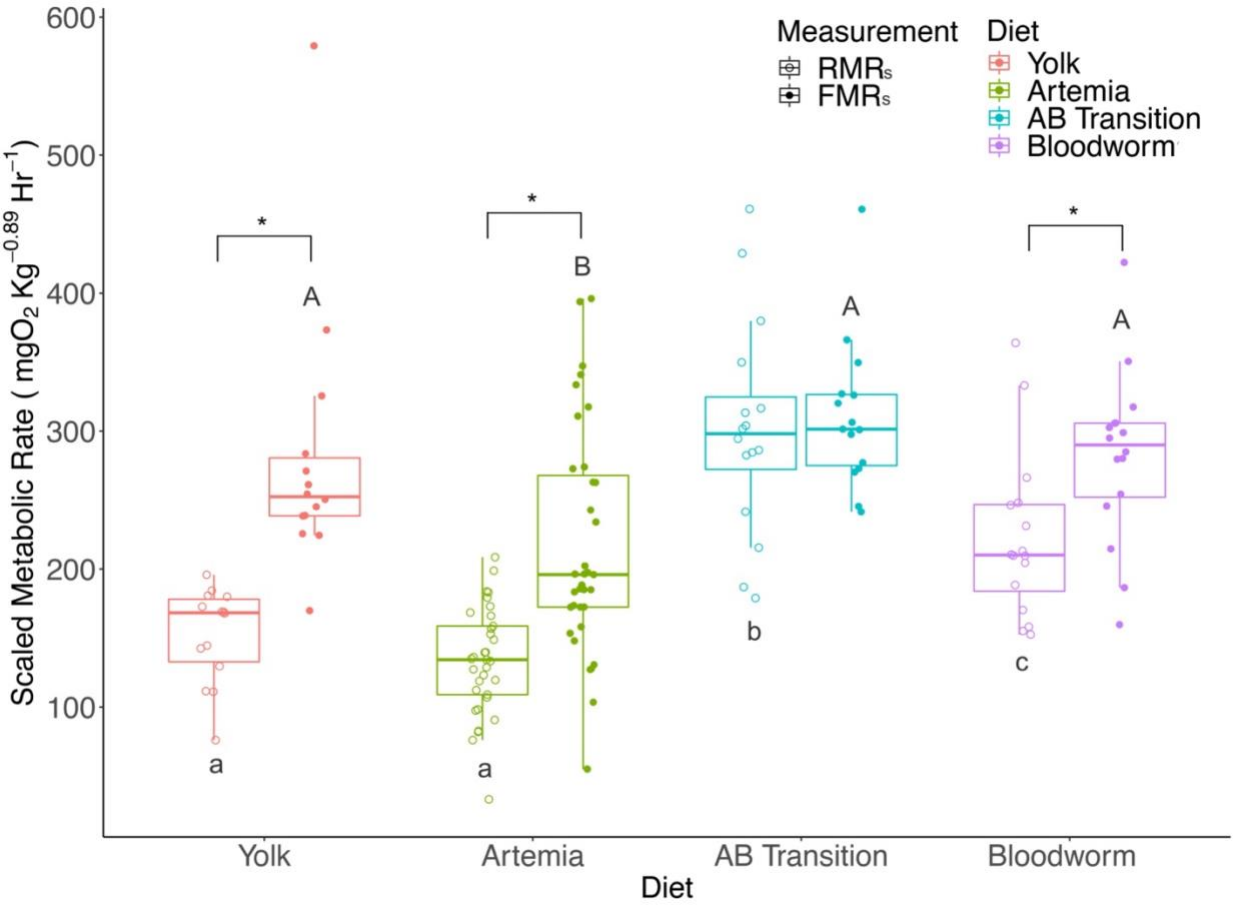

Figure 2.5. Scaled routine (RMR_s; open dots) and forced maximum metabolic rate (FMR_s; filled dots) in age-0 lake sturgeon (*Acipenser fulvescens*) by different diets. Metabolic rates were scaled to 1g of body mass using a mass scaling exponent of 0.89. Fish were reared from 0 to 76 days post hatch (dph) fed *Artemia* from 17 dph, transitioned from *Artemia* to bloodworm (AB Transition) from 49 - 67 dph and then fed completely on bloodworm from 67-76 dph. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots represent individual data points. Different letters denote significant difference within each metabolic rate by treatment, where capital letters denote differences between FMR_s and lowercase letters denote differences between RMR_s. * represent significant difference between RMR_s and FMR_s within diet types.

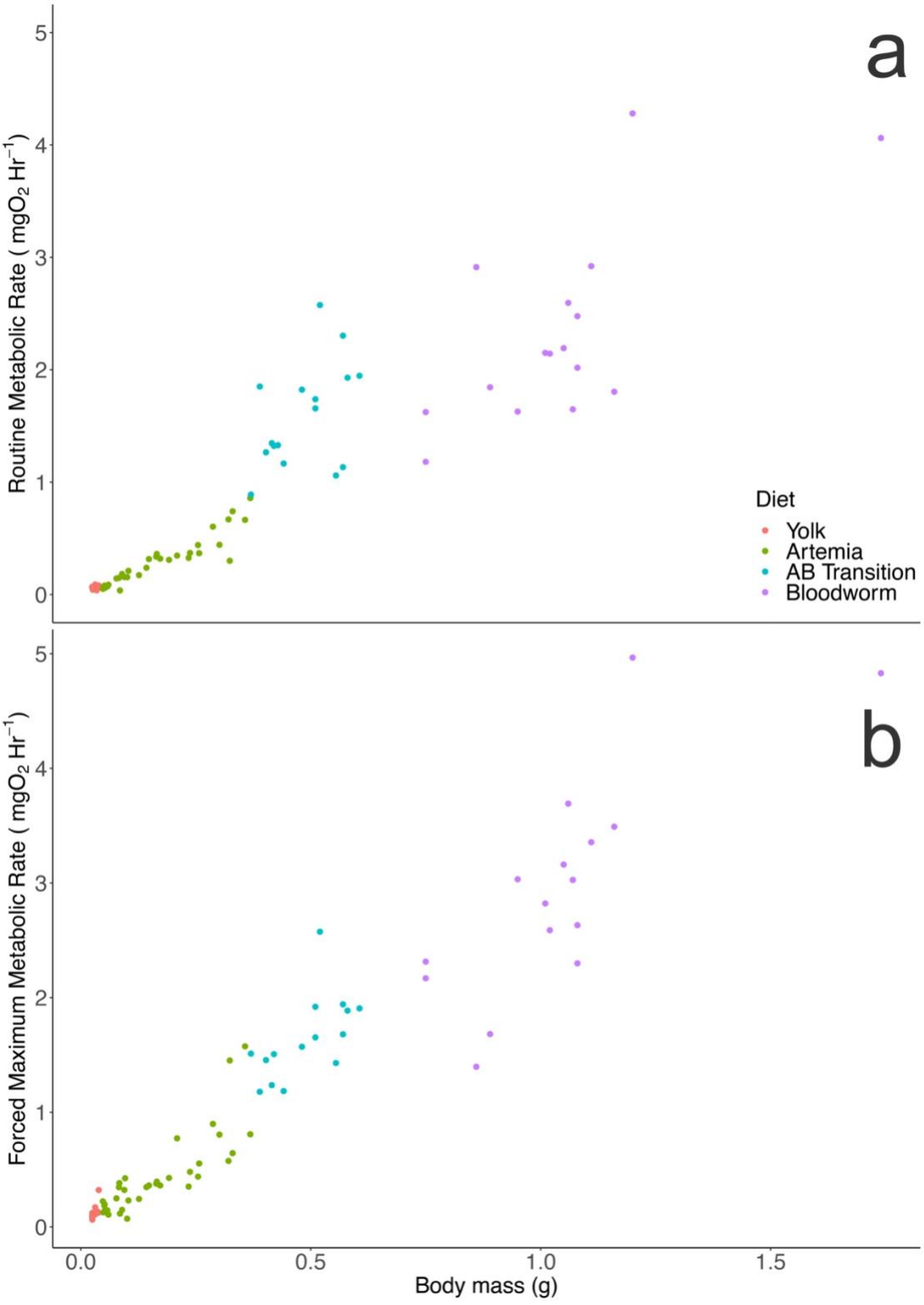

Figure 2.6. Relationship between routine and forced maximum metabolic rate and body mass (g) in age-0 lake sturgeon *Acipenser fulvescens*. Dots represent individuals data points.

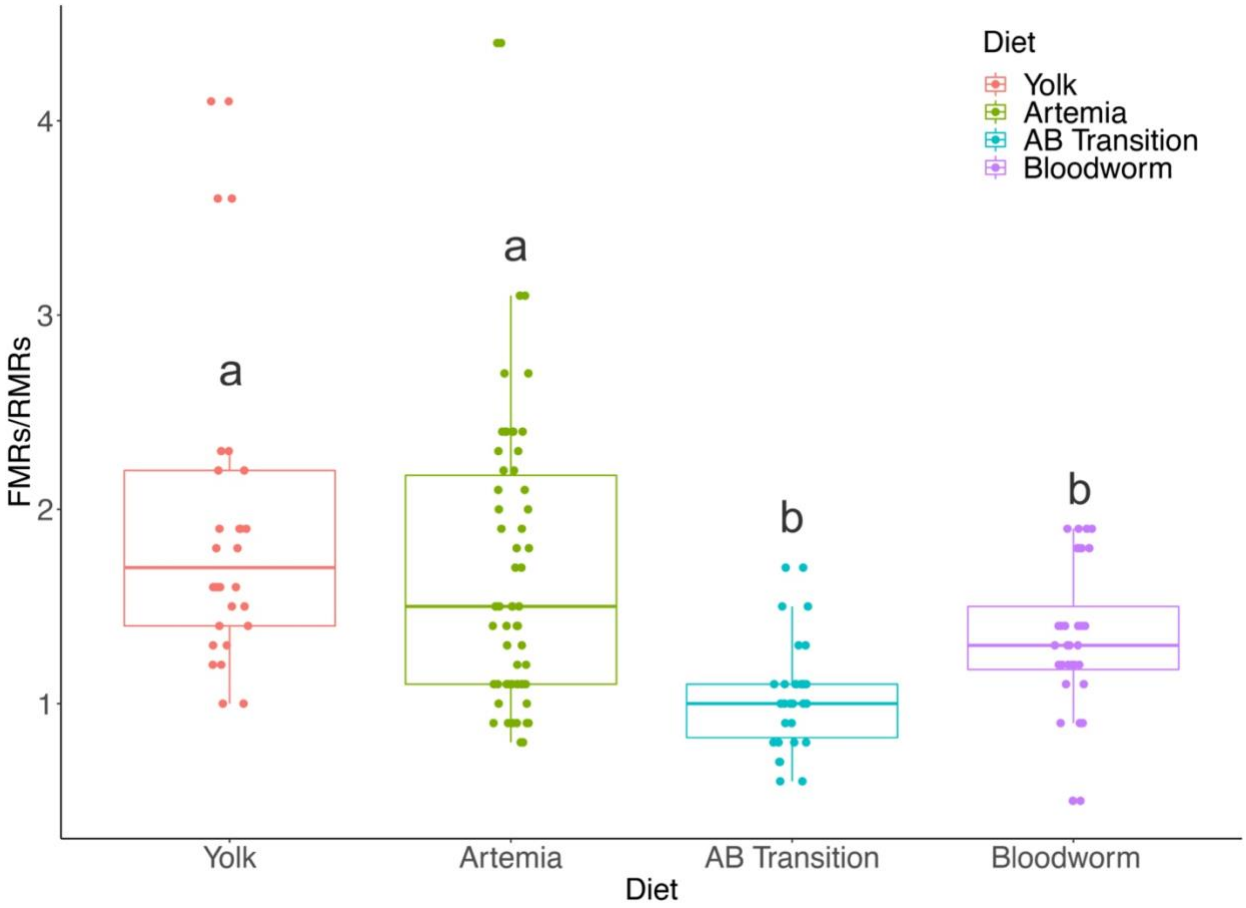

Figure 2.7. Ratio between scaled forced maximum metabolic rate (FMRs)/scaled routine metabolic rate (RMRs) in age-0 lake sturgeon *Acipenser fulvescens* reared from 0 to 76 days post hatch (dph) fed *Artemia* from 17 dph, transitioned from *Artemia* to bloodworm (AB Transition) from 49 - 67 dph and then fed completely on bloodworm from 67-76 dph. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots represent individual data points.

Chapter 2 demonstrated that metabolic rate, energy density and growth during early life are plastic traits that may be strongly influenced by diet. Chapter 3 examined how other metabolic phenotypes such as fatty acid profiles would change by temperature and diet.

3 Chapter 3: Effects of temperature and food availability on liver fatty acid composition and plasma cortisol concentration in age-0 lake sturgeon: support for homeoviscous adaptation

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3.1 Abstract

Overwintering survival in north temperate fishes involves a series of adaptive responses to multiple environmental stressors. Homeoviscous adaptation includes changes in membrane lipid composition in response to reduced environmental temperature, which may be driven by changes in hormones involved in the endocrine stress response. We examined how reduced temperature and food availability may act in concert to influence hepatic fatty acid composition of phospholipids and triglycerides, in addition to plasma concentration of cortisol in age-0 lake sturgeon (*Acipenser fulvescens*). At 153 days post hatch (dph), temperature was decreased from 16°C to 1°C at a rate of 0.5°C per day, and at 200 dph, fish were either fed every other day or deprived of food for 45 days to simulate an overwintering event. Liver fatty acid composition of phospholipids and triglycerides were assessed before temperature manipulation (16°C; 153 dph), when fish had been at 1°C for 16 days (199 dph), 25 days of overwintering (225 dph) and 45 days of overwintering (245 dph). Plasma cortisol concentration was assessed at 153, 225 and 245 dph. When temperature was decreased, both mono- and polyunsaturated fatty acids significantly increased in phospholipids and triglycerides. Total omega-6 fatty acids significantly increased in phospholipids while total omega-3 fatty acids did not. During the simulated overwintering, there was no obvious difference in fatty acids of phospholipids and triglycerides between diet treatments and no difference in circulating cortisol concentration between baseline and post-stressed fish in the fasted group. Our results provide support for homeoviscous adaptation to cold temperatures in lake sturgeon.

3.2 Introduction

Physiological adaptation to changing environments is particularly important in young of the year fish as it strongly dictates the subsequent cohort and population dynamics (Johnson et al., 2014; Sogard, 1997). The significance of this concept is well illustrated in temperate and sub-polar species as they approach the first winter of life (Fernandes and McMeans, 2019; Michaletz, 2010; Sogard and Olla, 2000). While physiological responses to cold environments have been reasonably well described (Donaldson et al., 2008), our knowledge is still lacking in terms of how extended periods of food deprivation, typical of a northern winter, can influence these responses. For example, scarcity in food during winter months may lead to β-oxidation of endogenous fatty acids to sustain vital cellular processes (Bar, 2014; Mommsen et al., 1999), however, this requirement often needs to be balanced with similar biochemical processes required for the synthesis of monounsaturated faaty acid (MUFA) and polyunsaturated fatty acids (PUFA) that play a key role in maintenance of membrane fluidity in response to cold temperatures (Homeoviscous adaptation; HVA) (Monroig et al., 2018).

In fishes, HVA has been well reported where exposure to colder environments results in an increase in MUFAs and PUFAs with concomitant decreases in saturated fatty acids (SATs) in both liver and muscle tissues (Hazel, 1995; Hsieh et al., 2003; Snyder et al., 2012; Wijekoon et al., 2021). Eicosapentaenoic acid (EPA, C20:5n3), arachidonic acid (AA, C20:4n6) and docosahexaenoic acid (DHA, C22:6n3) are long chain polyunsaturated fatty acids (LC-PUFAs) that are commonly found in phospholipids and thought to play a key role in HVA (Hazel, 1997; Parrish, 2009; Pernet et al., 2007; Sargent et al., 1993; Tocher and Sargent, 1990; Wallaert and Babin, 1994). During HVA, these LC-PUFAs could be supplied from dietary intake as fatty acids of triglycerides that are transferrable to phospholipids via acylation processes catalyzed by phospholipases, acyltransferases, and transacylases (Yamashita et al., 2014) or LC-PUFAs could be converted *in vivo* from either alpha-linolenic acid (C18:3n3) or linoleic acid (C18:2n6) through a series of desaturation, elongation and peroxisomal β-oxidation (Li et al., 2010; Monroig et al., 2018; Park et al., 2009; Sprecher, 2000; Tocher, 2010). Specifically, it has been shown that enzymatic activity levels of Δ 6 desaturase, a rate limiting enzyme for PUFA synthesis, increase in low temperature acclimated teleosts providing a mechanistic link between phospholipid restructuring and cold adaptation - HVA (Hagar and Hazel, 1985; Schünke and Wodtke, 1983; Tocher et al., 2004).

It is hypothesized that fish cannot synthesize C18:3n3 nor C18:2n6 due to a lack of Δ 12 and 15 desaturases (Monroig et al., 2018). Since *in vivo* production of LC-PUFAs is possible through substrate input of C18:3n3 and C18:2n6 from the diet (see Figure 3.1), reduced access to food sources during winter months may limit the capacity for appropriate cold adaptation. Food deprived fish catabolize fatty acids from triglyceride stores to support vital processes (Bar, 2014), but it has also been suggested that prolonged food deprivation may deplete such stores, leading to a breakdown of membrane lipids (Brückner and Heethoff, 2020). Fatty acids released from triglycerides could be metabolized to synthesize various molecular species of phospholipids such as phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine, all of which are known to play a key role in maintaining membrane fluidity in fish (Coleman and Lee, 2004; Tocher et al., 2008; Tocher and Sargent, 1990). As the capacity for HVA may depend on the availability of pre-existing substrates or PUFAs (Alhazzaa et al., 2013; Craig et al., 1995; Kelly and Kohler, 1999; Wijekoon et al., 2021), it is hypothesized that under cold, food deprived conditions, synthesis of LC-PUFAs in phospholipids may be facilitated by catabolism of fatty acids from triglycerides and the lipolysis of triglycerides may be mediated by cortisol, a glucocorticoid hormone involved in regulation of metabolites in response to a cold stress (Dave et al., 1979; de Gomez Dumm et al., 1979; Donaldson et al., 2008; Mommsen et al., 1999). Indeed, recent work in age-0 lake sturgeon (*Acipenser fulvescens*) showed that 28 days of fasting at 1°C resulted in a gradual decrease in plasma triglyceride concentrations followed by a significant increase in triglycerides post-fasting concomitant with a significant increase in whole body cortisol concentration (Deslauriers et al., 2018).

Every year northern fish populations experience periods of near 0°C and limited resources that can last for in excess of 5 months (Hrenchuk et al., 2017). Therefore, it is reasonable to expect that an appropriate HVA response would be particularly important for age-0 fish as they approach their first winter of life (Donaldson et al., 2008; Hazel, 1997; Hurst, 2007). Lake sturgeon is a species endangered or at risk across its natural range (Bruch et al., 2016) and conservation aquaculture has been in operation to support populations across its range (Osborne et al., 2020). In Manitoba, Canada, lake sturgeon fall fingerlings or spring yearlings are released annually to enhance existing populations, but estimated survival is substantially lower in fall fingerlings compared to spring yearlings (McDougall et al., 2020, 2014). One factor that likely reduces survival of fall fingerlings is the first winter of life. It has been shown that freshwater

alewife (*Alosa pseudoharengus*) exhibited increased mortality during winter months which may be the result of an inappropriate HVA response (Snyder et al., 2012; Snyder and Hennessey, 2003). Therefore, understanding how overwintering conditions could influence fatty acid metabolism and plasma cortisol concentration of age-0 lake sturgeon would inform current rearing practices and may ultimately result in improved post-release survival rates for fall fingerlings.

In this study, we examined the effects of reduced environmental temperature on hepatic fatty acid composition and tested the prediction that a reduced temperature would increase the proportion of MUFAs and PUFAs in phospholipids. We chose to measure fatty acids in liver tissue as liver is the central organ of fatty acid metabolism for phospholipids and triglycerides (Enjoji et al., 2018). We also examined the added effects of food deprivation and tested the prediction that fasted fish would have decreased hepatic MUFAs and PUFAs in triglycerides compared to fed fish during reduced environmental temperature, which may in part be driven by an increase in plasma cortisol concentration.

3.3 Material and Methods

3.3.1 Animal Husbandry and Environment Manipulation

Gamete collection and animal rearing were performed as previously described (Earhart et al., 2020) and 5 month-old fish were used for the present study. There were three flow-through fish tanks (11L; 27 × 18 × 40cm) per treatment. At 153 days post hatch (dph), the protocol for a simulated overwintering event was performed by decreasing water temperature from 16°C to 1°C by 0.5°C per day. All experiments were conducted in a controlled environment chamber (CMP 6050, Conviron, Manitoba, Canada) which allowed for cooling air temperature to near 0°C. Cold water inflow was at approximately 2°C; thus, a combination of reduced air temperature allowed us to maintain aquarium temperature at approximately 1°C for overwintering. When temperature reached 1°C, fish were either fed every other day to satiation with bloodworm (Hikari, California, USA) or transferred to one of three tanks to be fasted for 45 days. Fish were sampled for the measured variables on four separate occasions during the course of the study (1) at 153 dph, 16°C prior to any temperature manipulation or food deprivation; (2) at 199 dph when fish had been at 1°C for 16 days; (3) at 225 dph, 25 days after the initiation of

feeding/fasting protocol at 1°C; (4) at 245 dph, 45 days after the initiation of the feeding/fasting protocol at 1°C.

3.3.2 Sampling

At each sampling point, 8 fish were haphazardly captured from tanks by dipnet and sacrificed with an overdose of anesthetic (250mg·L^{-1}, MS-222, Syndel Laboratory, British Columbia, Canada) buffered with an equal amount of sodium bicarbonate. Then, total length and body mass were measured to 1mm and 0.001g, respectively. Following euthanization, whole blood was taken from fish by severing the tail and collecting blood with hematocrit tubes. Blood was transferred to 1.5mL Eppendorf tubes and centrifuged at 10,000 g for 5 minutes to separate plasma from red blood cells. The plasma was removed from the sample and frozen at -80°C until subsequent cortisol analysis. The liver was then removed from the abdominal cavity and again stored at -80°C for subsequent fatty acid analysis.

3.3.3 Fatty acid analysis

We analyzed total fatty acid composition of the diet (bloodworm) and fatty acid composition of phospholipids and triglycerides in the liver tissue. In brief, dietary fatty acid profile of bloodworm was assessed through the direct saponification and methylation method with slight modifications (Kang and Wang, 2005). In terms of fatty acid profile in phospholipids and triglycerides of the liver tissue, tissue was homogenized with 0.025% $CaCl_2$ and total lipids were extracted (n=8 per treatment) by chloroform:methanol (2:1, v/v) with butylated hydroxytoluene as an antioxidant (Folch et al., 1957). Analysis of fatty acids from phospholipid and triglyceride fractions were performed as previously described (Feltham et al., 2019). The extracted lipids were separated on silica gel G plates in a solvent system consisting of ether:diethylether:acetic acid in a ratio of 80:20:1 (by vol) to separate phospholipid and triglyceride. The corresponding lipid classes were visualized with 0.1% 8-anilino-1-naphthalene-sulfonic acid under UV light and scraped for saponification and methylation. Fatty acid methyl esters were prepared using 14% BF_3 (Suh et al., 2009). Fatty acid methyl esters were analyzed via gas chromatography (Vista 6010 GLC and Vista 402 data system; Varian Instruments, Ontario, Canada) with a microcapillary column (15 m × 0.1 mm inner diameter; BPX70; SGE

analytical science, North Carolina, USA). Hydrogen was used as a carrier gas at a flow rate of 0.5 $mL \cdot min^{-1}$. The initial oven temperature held at 130 °C and increased to 175 °C at 20 $°C \cdot min^{-1}$, held for 1 min then increased to 200°C at 6$°C \cdot min^{-1}$ with no hold, and finally increased to 280 °C at 30 $°C \cdot min^{-1}$. Peaks were identified using the fatty acid methyl ester standard mixture 461 (Nucheck Prep Inc., Minnesota, USA). It is important to note that for triglyceride fatty acid identification and quantification, we chose to report only major fatty acids from C14:0 to C18:3n3 because the small sample size combined with low concentrations of PUFAs did not allow us to reliably quantify LC-PUFAs. Internal standard of C23:0 was used, and fatty acid data was expressed in mol %.

3.3.4 Plasma cortisol concentration

We assessed baseline and peak plasma cortisol concentrations as previously described (Earhart et al., 2020; Zubair et al., 2012). In brief, baseline cortisol level was assessed by immediate collection (< 3min) as described above. Peak cortisol concentration was induced by stressing fish to elicit a cortisol response where fish were gently prodded in the tail with a plastic pipette for 5 min and left undisturbed for 30 minutes to recover. Following this period, fish were sacrificed by immersion in an overdose of MS-222 (250$mg \cdot L^{-1}$) buffered with equal volumes of sodium bicarbonate and blood was collected as described above. Plasma cortisol concentration was analyzed through radio immunoassay as previously established (Hare et al., 2015). All unknown cortisol values were determined by interpolating against a standard curve generated in each assay. Intra-assay variation was 11%, inter-assay variation was 9%, and extraction efficiency was 99.5 ± 1.3%.

3.3.5 Statistical analysis

Due to the non-factorial design and bimodal distribution of our data, we used a Student t-test to examine the temperature effect in total length, body mass and fatty acids from each lipid class between 153 and 199 dph. Also, we used two-way ANOVA to examine the effects of food deprivation and its duration on fatty acids from each lipid class between 225 and 245 dph. When ANOVA indicated a significance ($p<0.05$), a Tukey's HSD was performed to compare difference between individual treatments. With respect to plasma cortisol two-way ANOVA was performed

within baseline or peak concentrations as described above, and a t-test was used to compare difference between baseline and peak cortisol concentrations at each sampling time. Data were transformed through square root, log, cube root or Tukey power where the assumption of normality or homogeneity of variance was not met (Mangiafico, 2021). Assumptions of normality and homoskedasticity were visually assessed as previously described (Zuur et al., 2010). Full results of data analysis are provided as supplementary tables (Table A1- A5; access through the publisher). All data analysis was conducted in R (R Core Team, 2021).

All experiments were approved by the University of Manitoba Animal Care Committee under the requirements established by the Canadian Council for Animal Care under protocol number F015-007.

3.4 Results

3.4.1 Dietary fatty acid composition (bloodworm)

Overall, the most abundant fatty acid group in the diet was PUFA (39.41 ± 0.24 %; mean ± S.D) followed by SAT (34.02 ± 0.14 %) and MUFA (26.57 ± 0.13 %) (Table 3.1). Specifically, the most abundant SAT was C16:0 (15.53 ± 0.11%.) followed by C18:0 (10.29 ± 0.15 %). Among MUFA, the most abundant fatty acid was C18:1 (11.15 ± 0.13 %) followed by C16:1t (7.97 ± 0.16%). Also, C18:2n6 (23.81 ± 0.23%) was higher than C18:3n3 (8.54 ± 0.09 %), contributing to an omega-6 PUFA dominated diet. Lastly, we found that DHA (C22:6n3) was very low in the diet (0.02 ± 0.02 %).

3.4.2 Fatty acid composition in liver phospholipids and triglycerides

Overall, we saw that fatty acid compositions in phospholipids and triglycerides appear to be similar (Table 3.2, 3.3). For example, in phospholipids, the most abundant SAT was C16:0, MUFA was C18:1, and PUFA was DHA. Similarly, in triglycerides, C16:0 and C18:1 were the most abundant SAT and MUFA respectively.

When temperature decreased from 16 to 1°C between 153 and 199 dph, we observed that in phospholipid, total SATs significantly declined with a significant increase in total MUFAs ($p<0.001$; Table 3.2, Figure 3.2a,b) and total PUFAs also increased although this was statistically insignificant (Figure 3.2c). Specifically, C16:0 and C18:0 significantly decreased whereas C16:1, C18:1, C18:1n7c and C20:1 significantly increased ($p<0.05$; Table 3.2). Meanwhile, C20:4n6

significantly increased ($p<0.05$) whereas C20:5n3and C22:6n3 did not change (Table 3.2). The ratio of unsaturated fatty acids to saturated fatty acids (U/S Ratio) significantly increased with decreasing temperatures ($p<0.001$; Table 2, Figure 2d). Total omega-3 PUFAs did not change with temperature whereas total omega-6 PUFAs significantly increased as temperature decreased ($p<0.001$; Table 3.2, Figure 3.3 a,b). Finally, the unsaturation index did not change throughout the experiment (Figure 3.3c). We saw a similar trend in triglycerides where C14:0 significantly decreased when temperature decreased from 153 to 199 dph ($p<0.01$; Table 3.3) and C18:1, C18:1n7c, C18:3n6 and C18:3n3 significantly increased between 153 and 199 dph ($p<0.01$; Table 3.3).

During the overwintering event of food deprivation at 1°C for 45 days, we did not observe notable trends of changes in fatty acids in both phospholipids and triglycerides (Table 3.2, 3.3).

3.4.3 Plasma cortisol concentration

At 153 dph, there was no difference between baseline and peak cortisol concentration in the fed group ($p>0.1$; Figure 3.4). Between 225 and 245 dph, baseline plasma cortisol concentration was significantly influenced by both food availability and its duration ($p<0.001$; Figure 3.4). Specifically, within the fed group, baseline cortisol concentration was significantly higher at 245 dph compared to 225 dph ($p<0.001$; Figure 3.4). At 225 dph baseline plasma cortisol concentration did not differ between fed and fasted fish, but at 245 dph, baseline plasma cortisol concentration was significantly higher in the fed group compared to the fasted group ($p<0.001$; Figure 3.4). Our two-way ANOVA indicated a significant effect of food availability on peak cortisol concentration ($p<0.01$), but the *post hoc* analysis of individual group comparison did not support this conclusion ($p>0.1$; Figure 3.4). At 225 dph, there was significant differences between baseline and presumptive peak cortisol concentration in the fed group ($p<0.001$; Figure 3.4), but not in the fasted group. At 245 dph, there was no difference between baseline and peak cortisol in either the fed or fasted group ($p>0.05$, Figure 3.4,).

3.4.4 Body mass

Both student t-test and two-way ANOVA indicated that body mass did not significantly change throughout the study ($p>0.05$; Figure 3.5).

3.5 Discussion

We observed significant changes in hepatic fatty acids during cold adaptation, supporting the process of HVA in age-0 lake sturgeon entering their first winter of life. Specifically, we found significant increases in MUFAs of C16:1 and C18:1 in phospholipids from the liver in cold adapted fish. These increases may have been the result of selective retention of diet derived C16:1 and C18:1 during adaptation to colder temperatures or could be endogenous synthesis. Coupled with significant decreases in hepatic phospholipids of C16:0 and C18:0, our data suggests an increase in Δ9 desaturase activity. Δ9 desaturase (also known as stearoyl-CoA desaturase) is an enzyme involved in the synthesis of MUFAs, converting C16:0 and C18:0 to C16:1 and C18:1, and is the rate limiting step inserting a double bond (*cis* form) into the 9th carbon from the carboxyl end in the acyl chain (Miyazaki and Ntambi, 2003) prior to further conversion to C18:1n7c and C22:1 via fatty acid elongases (Burdge, 2018; Ferreri et al., 2020). In teleosts such as carp (*Cyprinus carpio*) and milkfish (*Chanos chanos*), cold exposure increased gene expression of Δ 9 desaturase (Hsieh et al., 2003; Trueman et al., 2000). Similarly, a recent study in our lab showed that age-0 lake sturgeon significantly increased hepatic gene expression of Δ 9 desaturase when environmental temperature was decreased to 3.5°C from 16°C (Bugg et al. *unpublished*).

Interestingly, we saw that when temperature decreased, total omega-6 PUFAs in phospholipids significantly increased whereas total omega-3 PUFAs did not. All of omega-6 PUFAs such as C20:3n6, C20:4n6 and C22:4n6 significantly increased. However, minor omega-3 PUFAs C20:3n3 and C22:5n3 significantly increased while the major C20:5n3 and C22:6n3 did not change. These results could mean that dietary retention of omega-3 LC-PUFAs may be limited in this species or that the process of elongation was not inhibited for both omega-3 and 6 fatty acids while desaturation capacity may be limited in the downstream production of omega-3 LC-PUFAs. The *in vivo* synthetic pathway for omega-3 and omega-6 fatty acids has been well established, and is hypothesized to be almost identical across all vertebrates (Li et al., 2010; Park et al., 2009; Sprecher, 2000; Tocher, 2010). Omega-3 or omega-6 LC-PUFAs could be synthesized from either C18:3n3 or C18:2n6. Downstream conversion is facilitated through a series of biochemical reactions with Δ 5 or 6 desaturases, fatty acid elongase (Elovl2 and Elovl5)

and a peroxisomal β-oxidation for shortening fatty acid chains. Δ5 and 6 desaturases are promiscuous with both omega-3 and omega-6 fatty acids (Guillou et al., 2010; Sprecher, 2000). For example, Δ6 desaturase can bind with one of two potential substrates - C18:3n3 or C18:2n6 (Park, 2018). Thus, despite a higher binding affinity to omega-3 fatty acids (Berger and German, 1990), relative substrate availability of these fatty acids may compete for Δ6 desaturase, which will significantly influence downstream production of AA (C20:4n6), EPA (C20:5n3) and DHA (C22:6n3) in mammals (De Antueno et al., 2001; Emken et al., 1994). In fish, effects of substrate availability of C18:3n3 and C18:2n6 on omega 3 and 6 fatty acid metabolism have shown to be equivocal. A previous study in rainbow trout (*Oncorhynchus mykiss*) demonstrated that substrate availability between C18:3n3 and C18:2n6 did not influence conversion to C18:4n3 and C18:3n6 (Emery et al., 2013). In contrast, in Atlantic salmon (*Salmo salar*), when fed the same ratio between C18:3n3 and C18:2n6, synthesis of omega-3 LC-PUFAs was 9 fold greater than omega-6 LC-PUFAs (Sprague et al., 2019). In the present study, it is hypothesized that the significant increase in AA, but not in EPA when fish were exposed to cold temperatures could be due to the relative amount of dietary C18:3n3 and C18:2n6. Future studies are suggested to understand how availability of C18:3n3 and C18:2n6 might influence LC-PUFA metabolism in sturgeon species during cold adaptation. We also saw a significant increase in AA in phospholipids when temperature decreased. In marine teleosts, it has been suggested that production of EPA and AA may be limited due to low activity levels of Δ5 desaturase, which converts C20:4n3 and C20:3n6 to EPA (C20:5n3) and AA (C20:4n6) respectively (Nakamura and Nara, 2004; Tocher, 2003; Tocher and Ghioni, 1999). It remains unclear if the increase in AA in cold adapted fish from the present study is due to increased activity and/or gene expression of Δ5 desaturase as similar to Δ9 desaturase described above or simply selective retention of AA from the diet.

We observed a similar trend in changes of saturated and unsaturated fatty acids in triglycerides. Specifically, we reported increases in both MUFAs and PUFAs of triglycerides in response to adaptation to decreased environmental temperature in age-0 lake sturgeon. These findings are, however, in disagreement with previous studies conducted in freshwater alewife (Snyder et al., 2012; Snyder and Hennessey, 2003). It is hypothesized that HVA may be less important in triglycerides because it is considered as a mechanism for storage of fatty acids where less PUFAs are found (Tocher, 2003; Tocher and Sargent, 1990). However, it was suggested that mobilization of fatty acids in triglycerides at low temperatures may be influenced

by a fluid state of fatty acids that vary with fatty acid composition (Florant, 1998; Frank, 1992), suggesting substantial relevance of HVA in triglyceride metabolism. For example, in both blue mussels (*Mytilus edulis*) and oyster (*Crassostrea virginica*), exposure to cold temperatures resulted in significant increases of PUFAs such as EPA in triglycerides (Pernet et al., 2007). It is important to note that Pernet et al. (2007) used algae (*Chaetoceros muelleri* and *Isochrysis galbana*) that contained high levels of PUFAs, which both bivalves may have accumulated from their diet during winter.

In the fed group, we saw that there was no clear pattern of changes in MUFAs or PUFAs at 225 and 245 dph. This result suggests that fish may have been adapted to a cold environment between 153 and 199 dph and required less to regulate MUFAs and PUFAs under 1°C. A short term response to adjust biological membranes such as changes in head groups could occur within 24 hours of temperature exposure, but a long-term response such as changes in LC-PUFAs could be completed within two weeks of exposure (Hazel, 1997; Hazel and William, 1990). Thus, 16 days of acclimation to 1°C may have been sufficient for age-0 lake sturgeon to complete HVA and survive at 1°C. Also, we did not observe any change of fatty acids in the fasted group at 225 and 245 dph, which could be due to a lack of changes in body mass that did not trigger any changes in fatty acids alongside plasma cortisol concentration during the simulated overwintering event (see below).

We observed higher baseline cortisol in the fed group at 153 and 245 dph. It remains unknown what drove higher baseline cortisol in the fed group. Although we did not quantify food intake during overwintering, we occasionally found food in the gut of fish from the fed group during dissection, suggesting fish were actively foraging at 1°C. Thus, it is possible that lack of growth in the fed group despite presence of food in the gut during overwintering could be attributable to reduced digestive capacities at low environmental temperatures. It was suggested that increased plasma cortisol concentration may not necessarily indicate distressed individuals as cortisol plays numerous roles in homeostasis (MacDougall-Shackleton et al., 2019; Romero and Beattie, 2021) such as nutrient uptake across the intestine (Rosengren et al., 2018; Tanaka et al., 1995). Our baseline cortisol data in the fasted group disagrees with a previous study conducted in age-0 lake sturgeon where whole-body plasma cortisol concentration significantly increased after 30 days of fasting at 1°C (Deslauriers et al. 2018). The discrepancy is likely due to reporting plasma cortisol in the present study compared to whole-body cortisol (Mommsen et

al., 1999; Romero and Beattie, 2021). We also found that baseline and peak cortisol concentrations did not differ in the fasted group during the overwintering event. Although baseline and peak cortisol concentrations in sturgeon species are influenced by environmental temperature (Bates et al., 2014; Zubair et al., 2012), limited production capacity of cortisol in fasting may be detrimental for age-0 fish as cortisol plays an important role in energy mobilization that can be used to forage or escape predation (Bar, 2014; Barcellos et al., 2010; Piccinetti et al., 2015).

3.6 Conclusion

This research demonstrated that cold exposure resulted in significant increases in both MUFAs and PUFAs of phospholipids and triglycerides in age-0 lake sturgeon supporting homoeviscous adaptation in this species. We also observed evidence of upregulated omega-6 fatty acid synthesis but not omega-3 fatty acid in the liver tissue possibly due to substrate availability from the diet. We saw no difference between baseline and peak plasma cortisol concentrations in fasted fish, which may influence survival during winter months. The present study aids in our understanding of mechanisms needed to promote survival during the first winter of life in age-0 lake sturgeon, which would inform the current conservation aquaculture programs for lake sturgeon.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Credit Author Statement

Gwangseok R Yoon: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, funding acquisition; Madison Earhart: methodology, data curation, resources, writing- review & editing; Yidi Wang: methodology, data curation, writing- review & editing; Miyoung Suh: conceptualization, methodology, validation, investigation, resources, writing- review & editing, supervision; W. Gary Anderson: conceptualization, methodology, validation, investigation, writing- review & editing, supervision, funding acquisition

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Table 3.1. Summary of fatty acid profile in diet (bloodworms; n=6) used in the present study. Data are expressed in mean ± S.D.

Fatty acid	mol %
C14:0	2.2 ± 0.19
C14:1	1.82 ± 0.1
C15:0	1.54 ± 0.04
C16:0	15.53 ± 0.11
C16:1	1.12 ± 0.11
C16:1t	7.97 ± 0.16
C17:0	2.58 ± 0.03
C18:0	10.29 ± 0.15
C18:1	11.15 ± 0.13
C18:1n7c	4.17 ± 0.05
C18:2n6	23.81 ± 0.23
C18:3n6	0.84 ± 0.06
C18:3n3	8.54 ± 0.09
C20:0	1.43 ± 0.05
C20:1	0.13 ± 0.03
C20:2n6	0.12 ± 0.03
C20:3n6	0.14 ± 0.01
C20:3n3	0.15 ± 0.02
C20:4n6	3.50 ± 0.06
C20:5n3	2.26 ± 0.07
C22:0	0.32 ± 0.02
C22:1	0.22 ± 0.03
C22:4n6	0.03 ± 0.02
C22:6n3	0.02 ± 0.02
C24:0	0.13 ± 0.02
∑ SAT	34.02 ± 0.14
∑ MUFA	26.57 ± 0.13
∑ PUFA	39.41 ± 0.24
∑ n3	10.97 ± 0.21
∑ n6	28.44 ± 0.25
U/S Ratio	1.94 ± 0.01

Table 3.2. Summary of fatty acid profile in phospholipid of age-0 Lake sturgeon (*Acipenser fulvescens*) during a simulated overwintering event. * represents significant difference in values between 153 and 199 days post hatch (dph) whereas different letters indicate significant difference in values between 225 and 245 dph. Fatty acids equal or less than 0.1% were not listed (C22:0, C22:1, C24:0 and C24:1). Data are expressed in mol % (mean ± S.D.). N=5-8.

Fatty acid (mol %)	153dph (16°C)	199 dph (1°C)	225 dph (1°C)		245 dph (1°C)	
	Fed		Fed	Fasted	Fed	Fasted
C14:0	1.48 ± 0.66	0.53 ± 0.06*	0.5 ± 0.07	0.55 ± 0.27	0.51 ± 0.1	0.59 ± 0.08
C14:1	0.25 ± 0.19	0.32 ± 0.04	0.27 ± 0.04	0.32 ± 0.24	0.26 ± 0.09	0.33 ± 0.08
C15:0	0.76 ± 0.12	0.71 ± 0.05	0.72 ± 0.11	0.74 ± 0.09	0.73 ± 0.06	0.76 ± 0.08
C16:0	34.99 ± 6.1	27.07 ± 2.66*	29.85 ± 2.15ab	30.82 ± 2.79a	31.38 ± 3.34ab	31.92 ± 4.12b
C16:1	0.38 ± 0.11	0.54 ± 0.08*	0.69 ± 0.16	0.67 ± 0.08	0.65 ± 0.11	0.7 ± 0.05
C16:1t	1.59 ± 0.48	2.13 ± 0.38*	2.47 ± 0.42	2.07 ± 0.81	2.68 ± 0.57	2.73 ± 0.27
C17:0	1.08 ± 0.28	1.06 ± 0.44	0.72 ± 0.13a	1.06 ± 0.26b	0.62 ± 0.28a	0.76 ± 0.16ab
C18:0	15.33 ± 2.86	8.33 ± 1.52*	7.22 ± 1.36	10.03 ± 2.17	7.99 ± 2.47	7.09 ± 2.58
C18:1	9.89 ± 0.48	12.09 ± 0.74*	10.85 ± 1.11	10.98 ± 1.53	11.14 ± 2.26	10.72 ± 0.54
C18:1n7c	4.42 ± 0.46	9.43 ± 0.7*	9.44 ± 0.67a	8.98 ± 0.36ab	8.27 ± 0.92b	9.00 ± 0.45ab
C18:2n6	3.13 ± 0.28	3.71 ± 0.35*	3.3 ± 0.35	3.23 ± 0.55	3.67 ± 0.66	3.35 ± 0.27
C18:3n6	0.25 ± 0.05	0.94 ± 0.06*	0.95 ± 0.08	0.96 ± 0.11	0.85 ± 0.1	0.95 ± 0.11
C18:3n3	0.46 ± 0.31	1.02 ± 0.2*	0.74 ± 0.14ab	0.52 ± 0.38a	0.84 ± 0.19ab	0.93 ± 0.27b
C20:0	0.35 ± 0.22	0.42 ± 0.11	0.44 ± 0.1ab	0.54 ± 0.13a	0.39 ± 0.11b	0.44 ± 0.09ab
C20:1	1.25 ± 0.23	2.02 ± 0.33*	1.97 ± 0.36	2.03 ± 0.36	1.63 ± 0.2	1.9 ± 0.28
C20:2n6	0.89 ± 0.26	1.20 ± 0.16*	1.10 ± 0.16	0.98 ± 0.16	1.52 ± 1.6	0.94 ± 0.15
C20:3n6	0.61 ± 0.18	1.84 ± 0.66*	1.37 ± 0.39ab	1.15 ± 0.17a	1.52 ± 0.29ab	1.64 ± 0.39b
C20:3n3	0.37 ± 0.09	0.69 ± 0.09*	0.56 ± 0.09	0.57 ± 0.14	1.04 ± 1.39	0.52 ± 0.07

C20:4n6	4.16 ± 1.81	6.73 ± 0.77*	7.41 ± 0.68	6.84 ± 0.78	6.67 ± 1.16	7.00 ± 1.19
C20:5n3	3.79 ± 1.61	2.51 ± 0.46	1.93 ± 0.19	1.67 ± 0.48	2.21 ± 0.49	2.23 ± 0.66
C22:4n6	0.09 ± 0.03	0.24 ± 0.07*	0.25 ± 0.04	0.29 ± 0.06	0.25 ± 0.02	0.24 ± 0.06
C22:5n3	1.08 ± 0.36	1.67 ± 0.39*	1.50 ± 0.22	1.40 ± 0.28	1.57 ± 0.13	1.77 ± 0.59
C22:6n3	12.99 ± 7.09	14.51 ± 3.00	15.44 ± 2.64	13.18 ± 2.99	13.27 ± 4.73	13.14 ± 2.97
∑ SAT	54.29 ± 9.85	38.31 ± 4.65*	39.63 ± 3.58	44.03 ± 5.07	41.86 ± 5.63	41.8 ± 5.93
∑ MUFA	17.89 ± 1.18	26.64 ± 1.11*	25.82 ± 1.1	25.17 ± 2.49	24.73 ± 1.91	25.49 ± 0.99
∑ PUFA	27.81 ± 10.3	35.05 ± 4.53	34.55 ± 3.66	30.8 ± 4.44	33.4 ± 6.76	32.71 ± 5.69
∑ n3	18.69 ± 8.75	20.39 ± 3.47	20.17 ± 3.04	17.34 ± 3.54	18.92 ± 6.25	18.58 ± 3.92
∑ n6	9.13 ± 2.13	14.66 ± 1.37*	14.38 ± 0.77	13.46 ± 0.99	14.48 ± 1.57	14.13 ± 1.82
U/S Ratio	0.89 ± 0.31	1.64 ± 0.28*	1.54 ± 0.24	1.3 ± 0.25	1.43 ± 0.37	1.43 ± 0.34
UI	150.26 ± 55.37	185.73 ± 23.72	185.93 ± 19.86	166.2 ± 23.93	174.04 ± 35.62	173.98 ± 28.26

Table 3.3. Analysis of fatty acid profile in triglyceride of age-0 Lake sturgeon (*Acipenser fulvescens*) during a simulated overwintering event. * represents significant difference in values between 153 and 199 days post hatch (dph) whereas different letters indicate significant difference in values between 225 and 245 dph. For triglyceride fatty acid identification and quantification, only major fatty acids from C14:0 to C18:3n3 were reported because the small sample size combined with low concentrations of polyunsaturated fatty acids (PUFAs) did not allow us to reliably quantify long-chain PUFAs. Data are expressed in mol % (mean ± S.D.). N=5-8.

Fatty acid (mol %)	153 dph (16°C)	199dph (1°C)	225dph (1°C)		245dph (1°C)	
	Fed		Fed	Fasted	Fed	Fasted
C14:0	8.24 ± 1.36	2.79 ± 0.39*	2.93 ± 0.51	3.5 ± 1.06	2.94 ± 0.21	2.69 ± 0.26
C14:1	1.76 ± 1.3	2.77 ± 0.27*	2.08 ± 0.64ab	1.47 ± 0.6a	2.25 ± 0.57ab	2.50 ± 0.38b
C15:0	1.39 ± 0.46	1.40 ± 0.14	1.28 ± 0.34	1.43 ± 0.58	1.32 ± 0.21	1.13 ± 0.1
C16:0	31.17 ± 4.66	27.45 ± 2.98	32.15 ± 9.25ab	38.3 ± 5.28a	32.67 ± 6.93ab	25.85 ± 4.47b
C16:1	1.12 ± 0.3	1.06 ± 0.18	1.2 ± 0.54ab	0.79 ± 0.71a	0.99 ± 0.35ab	1.24 ± 0.28b
C16:1t	10.05 ± 2.23	8.77 ± 1.45	6.57 ± 3.22	3.90 ± 3.64	7.59 ± 3.71	10.32 ± 2.45
C17:0	1.67 ± 1.29	1.16 ± 0.25	2.04 ± 2.21	3.75 ± 4.11	2.38 ± 1.94	1.06 ± 0.58
C18:0	7.43 ± 5.97	6.38 ± 2.34	13.93 ± 9.81	28.06 ± 8.26	14.15 ± 10.66	5.22 ± 6.34
C18:1	21.02 ± 4.87	25.46 ± 2.28*	20.37 ± 9.09ab	9.13 ± 7.54a	18.96 ± 8.35ab	26.21 ± 4.42b
C18:1n7c	5.70 ± 1.39	11.36 ± 1.29*	9.08 ± 5.17ab	4.19 ± 3.47a	7.91 ± 4.35ab	12.74 ± 2.01b
C18:2n6	8.15 ± 1.72	7.40 ± 1.49	4.91 ± 2.24a	1.95 ± 1.27b	5.19 ± 1.83a	6.90 ± 1.67a
C18:3n6	0.59 ± 0.12	1.00 ± 0.17*	0.77 ± 0.33a	0.42 ± 0.47a	0.87 ± 0.28ab	1.28 ± 0.17b
C18:3n3	1.3 ± 0.7	2.37 ± 0.88*	1.53 ± 0.81a	0.16 ± 0.25b	1.44 ± 0.91a	2.46 ± 1.02a

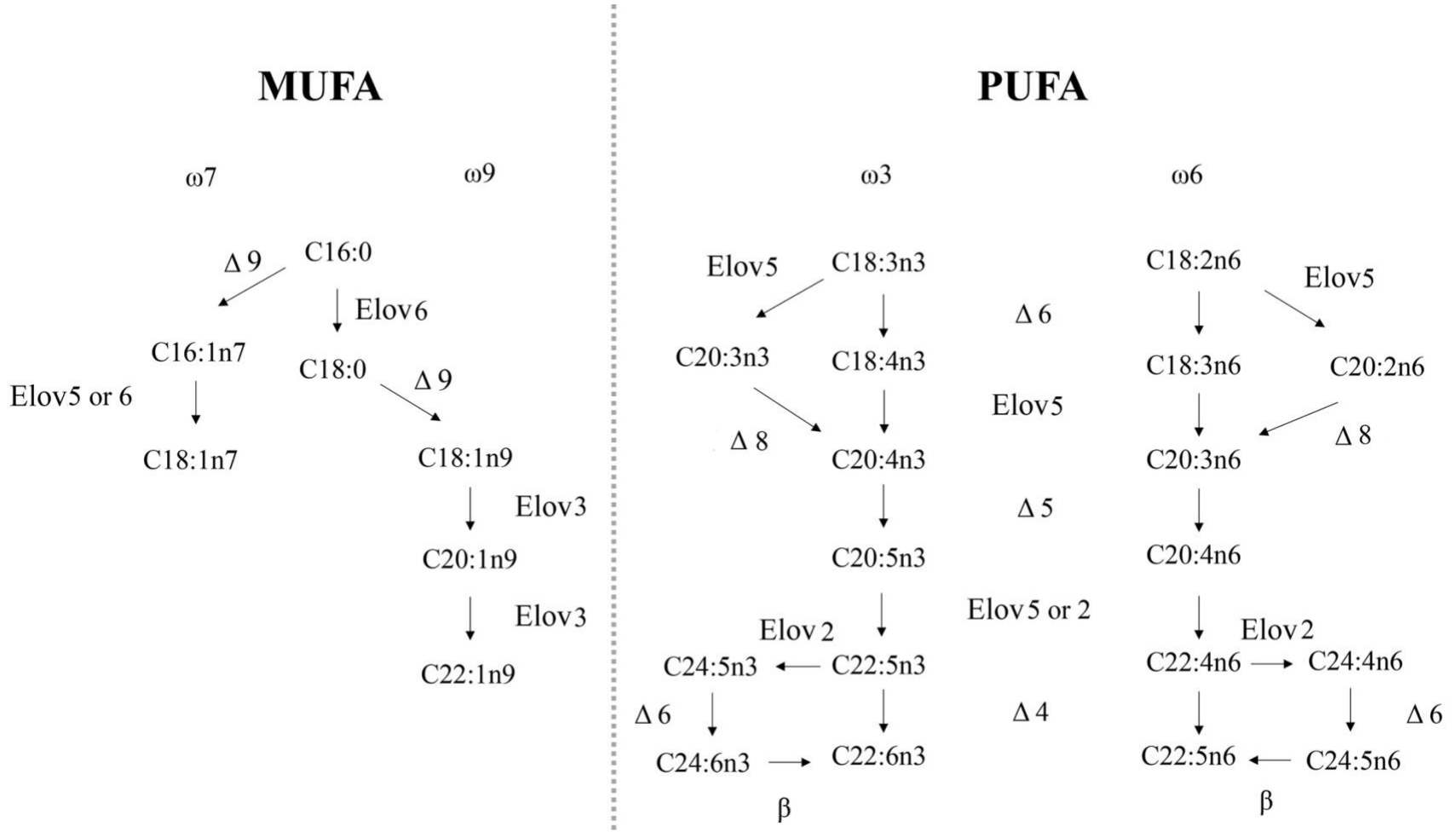

Figure 3.1. *In vivo* mono (MUFA) and polyunsaturated fatty acids (PUFA) pathway in vertebrates. Elov 2, 3, 5 and 6 denote fatty acid elongase 2, 3, 5 and 6. Δ 4, 5, 6, 9 represent Δ 4, 5, 6, 9 desaturase, respectively. β is for peroxisomal oxidation for shortening fatty acid chain. Reference of Δ 4 desaturase in fish: Li et al. (2010).

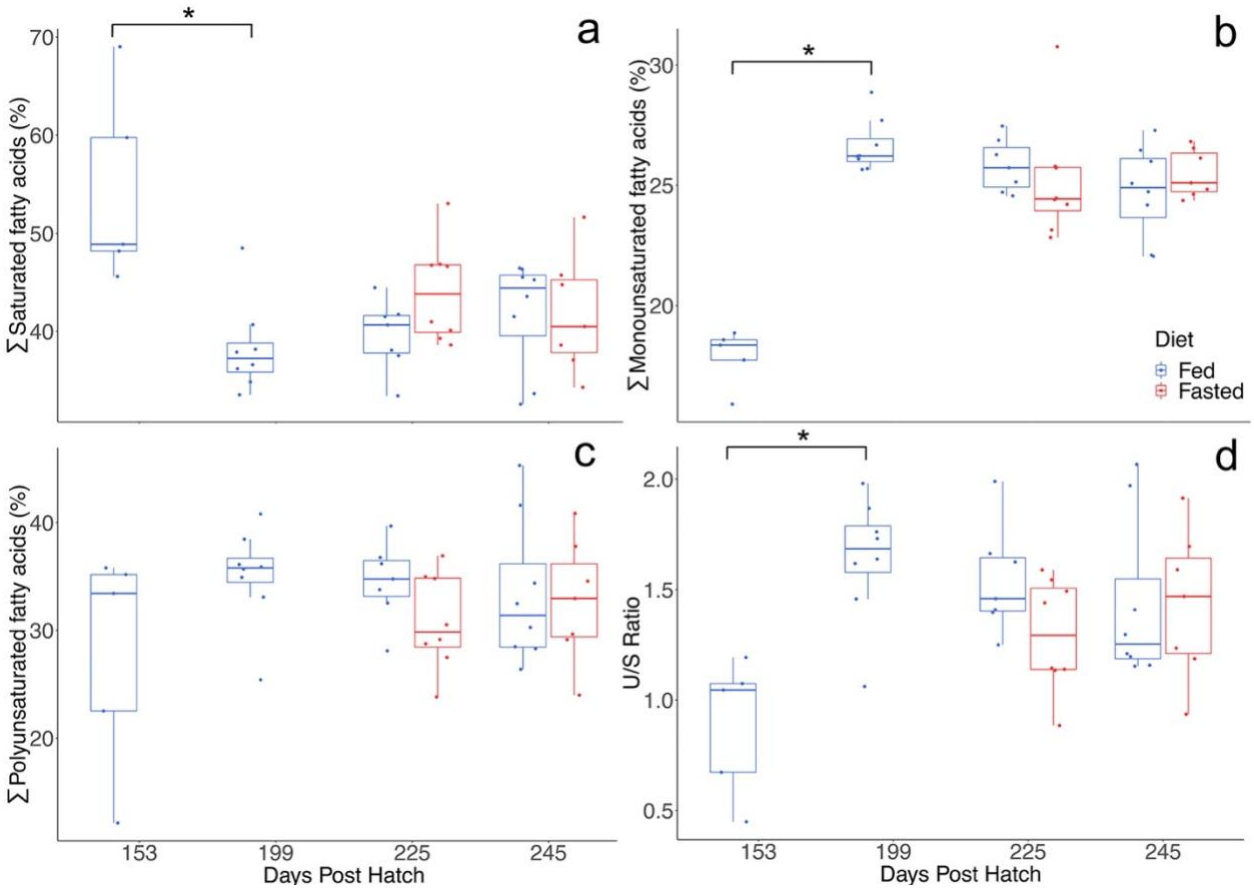

Figure 3.2. Changes in saturated (a), monounsaturated (b), polyunsaturated fatty acid (c) and unsaturated to saturated fatty acids ratio; U/S ratio (d) in the phospholipid of liver tissue of age-0 lake sturgeon (*Acipenser fulvescens)*. From 200 days post hatch (dph), fish were either fed every other two days (blue) or fasted for 45 days (red). * denotes significant difference between 153 and 199 dph (t.test; $p<0.001$). Data are not transformed for the graphical purpose. n=5-8. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represents data distribution. Dots represent individual data points.

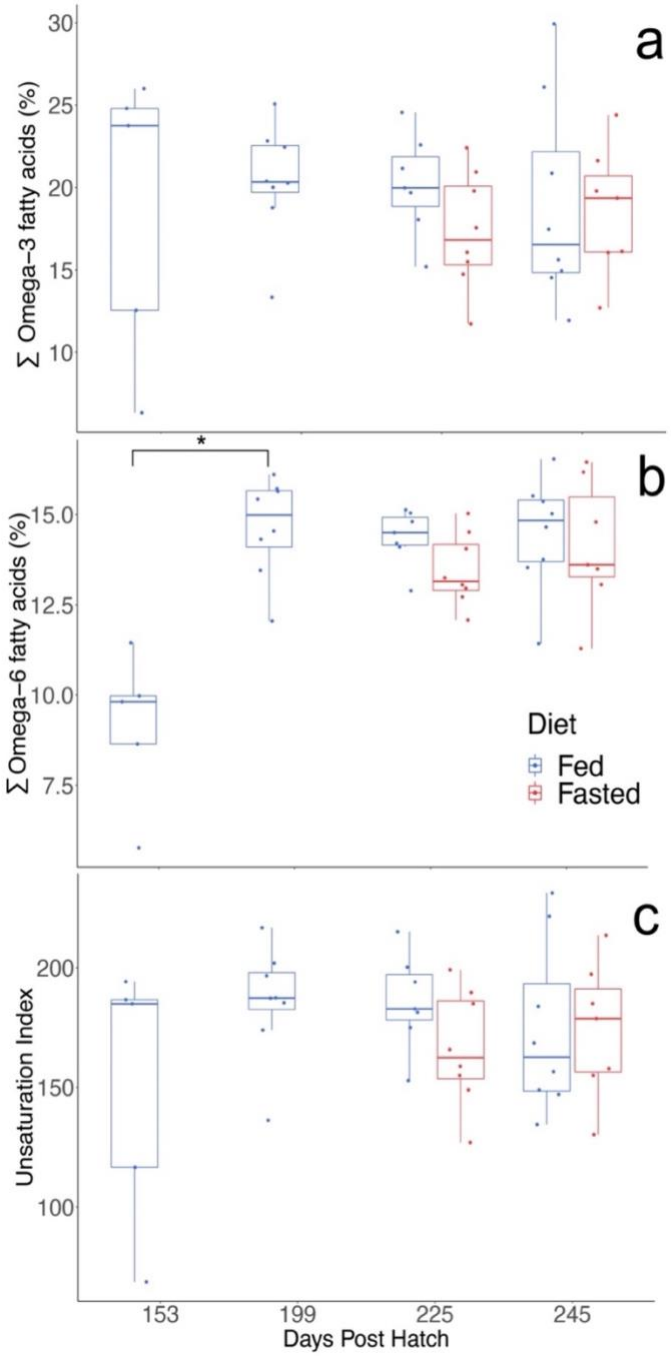

Figure 3.3. Changes in omega 3 (a), 6 fatty acids (b) and unsaturation index (c) in the phospholipid of liver tissue of age-0 lake sturgeon (*Acipenser fulvescens)*. From 200 days post hatch (dph), fish were either fed every other two days (blue) or fasted for 45 days (red). * denotes significant difference between 153 and 199 dph (t.test; $p<0.001$). Data are not transformed for the graphical purpose. n=5-8. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represents data distribution. Dots represent individual data points.

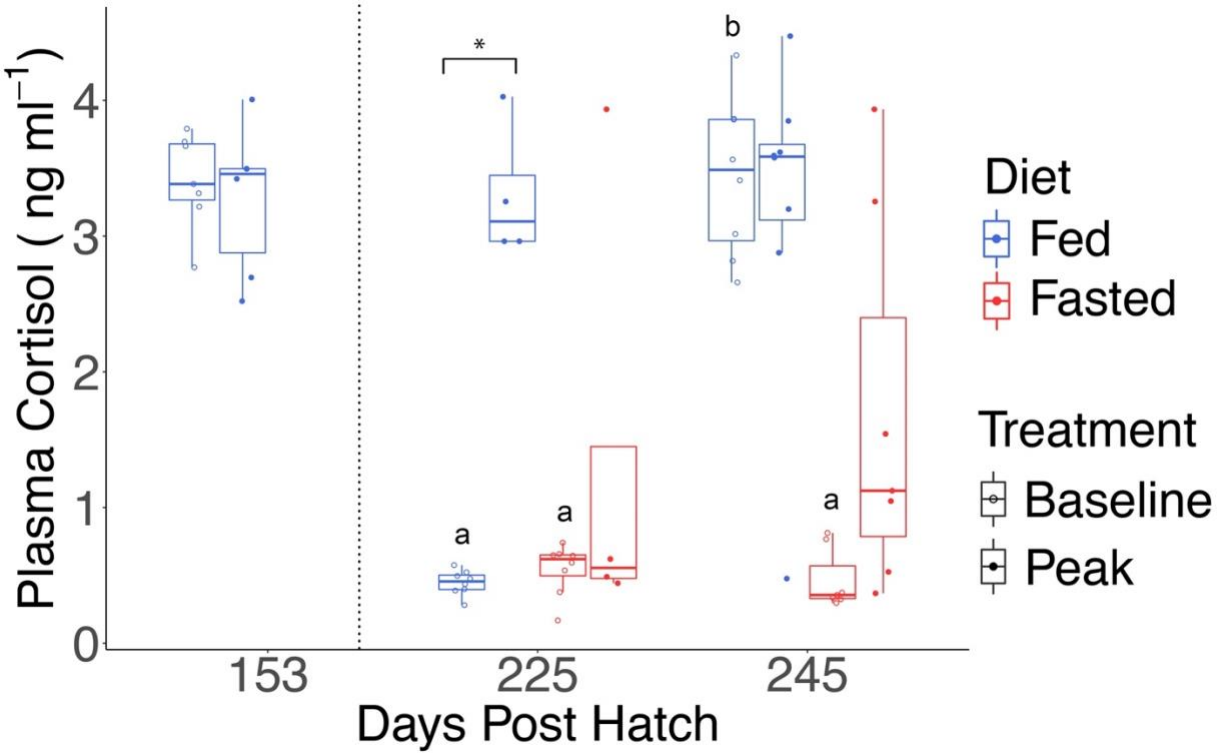

Figure 3.4. Changes in plasma cortisol concentration in age-0 lake sturgeon (*Acipenser fulvescens)*. Baseline (open dots) was assessed by immediate collection of blood from the caudal vein whereas peak (filled dots) was induced by chasing fish 5 minutes and 30 minutes of recovery. From 200 days post hatch (dph), fish were either fed every other two days (blue) or fasted for 45 days (red). * denotes significant difference between baseline and peak concentrations (t.test; $p<0.001$). Different letters indicate significant difference within baseline group between 225 and 245 dph (two-way ANOVA, $p<0.001$). Data are not transformed for the graphical purpose. n=4-8. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represents data distribution. Dots represent individual data points.

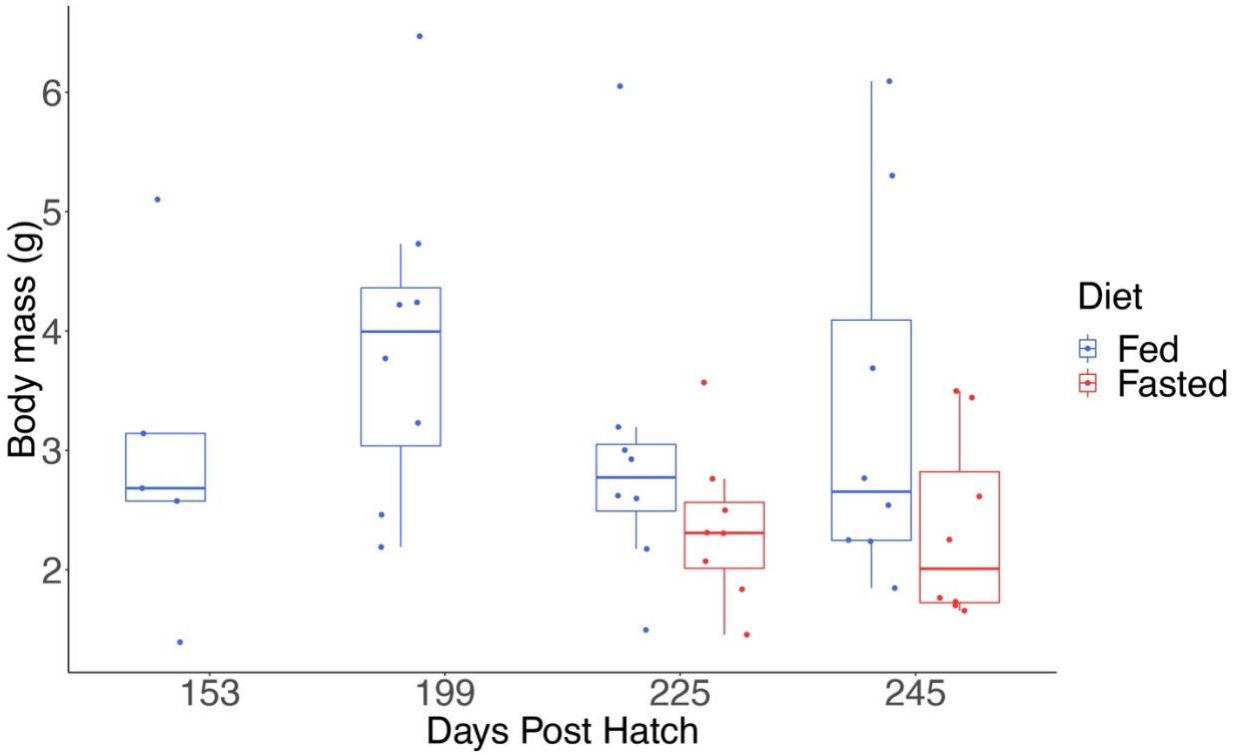

Figure 3.5. Changes in body mass of lake sturgeon (*Acipenser fulvescens*). From 200 days post hatch (dph), fish were either fed every other two days (blue) or fasted for 45 days (red). Dots represent individual data points. Data are not transformed for the graphical purpose. n=5-8. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represents data distribution. Dots represent individual data points.

Both Chapters 2 and 3 demonstrated that metabolic phenotypes may be plastic traits that are strongly influenced by temperature and diet. The following Chapters 4, 5 and 6 examined how environment during early life could influence phenotypic development within the first year of life. Chapter 4 focused on the effects of temperature during early life on growth and energy metabolism in age-0 lake sturgeon.

4 Chapter 4: Long-term effects of temperature on growth, energy density, whole-body composition and aerobic scope of age-0 Lake sturgeon (*Acipenser fulvescens*)

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Keywords: developmental plasticity, critical window, tradeoff, temperature, conservation aquaculture

4.1 Abstract

Fish are known to show high intraspecific phenotypic variation during early life history. Specifically environmental temperature during early life can result in developmental plasticity, which will influence developmental trajectory and ultimately individual fitness. In the present study, we examined long-term effects of early rearing temperature on growth, energy density, body composition (i.e., whole body glucose, triglyceride and protein concentration) and aerobic scope of age-0 Lake Sturgeon (*Acipenser fulvescens*) to determine if temperature manipulation after yolk absorption would have a long-term impact on these traits. At 58 days post hatch, fish were subjected to one of three temperature manipulations (16°C; control, 18°C and ambient river temperature in a range of 14.0 – 19.4°C; Ambient) for 35 days after which all fish were raised in Ambient conditions until 360 days post hatch, including 4 months of natural winter temperatures. We hypothesized that temperature conditions experienced before the first winter of life would result in short-term effects of improved growth and energy reserves. Our results showed that temperature manipulation resulted in a short-term, reversible effect of improved growth prior to overwintering without a longer-term effect on growth. Enhanced somatic growth of total length and body mass prior to winter did not translate into improved energy reserves, and observed differences in growth rate between treatments did not correlate with aerobic scope. Our data demonstrate that a subtle change in temperature during early life history in Lake Sturgeon may result in short term positive effects on growth rate. These data may enhance current conservation aquaculture operations to promote winter survival in fall released fingerlings without long-term implications on growth phenotypes.

4.2 Introduction

During early life history, fish are known to exhibit particularly high plasticity compared to other life stages. Depending on the developmental stage and magnitude of change in the environment, conditions experienced during early life can have irreversible and long-term consequences for individual phenotypes (Jonsson and Jonsson, 2014; Monaghan, 2008; Vagner et al., 2019). Specifically, environmental manipulation during a critical window can result in developmental plasticity that strongly influences many morphological and physiological traits (Burggren, 2020). Thus, developmental plasticity has received a great deal of attention due to its ecological implications for understanding population dynamics (Ghalambor et al., 2007; Schulte, 2014); evolutionary adaptation in climate change (Pigliucci, 2005; Vagner et al., 2019); relationship with growth in industrial aquaculture operations (Finstad and Jonsson, 2012; Garcia de la serrana et al., 2012; Imsland et al., 2007; Vagner et al., 2009) and promotion of wild phenotypes in conservation aquaculture operations (Jonsson and Jonsson, 2014; Roberts et al., 2011).

Temperature is a pervasive abiotic factor that strongly influences physicochemical reactions and thus metabolism (Clarke and Fraser, 2004; Vagner et al., 2019). As body size in juvenile fish is typically correlated with survival and energy reserves (Arendt, 1997), studies in conservation aquaculture have focused on relatively short-term responses to changing environmental temperature such as growth rate and nutrient uptake (Boucher et al., 2014; Bugg et al., 2020; Hung et al., 1993); however, temperature effects can have a long-lasting effect on growth and metabolism (Vagner et al., 2019). For example, manipulation of temperature during early development has been shown to have fixed effects on muscle cellularity (Johnston, 2006) and growth (Garcia de la serrana et al., 2012), but in Sablefish (*Anoplopoma fimbri*), while higher temperatures improved growth performance during larval development, long-term consequences included increased rates of jaw deformity, and thus reduced growth rate later in life (Lee et al., 2017). In sturgeon, increased temperature during early life can result in improved growth performance (Wang et al. 1985), but also significantly influence morphology (Chebanov and Galich, 2013; Jay et al., 2020; Linares-Casenave et al., 2013) and behaviour (Wassink et al., 2019) that one assumes may impact individual fitness later in life (Dammerman et al. 2016; Wassink et al. 2019).

Balancing energy between use (i.e. growth) and storage (i.e. lipid reserves) is critical for survival in all organisms, especially when periods of starvation are anticipated. This balance is well-illustrated in north temperate species as young of the year fish approach the first winter of life when access to food is limited for extended periods of time (Fernandes and McMeans, 2019; Hurst, 2007; Post and Parkinson, 2001). Overwinter survival is predicted by interactions between an individual's energetic status and duration of winter months with severity (Hurst, 2007). As hepatosomatic index (relative liver mass to whole-body mass), energy density and whole-body composition may represent energy metabolism toward somatic growth and energy reserves (Breck, 2014), understanding how early environment influences these metrics would provide insight into the general nutritional status that may influence survival rate during winter months.

Metabolic costs for growth can be estimated through measuring metabolic rate. In fish, it is indirectly estimated through measuring whole-body oxygen consumption rate. Aerobic scope (AS) is defined as the difference between standard metabolic rate (SMR) and maximum metabolic rate (MMR) (Clark et al., 2013). AS has numerous ecological implications as it is considered as the metabolic capacity available for growth and is thought to fuel routine activities such as locomotion, foraging and digestion (Clark et al., 2013; Metcalfe et al., 2016). In larval fish, AS is known to be constrained or smaller than those at juvenile or adult stages due to high metabolic demands for development and growth (Glazier, 2005; Killen et al., 2007; Wieser, 1995; Yoon et al., 2021). AS may be further constrained in larval fish where the relationship between SMR and MMR is influenced by temperature (Pörtner, 2010), and its effect may result in a fixed metabolic phenotype later in life (Donelson et al., 2018; Lee et al., 2020; Ryu et al., 2020).

The importance of understanding longer term effects of developmental temperature should be highlighted in conservation aquaculture where managing a sustainable population is the key to success (Froehlich et al., 2017). Interestingly, conservation programs for Lake Sturgeon have been ongoing for a number of decades throughout the range of the species, however, Lake Sturgeon populations have repeatedly demonstrated low recruitment rates (Bruch et al., 2016; Osborne et al., 2020). As such, fisheries managers have used conservation aquaculture as one of a number of tools to enhance Lake Sturgeon stocks, however, success is limited in part owing to lower survival of age-0 stocked fall fingerlings compared to age-1+ juveniles (McDougall et al., 2020). Despite this, release of fall fingerlings is often required due

to logistical and financial constraints on hatcheries (Brown and Day, 2002). Previous work in our lab has demonstrated a positive relationship between temperature and growth in Lake Sturgeon during the first year of life (Brandt et al., 2022; Bugg et al., 2020). Specifically, the transient increase in temperature in the first month of life was shown to have a persistent effect on growth in Lake Sturgeon approximately one year later (Brandt et al., 2022). Therefore, understanding longer-term effects of temperature on overwintering performance in age-0 lake sturgeon would inform the current conservation aquaculture and may ultimately increase post-release survival rate of fall fingerlings.

In the present study, we were interested in examining the effects of temperature later in life, testing the hypothesis that the effects of temperature on growth phenotypes would be related to a distinct developmental window. Specifically, we chose to manipulate temperature in developing Lake Sturgeon at two months old based on results of recent work in our lab demonstrating persistent temperature effects of the first month on growth phenotypes towards age1+ (Brandt et al., 2022). Based on the assumption that the critical window for the effect of temperature on development of growth phenotypes in lake sturgeon may be in the first month of life, we examined how changes in temperature after the first month might influence energy balance in relation to growth prior to the first winter of life. We predicted that temperature manipulation at two months would result in a transient effect on growth (i.e. phenotypic plasticity), but it would not persist for an appreciable length of time beyond the temperature manipulation period (i.e. developmental plasticity).

4.3 Material and Methods

4.3.1 Animal Husbandry and Environmental Manipulation

Animal rearing and temperature manipulation were performed as previously described with some modifications (Brandt et al., 2022). Fish were initially raised in Grand Rapids Fish Hatchery, Manitoba. At 58 days post hatch (dph), 720 Lake Sturgeon were evenly distributed to three equally sized troughs (L488 × W61 × H25 cm). Holding troughs varied in temperature and were held at either 16°C (16.1 ± 0.6°C; mean ± S.D), as the control group representing the hatchery condition, 18°C (18.0 ± 0.6°C) as the future operation protocol in Manitoba, or ambient river temperature (in a range of 14.0 – 19.4°C; Ambient). For 16 and 18°C, temperature was

controlled by a heater in the fish hatchery whereas Ambient was the uncontrolled ambient river temperature.

Fish were maintained in these different temperature environments for the following 35 days (see Figure 4.1a). At 93 dph fish (n = 8-10 per treatment) from each group were sampled from their holding tanks to determine growth, whole-body composition and metabolic rate and the remaining fish were transferred to a common garden setup with six identical holding tanks so that each temperature manipulation treatment was held in duplicate. At subsequent sampling timepoints, equal numbers of fish were haphazardly selected from duplicate tanks for a total of 8-10 fish per sampling timepoint. At 93 dph, tanks were supplied by flow-through river water drawn from the nearby Saskatchewan river as described above until the end of experiment (see Figure 1b). Fish were fed *ad libitum* twice daily with bloodworm (Hikari USA, Hikari, California, USA), and any uneaten food was removed after 1 hour. At each feeding, any mortality was removed and recorded. Water temperature was monitored to the nearest 0.1°C using a digital thermometer twice a day at each feeding, and water chemistry was assessed to monitor the levels of pH, dissolved oxygen, ammonia and nitrite weekly. The ranges of pH, dissolved oxygen, ammonia and nitrite were 7.43 – 8.80, 7.17 – 15.71mg.L^{-1}, 0.00 – 0.20ppm and 0.00 and 0.05ppm respectively throughout the entire study and did not significantly differ between treatment tanks. Feeding was performed as previously described to mimic reduced resources prior to and over winter (Brandt et al., 2022). Briefly, at 128 dph when water temperature was 7°C the frequency of feeding was gradually reduced by increasing intervals between feeding days until 174 dph, when food was withheld from all fish for the following 122 days (see Figure 4.1b). Food was reintroduced to fish in all treatments the following spring when daily water temperature was greater than 2°C for three consecutive days.

4.3.2 Growth

Total length and body mass were measured every month to monitor patterns of growth. In brief, fish (n=10) were haphazardly captured from each treatment and sacrificed by immersion in an overdose of anaesthetic buffered with an equal amount of sodium bicarbonate (MS-222, 250mg.L^{-1}, Syndel Laboratory, Vancouver, British Columbia, Canada). Then, fish were patted dry on a paper towel and measured to the nearest 1mm and 0.01g. Fulton's condition factor was calculated with the following equation: $K = g \cdot 100/cm^3$. It is important to note that at 93, 251,

306 and 360 dph, growth data was taken from fish used for whole-body composition and energy density (see below; n=16-20).

4.3.3 Hepato- and gutsomatic index, energy density and whole-body composition

Fish (n=8-10) were haphazardly sacrificed at 93, 251, 306 and 360 dph as described above. Whole liver, and gastrointestinal tract (GIT, from esophagus to anus) were carefully removed, the GIT was opened longitudinally and any remaining food was gently rinsed off and the mass of each organ was measured to the nearest 0.001g. The mass of liver and GIT ranged from 0.0156 to 0.3380g and from 0.0507 to 0.6033g throughout the samples, respectively. Hepatosomatic and gutsomatic index (HSI and GSI; n=8-10) were calculated with the following equation: HSI or GSI = Tissue mass (g) / body mass (g) × 100.

Then, organs were returned to the cavity and combined for measurement of whole-body composition as previously described with some modifications (Deslauriers et al., 2018). In brief, whole carcasses were dried at 60°C for 48 hours or constant weight was recorded. Then, dried carcasses were homogenized using a TissueLyser II (QIAGEN, Hilden, Germany) at 30 Hz for 10 min with a ratio of 1:14 (0.01g of dried sample in 0.14mL of 50mM imidazole buffer at pH 7.5). Sample was subsequently transferred to a 1.5mL tube and centrifuged at 13,000 RPM for 10 min. Supernatant was used to assess whole-body glucose (LabAssay™ Glucose; Wako, Osaka, Japan), triglyceride (LabAssay™ Triglyceride; Wako, Osaka, Japan) and protein (Bradford Protein Assay Kit; Bio-Rad, California, USA). It is important to note that the supernatant was further diluted three times with deionized water for protein measurement to ensure that readings fell within the range of the standard curve. All biochemical assays were conducted using a PowerWave HT Microplate Spectrophotometer (BioTek, Vermont, USA) and Gen5 (v. 1.11).

A second group of fish (n=8-10) were sacrificed using the same method and at the same timepoints as above, and total length and body mass were recorded. Dried carcasses were prepared as described above to calculate dry to wet mass ratio (DWR), which was used to estimate whole-body energy density (ED, $J \cdot g^{-1}$ of wet mass; n=8-10) using the previously established model: $ED = 23{,}965.7 \times DWR - 433.1$; $p < 0.001$, $r^2 = 0.96$ (Yoon et al., 2019).

4.3.4 Whole-body metabolic rate

There were three sampling points for measurement of metabolic rates taken at 88-93, 247-251 and 357-360 dph, which will be abbreviated to 93, 251 and 360 dph hereafter. At each sampling point, whole-body metabolic rate ($\dot{M}O_2$, n=7-8) was assessed using intermittent flow respirometry. It is important to note that fish were at different environmental temperatures for measurement of metabolic rate at 93 dph (16, 18 and 14°C for the ambient group) but the same temperature at 251 (1.3°C) and 360 dph (11°C). At each sampling date, fish were captured by dipnet, total length and body mass were measured, and the fish was placed in a metabolic chamber. We used two different sizes of chambers and tubing to accommodate fish as they grew throughout the study period. At 93 dph, we used borosilicate glass chambers with a volume of 43.43 ± 0.84mL (mean ± S.D.) and tubing with a volume of 3.15 ± 0.19mL. At 251 and 360 dph, we used transparent acrylic chambers with a volume of 540.72 ± 1.73mL and tubing with a volume of 90.09 ± 8.64 mL. The respirometry setup was comprised of metabolic chambers in a water bath with Witrox 4 Oxygen meters to which oxygen probes were connected (PreSens, Regensburg, Germany). At 93 dph, oxygen sensor spots were placed on the surface of borosilicate chambers whereas at 251 and 360 dph, oxygen dipping probes were situated in flow-through-vessels (PreSens, Regensburg, Germany). Oxygen levels were calibrated at 0 and 100% with 2% sodium sulfite and fully saturated water, respectively. Tubing was non-oxygen permeable Tygon™. Flush and recirculation pumps were provided from the manufacture of the respirometry equipment (Loligo Systems, Viborg, Denmark). Flow rate was sufficient to exchange water, but not to cause physical disturbance and a black curtain surrounded the chambers to avoid any visual disturbance. SMR was measured for 16 hours following 6 hours of acclimation (Chabot et al. 2016). Following the measurement of SMR, fish were removed from the chamber and a standardized chase protocol with gentle prodding of the tail was performed for 15 minutes; fish were returned to the chambers and oxygen consumption was measured for 3 measurement cycles to estimate MMR. Background respiration (BR) of each chamber was measured without fish before and after each experiment, and assuming a linear increase in BR over the experimental time, measured BRs were used to interpolate a linear regression in order to correct measured $\dot{M}O_2$ (Rodgers et al., 2016). We took two approaches for analyzing SMR data due to relatively low R^2 values at 251 dph compared to the other sampling points. At 93 and 360 dph, $\dot{M}O_2$ slopes with $R^2 \geqq 0.85$ were chosen while ensuring linearity (Chabot et al., 2021), but $R^2 > 0.5$ was used at 251 dph due to the low respiration rates of fish at the low temperatures, relatively high signal-to-noise ratio and higher

organism:chamber volume ratio at 1.5°C (Chabot et al., 2021; Kissinger et al., 2017; Svendsen et al., 2016). Although $R^2 > 0.5$ may not be an ideal criteria, it is important to note that both SMR and MMR values at 251 dph in the present study (1.3°C) were comparable to a previous study measured at 1.0°C with the criteria of $R^2 > 0.9$ (Deslauriers et al., 2018). SMR was estimated by $q_{0.2}$ (20th quantile) method as previously outlined (Chabot et al., 2016) and MMR was taken as the highest $\dot{M}O_2$ following the standardized chase protocol. Absolute aerobic scope (AAS) was calculated by subtracting SMR from MMR whereas factorial aerobic scope (FAS) was calculated by dividing MMR by SMR.

4.3.5 Statistical analysis

Our initial analysis did not indicate a significant effect of rearing tank; thus, we assumed that duplicate rearing tanks did not violate the assumption of independence and removed it from our data analysis. We performed two-way ANOVA to assess specific effects of temperature manipulation on measured variables at each sampling time (dph). Specifically, we used a type II analysis to account for unbalanced data across time points (Langsrud, 2003). When significance was indicated ($p < 0.05$), a *post hoc* Tukey's test was used to compare individual treatments. Assumptions of normality and homoskedasticity were visually assessed as previously outlined (Zuur et al., 2010). Log, square root or cube root was performed on data where necessary. All data analysis was performed on R 4.0.0 (R Core Team, 2020).

4.4 Results

4.4.1 Temperature manipulation

During the temperature manipulation from 58 to 93 dph, cumulative daily temperatures for Ambient, 16°C and 18°C were 595.1, 579.1 and 646.3°C·days, respectively (Figure 4.1).

4.4.2 Mortality

Nine mortalities were recorded in the 18°C group during the temperature manipulation from 58 to 93 dph. Afterwards, only a single mortality occurred in the Ambient group when fish were in the common garden setup at 308 dph.

4.4.3 Growth

Two-way ANOVA showed that total length, body mass and K were significantly influenced by temperature manipulation and time (p<0.01; Table 4.1). Specifically, immediately following temperature manipulation at 93 dph, there was no difference in total length between any treatments (Figure 4.2a). From 93 to 123 dph, total length of all groups significantly increased (p<0.05; Figure 4.2a) and did not change within groups for the rest of study period. At 184 and 251 dph, the 18°C group showed a higher total length than 16°C group (p<0.05; Figure 4.2a).

Body mass showed a similar trend as total length where the 18°C fish were significantly heavier than the 16°C group at 184 dph (p<0.01; Figure 4.2b). During the winter months, we saw a trend of a decrease in body mass; however, significant changes were only found in the Ambient and 18°C groups. Specifically, within the same group 18°C group showed lower body mass at 251 and 306 dph compared to those at 184 dph (p<0.05; Figure 4.2b). Similarly, within the Ambient group, fish had a lower body mass at 306 dph compared to those at 184 dph (p<0.05; Figure 4.2b). We observed a trend of higher body mass in 18°C group compared to 16°C at 251 and 306 dph although it was statistically insignificant. From 306 to 337 dph, within the Ambient and 16°C groups fish showed a significant increase in body mass (p<0.05; Figure 4.2b) while 18°C did not (p=0.5; Figure 4.2b). At 360 dph, all three groups showed higher body mass compared to fish at 306 dph (p<0.001; Figure 4.2b).

At 93 dph, the Ambient group showed a significantly higher K than the 18°C group (p<0.001; Figure 4.2c), but there was no difference in K among groups throughout the rest of the study period. From 93 to 123 dph, within the groups, K significantly increased (p<0.05; Figure 4.3c) and remained unchanged until 214 dph. From 214 to 251 dph, only fish within Ambient and 16°C groups showed a lower K (p<0.001; Figure 4.2c) while fish in the 18°C group did not. After overwintering, all groups showed a significant increase in K at 337 dph compared to those found at 306 dph (p<0.001; Figure 4.2c). However, within 16°C and 18°C groups fish showed a significant decrease in K at 360 dph compared to those at 337 dph (p<0.05; Figure 4.2c) while Ambient group did not.

4.4.4 Hepatosomatic index, gutsomatic index and whole-body energy density

Our results indicated that HSI was significantly influenced by time (p<0.001; Table 4.1). Also, results indicated that GSI was influenced by temperature manipulation (p<0.01; Table 4.1),

but our *post hoc* analysis did not support this ($p>0.05$). ED was influenced by both temperature manipulation and time ($p<0.001$; Table 4.1). At 93 dph, the Ambient group showed a trend of higher HSI compared to the 18°C group albeit statistically insignificant (Figure 4.3a). During the overwintering event, HSI of all groups did not change and no difference among groups was found. However, HSI of all groups substantially increased at 360 dph ($p<0.01$; Figure 4.3a) with again no difference among groups. Conversely, GSI did not change appreciably throughout the study period and there was no difference among groups ($p>0.05$; Figure 4.3b). At 93dph, ED of Ambient group was significantly higher than the other two groups ($p<0.01$; Figure 4.3c). ED for all groups did not change during the overwintering event and significantly increased post-winter ($p<0.01$; Figure 4.3c).

4.4.5 Whole body composition

Our results indicated that both whole-body glucose and triglyceride concentrations were influenced by temperature manipulation and time ($p<0.01$; Table 4.2). Although the initial analysis indicated that whole-body protein concentration was significantly influenced by the interaction between temperature and time, our *post hoc* analysis did not support this ($p>0.05$).

At 93 dph, there was no difference in whole-body glucose concentration among groups (Figure 4.4a). However, at 251 dph, fish in the 16°C group showed significantly higher whole-body glucose than fish raised at 18°C ($p<0.05$; Figure 4.4a). After winter, within group analysis found that only the Ambient group showed a significant increase in whole-body glucose concentration ($p<0.01$; Figure 4.4a). Whole-body triglyceride concentration did not differ among groups at 93 dph (Figure 4.4b). At 251 dph, all groups showed significant higher whole-body triglyceride concentration than those found at 93 dph ($p<0.01$; Figure 4.4b) while there was no difference among groups. At 306 dph, the Ambient group showed a significant reduction in whole-body triglyceride concentration compared to 251 dph ($p<0.05$; Figure 4.4b) while other groups did not ($p>0.1$). Unlike whole-body glucose and triglyceride, whole-body protein concentration did not differ significantly throughout the study period (Figure 4.4c).

4.4.6 Whole-body metabolic rate

Our results indicated that SMR was influenced by time and the interaction between temperature manipulation and time ($p<0.05$; Table 4.3). Also, MMR and AAS were only influenced by time ($p<0.001$; Table 4.3), and there was no significant effect on FAS. At 93 dph, fish in the 18°C group showed higher SMR compared to the Ambient group ($p<0.05$; Figure 4.5a). SMR substantially decreased in the middle of winter and increased to pre-winter levels post-winter, and there was no difference among groups. MMR and AAS showed a similar trend across time but there was no difference between groups ($p>0.1$; Figure 4.5b). FAS did not differ among groups nor change throughout the study (Figure 4.5c).

4.5 Discussion

Our research showed that 35 days of temperature manipulation at two months old result in a short-term effect on growth and energy metabolism prior to the overwintering, but there was no long-term effect in age-0 Lake Sturgeon.

We saw a period of improved growth in the fish held at 18°C at the beginning of overwintering, which may be attributable to thermal history experienced during the early life stage. Specifically, the 18°C group experienced the highest cumulative daily temperatures (646.3°C·days) followed by Ambient (595.1°C·days) and 16°C (579.1°C·days). An ample amount of studies have suggested that environmental change during the early developmental stages may often result in irreversible phenotypes that can have long-lasting effects later in life (i.e. developmental plasticity) (Hou and Fuiman, 2020; Monaghan, 2008; Vagner et al., 2019). Specifically, it is well documented that increased temperature during early development could result in a persistent effect on growth in fish (Garcia de la serrana et al., 2012; Imsland et al., 2007; Macqueen et al., 2008; Martell et al., 2005; Schnurr et al., 2014; Scott and Johnston, 2012). However, the lack of persistent temperature effects on growth trajectory in the present study might be due to the timing of temperature manipulation being already past the critical window. The critical window for phenotypic development has been documented in virtually all taxa such as humans (Gluckman and Hanson, 2004), plants (Sultan, 2000), reptiles (Refsnider et al., 2019; Singh et al., 2020), insects (Tzschentke and Halle, 2009), birds (Durant et al., 2013) and fishes (Jonsson and Jonsson, 2019). In oviparous species, the critical window is hypothesized to be during embryonic development (Durant et al., 2013; Jonsson and Jonsson, 2019; Melendez and Mueller, 2021; Singh et al., 2020). Therefore, much of the previous research about developmental plasticity in fish was focused on earlier stages of development (i.e.

embryogenesis). For example, environmental temperature during embryogenesis has a profound effect on development of morphological and physiological phenotypes (Chebanov and Galich, 2013; Jay et al., 2020; Schnurr et al., 2014; Scott and Johnston, 2012; Wassink et al. 2019) that may ultimately influence growth trajectory. When an appropriate environmental context is provided, fish may show persistent enhanced growth and swimming performance (Finstad and Jonsson, 2012). Recent work in our lab demonstrated that a transient increase in temperature during the first month of life could result in persistent improved growth phenotypes toward age1+ in Lake Sturgeon (Brandt et al., 2022). Thus, in the present study temperature manipulation at two months old may be less profound than during the earlier developmental stages (Burggren, 2015), leading to a period of improved growth in age-0 Lake Sturgeon but without longer-term effects.

Our results indicated that temperature manipulation did not result in immediate improvements in total length and body mass, but rather reduced K. Similar studies in Lake Sturgeon have demonstrated that higher temperatures during early life could significantly influence development of morphology, physiology and behaviour (Bugg et al. 2020; Dammerman et al. 2016; Jay et al. 2019; Wassink et al. 2019). For instance, larvae Lake Sturgeon raised at 18°C showed significantly increased body mass compared to those raised at 10°C, but also showed higher body activity with no difference in cortisol level between baseline and post-stress (Wassink et al. 2019). When raised at 20°C, age-0 Lake Sturgeon consistently showed reduced K than those raised at 16°C (Bugg et al. 2020). It remains unclear how an increased regime of temperature could result in reduced K in the present study, but it may be due to optimum temperature for growth varying with individual developmental trajectories and life stages (Kappenman et al., 2009; Killen, 2014) or the natural temperature regime that fish may experience in the wild. For example, based on the temperature profile of the confluences where gametes were collected, presumptive temperatures that this age class of fish may experience in the wild would be a gradual decline from 15 to 11°C (Bugg et al. 2020). Our results indicate that 18°C may not be optimal for growth at this age class, which is also supported by increased mortalities during the temperature manipulation and lower ED at 93 dph. At temperatures higher than optimum for growth, food intake may be reduced in order to decrease metabolic costs for digestion (i.e. specific dynamic action) that may limit other routine activities in fish (Jutfelt et al., 2021). Thus, it is possible that reduced food intake at higher temperatures may have led to

reduced K in fish held at 18°C. Interestingly, the 18°C group later showed significantly higher total length and body mass than 16°C group at 184 dph. This could be explained by a trend of increased growth performance in 18°C compared to 16°C prior to overwintering. It remains undetermined what drove improved growth performance of the 18°C group in our study (see below) but other metabolic measures such as changes in enzymatic activities for oxidative phosphorylation (Schnurr et al., 2014) and/or muscle cellularity (Garcia de la serrana et al., 2012; Johnston, 2006; Martell and Kieffer, 2007) likely played a role.

We also found that difference in growth trajectory between 16 and 18°C appears to have become diminished by the end of overwintering. Specifically, we saw that only the 18°C group showed a significant reduction in body mass at 251 dph while other groups did not, and that there was no difference in body mass afterwards. Again, this result might be a due to the timing of our temperature manipulation that may be already past the critical window (see above); however, it may be explained by a potential trade-off with the higher growth rate and/or reversibility of improved growth phenotypes during winter. Studies have shown that environmental manipulation during the critical window may result in irreversible changes in growth rate, which remain for the rest of life (Donelson et al., 2018; Jonsson and Jonsson, 2019; Moore and Martin, 2019; Singh et al., 2020; Vagner et al., 2019; Wells, 2011). However, it has been suggested that growth rate may be a plastic trait that is strongly influenced by environmental conditions and developmental stages (Arendt, 1997; Auer et al., 2015b; Dmitriew, 2011; Metcalfe and Monaghan, 2001; Monaghan, 2008). Specifically, it was proposed that growth rate typically does not stay at the enhanced level due to ecological and physiological prices (Ali et al., 2003; Hector and Nakagawa, 2012). Improved growth phenotypes may be beneficial where resources are abundant; however, they are thought to be metabolically very expensive, which could be detrimental where periods of starvation are expected (Dmitriew, 2011; Hurst, 2007; Johnsson and Bohlin, 2006). Thus, under suboptimal conditions for growth, fish may regulate feeding amount and/or frequency to retain metabolic capacity to support routine activities (Jutfelt et al., 2021; Norin and Clark, 2017). Lower levels of whole-body glucose and protein in the 18°C group compared to 16°C at 251 dph suggest that improved growth phenotypes of the 18°C treated fish may have not efficiently adapted to lower resources at the start of winter compared to fish in the 16°C group, which could have resulted in significant reduction in body mass between 184 and 251 dph. However, as winter proceeded, improved growth phenotypes may have been

reversed or corrected to survive wintering conditions (Burggren, 2020), which would give rise to no difference in growth among treatments post-winter.

Our study showed a trend of HSI, ED and whole-body triglyceride that did not correlate with growth throughout the study period. This may indicate that increased somatic growth performance did not necessarily translate into improved energy storage in age-0 fish. Also, we found a lack of correlation between growth and GSI throughout the study period. In Rainbow Trout, a positive correlation between growth rate and GIT length was reported (Allen et al., 2016). However, a bigger size or length of intestine may not necessarily translate into improved efficiency of nutrient uptake and thus growth because intensity of digestive enzyme (Caviedes-Vidal et al., 2008), morphology of intestine such as length of microvilli (Zeng et al., 2012) and metabolic cost for basal maintenance (Allen et al., 2016; Chabot et al., 2016) all influence growth rate in fish.

We also saw that HSI, GSI and ED did not significantly decrease during the winter. During conditions of starvation in winter months, endogenous energy reserves in the hepatic tissue are known to be mobilized to sustain vital processes (Bar, 2014; Foster and Moon, 1991), which typically leads to a decrease in HSI (Collins and Anderson, 1995). Further, a prolonged starvation can result in atrophy of intestinal tissue and thus decreased GSI (Furne and Sanz, 2017; Secor, 2008; Secor et al., 2012). It remains unknown why we saw lack of changes in HSI, GSI and ED throughout winter. However, it is possible that fish may have had sufficient access to a maintenance diet from surface river water supplying the holding tanks. Although we did not quantify gut contents in any of our fish sampled throughout the study, we would occasionally remove invertebrates from gut tissue in fish sampled mid-winter suggesting that fish had access to food sufficient to maintain HSI, GSI and ED for the duration of winter. It is worth noting that given that holding tanks were supplied by surface river water, this would be analogous to overwintering in the natural river environment.

We observed difference in SMR between groups at 93 dph. The difference in SMR could likely be a result of temperatures used in the respirometry experiment due to corresponding temperatures at that time. Although we did not quantify swimming behaviour, we observed that body movement significantly decreased during overwintering, which may explain significant reductions in SMR, MMR and AAS at colder temperatures. Interestingly, we found lack of changes in FAS despite a substantial change in AAS throughout the experiment. Aerobic scope

can be reported in AAS and/or FAS, which represent absolute metabolic capacity that support routine activities and relative capacity of oxygen transport systems within the same individual, respectively (Halsey et al., 2018). Depending on experimental context and variability in SMR or MMR, the results may show different patterns, so it is recommended that both AAS and FAS are reported (Halsey et al., 2018; Norin et al., 2014). Our results suggest that although fish in the middle of overwintering may show a reduced metabolic capacity to perform routine activity due to cold temperature at 1.5°C, the capacity of oxygen transport was not affected in the middle of overwintering. It remains unknown why this did not change, but it is possibly due to our observation of prey in the gut from the mid-winter sampling period (see above).

We also found that differences in growth trajectory did not coincide with differences in AAS nor FAS, which agrees with previous results (Grans et al., 2014; Jutfelt et al., 2018; Messmer et al., 2017; Norin et al., 2014). Theoretically AS can be interpreted as the metabolic capacity for growth in fish (Clark et al., 2013; Metcalfe et al., 2016), however, recent studies suggest that the relationship between AS and growth is less clear (Auer et al., 2015a; Clark et al., 2013; Grans et al., 2014; Jutfelt et al., 2021; Leal et al., 2021; Metcalfe et al., 2016; Norin and Clark, 2017). This is probably because physiological processes are optimized at different temperatures (Eliason and Farrell, 2016; Hood et al., 2018; McKenzie et al., 2020; Schulte et al., 2011). For example, temperature where AS is maximized does not match with the optimum temperature for growth (Farrell, 2009; Schulte et al., 2011). Also, optimum temperatures for growth and feed conversion may change with developmental stages (Killen, 2014). Thus, it was suggested that more caution is needed to fully understand the relationship between AS and physiological performance or growth in fish as it is context and species specific (Jutfelt et al., 2018; Pörtner et al., 2018).

4.6 Conclusion

This research revealed that temperature manipulation at two months old may have a short-term effect of improved growth prior to the overwintering without having a long-term effect on growth. Enhanced somatic growth rate did not directly translate into improved energy reserves in age-0 Lake Sturgeon, and difference in growth rate did not correlate with aerobic scope. Thus, we recommend that the practice of temperature manipulation at two months old could benefit conservation aquaculture of Lake Sturgeon as the goal is to promote increased

post-release survival rates. Our research may aid in improved rearing protocols in species of concern for conservation purposes.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Credit Author Statement

Gwangseok R Yoon: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, funding acquisition; Laura Groening: methodology, data curation, resources; Catherine Brandt: conceptualization, methodology, data curation, writing- review & editing; Cheryl Klassen: conceptualization, methodology, investigation, resources, writing- review & editing, supervision; W. Gary Anderson: conceptualization, methodology, validation, investigation, writing- review & editing, supervision, funding acquisition

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Table 4.1. Summary of two-way ANOVA for total length (TL), body mass (BM), condition factor (K), hepatosomatic index (HSI), gutsomatic indec (GSI) and energy density (ED) of age-0 Lake Sturgeon (*Acipenser fulvescens*) raised in different rearing temperatures. Data were transformed by log (L) or square root (S) where appropriate to meet the assumptions of normality and homoskedasticity. Temperature is temperature manipulation and DPH is days post hatch.

	Variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Note
TL	Temperature	2	4588	2294.1	29.785	<0.001	
	DPH	9	23329	2592.1	33.654	< 0.001	
	Temperature × DPH	18	1828	101.6	1.319	0.172	
	Residuals	378	29114	77			
BM	Temperature	2	3.060	1.531	25.081	<0.001	S
	DPH	9	32.37	3.596	58.914	<0.001	
	Temperature × DPH	18	1.590	0.088	1.446	0.107	
	Residuals	378	23.070	0.061			
K	Temperature	2	0.112	0.0558	5.584	0.004	L
	DPH	9	3.933	0.437	43.695	<0.001	
	Temperature × DPH	18	0.367	0.020	2.036	0.008	
	Residuals	378	3.780	0.010			
HSI	Temperature	2	0.550	0.275	3.028	0.0528	L
	DPH	3	14.745	4.915	54.109	<0.001	
	Temperature × DPH	6	1.572	0.262	2.884	0.0123	
	Residuals	102	9.265	0.091			
GSI	Temperature	2	13.430	6.715	5.616	0.0056	
	DPH	3	3.120	1.039	0.869	0.45984	
	Temperature × DPH	6	10.270	1.711	1.431	0.20991	
	Residuals	102	121.970	1.196			
ED	Temperature	2	861844	430922	10.902	<0.001	
	DPH	3	22295533	7431844	188.018	<0.001	
	Temperature × DPH	6	590383	98397	2.489	0.027	
	Residuals	102	4031795	39527			

Table 4.2. Summary of two-way ANOVA whole body glucose, triglyceride and protein concentrations of age-0 Lake Sturgeon (*Acipenser fulvescens*) raised in different rearing temperatures. Data were transformed by log (L) or square root (S) where appropriate to meet the assumptions of normality and homoskedasticity.

	Variable	Df	Sum Sq	Mean Sq	F value	p	Note
Glucose	Temperature	2	3.924	1.962	10.978	<0.001	L
	DPH	3	20.828	6.943	38.845	<0.001	
	Temperature × DPH	6	1.142	0.19	1.065	0.389	
	Residuals	99	17.694	0.179			
Triglyceride	Temperature	2	7.81	3.903	6.934	0.002	S
	DPH	3	79.46	26.488	47.052	<0.001	
	Temperature × DPH	6	11.1	1.851	3.287	0.005	
	Residuals	102	57.42	0.563			
Protein	Temperature	2	1.15	0.5763	0.704	0.497	S
	DPH	3	3.96	1.319	1.612	0.191	
	Temperature × DPH	6	12.74	2.1239	2.596	0.022	
	Residuals	99	81	0.8182			

Table 4.3. Summary of two-way ANOVA for standard metabolic rate (SMR) and maximum metabolic rate (MMR), absolute aerobic scope (AAS) and factorial aerobic scope (FAS) of age-0 Lake Sturgeon (*Acipenser fulvescens*) raised in different rearing temperatures. Data were transformed by log (L) or cube root (C) where appropriate to meet the assumptions of normality and homoskedasticity.

	Variable	Df	Sum Sq	Mean Sq	F value	p	Note
SMR	Temperature	2	4605	2303	2.632	0.080	
	DPH	2	122265	61132	69.882	<0.001	
	Temperature × DPH	4	9283	2321	2.653	0.041	
	Residuals	61	53362	875			
MMR	Temperature	2	680	340	1.477	0.236	C
	DPH	2	69688	34844	151.304	<0.001	
	Temperature × DPH	4	796	199	0.864	0.491	
	Residuals	62	14278	230			
AAS	Temperature	2	38	19	0.115	0.891	C
	DPH	2	20000	10000	61.070	<0.001	
	Temperature × DPH	4	288	72	0.440	0.779	
	Residuals	60	9825	164			
FAS	Temperature	2	0.122	0.06105	0.984	0.380	L
	DPH	2	0.187	0.09325	1.503	0.231	
	Temperature × DPH	4	0.397	0.09932	1.601	0.186	
	Residuals	60	3.723	0.06205			

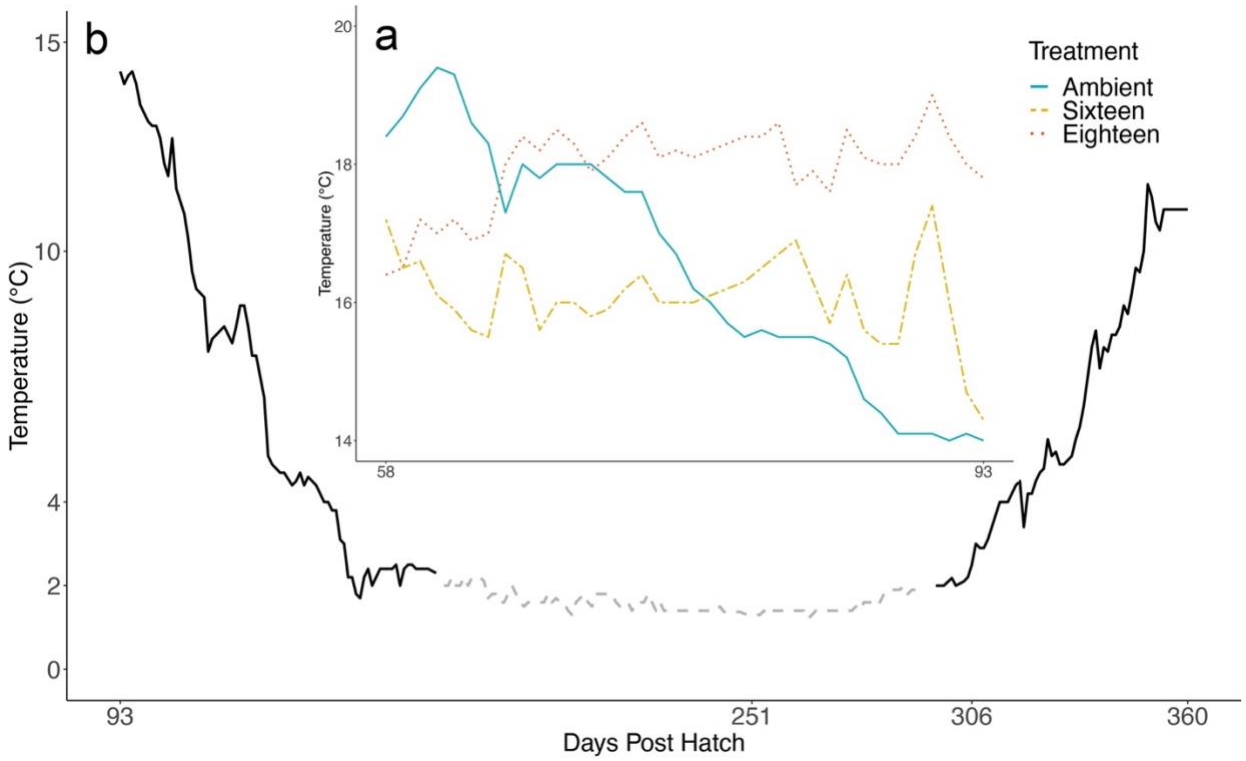

Figure 4.1. Ambient river temperature used in the present study. Temperature manipulation (a) was performed between 58 and 93 days post hatch after which all fish were reared at ambient river temperature until the end of study (b). Grey dashed line represents an overwintering event from 174 to 296 days post hatch where food was deprived for 122 days at 2°C.

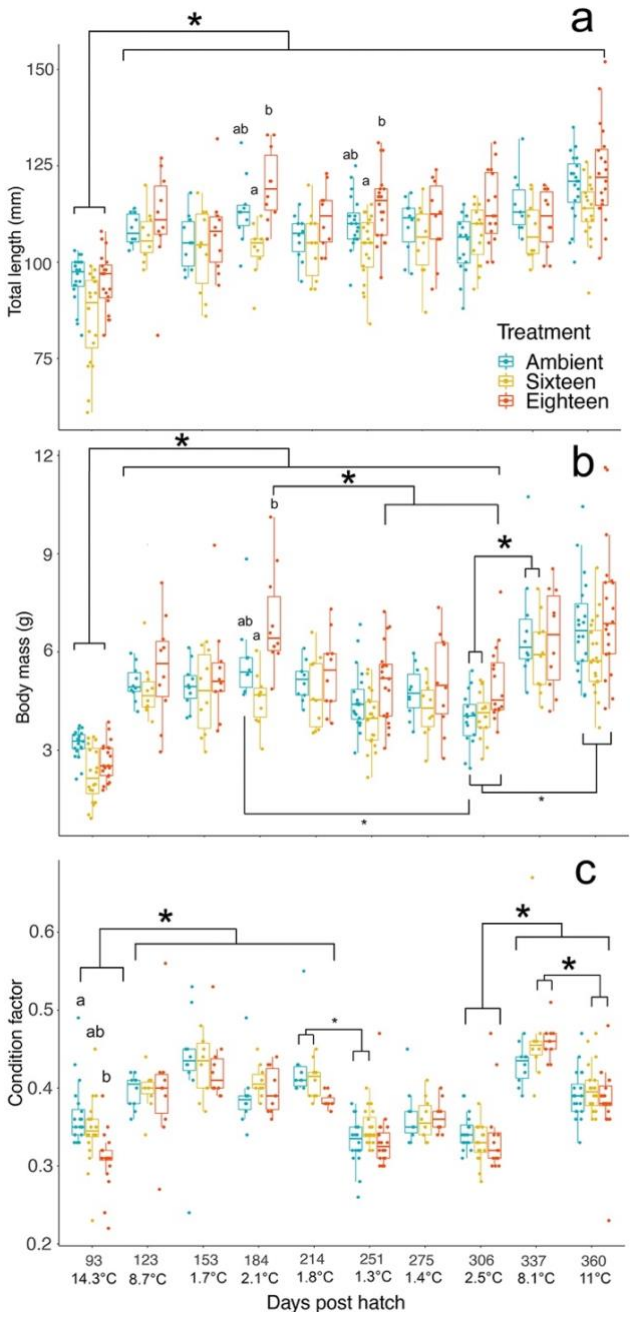

Figure 4.2. Total length (a), body mass (b) and condition factor (c) of age-0 Lake Sturgeon (*Acipenser fulvescens*) exposed to temperature manipulation from 58 days post hatch (dph) to 93 dph. Following the temperature manipulation, all fish were raised in ambient river temperature the rest of experiment period. At 173 dph, food was deprived until 294 dph after which all fish were fed *ad libitum*. Ambient: ambient river temperature. Sixteen: 16°C. Eighteen: 18°C (n=10-20). Data are not transformed for graphical purpose. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots represent individual data points. * represents significant changes within Temperature whereas letters denote significant differences between temperatures at each sampling point (Two-way ANOVA; $p<0.001$). Temperatures on the X-axis are measured water temperatures at each sampling point.

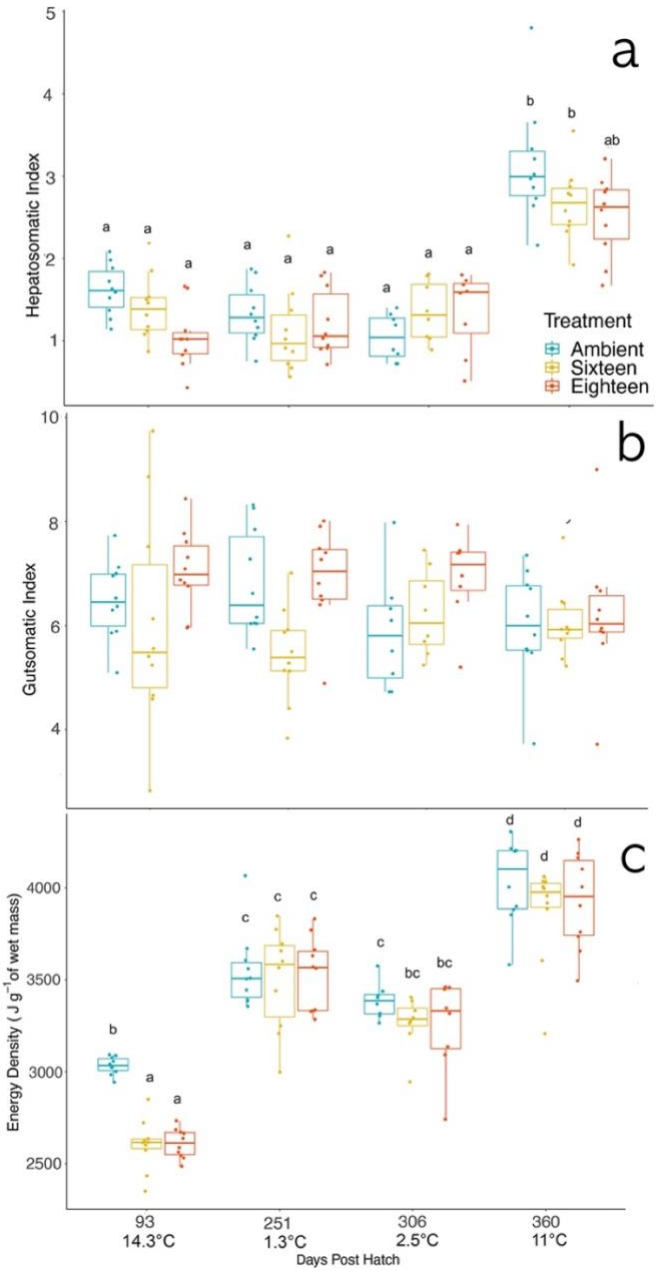

Figure 4.3. Hepatosomatic (a), gutsomatic index (b) and energy density (c) of age-0 Lake Sturgeon (*Acipenser fulvescens*) raised in different rearing temperature. Letters manipulation from 58 days post hatch (dph) to 93 dph. Following the temperature manipulation, all fish were raised in ambient river temperature the rest of experiment period. At 173 dph, food was deprived until 294 dph after which all fish were fed *ad libitum*. Ambient: ambient river temperature. Sixteen: 16°C. Eighteen: 18°C (n=10-20). Data are not transformed for graphical purpose. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots represent individual data points. * represents significant changes within Temperature whereas letters denote significant differences between Temperatures at each sampling points (Two-way ANOVA; $p<0.001$). Temperatures on the X-axis are measured water temperatures at each sampling point.

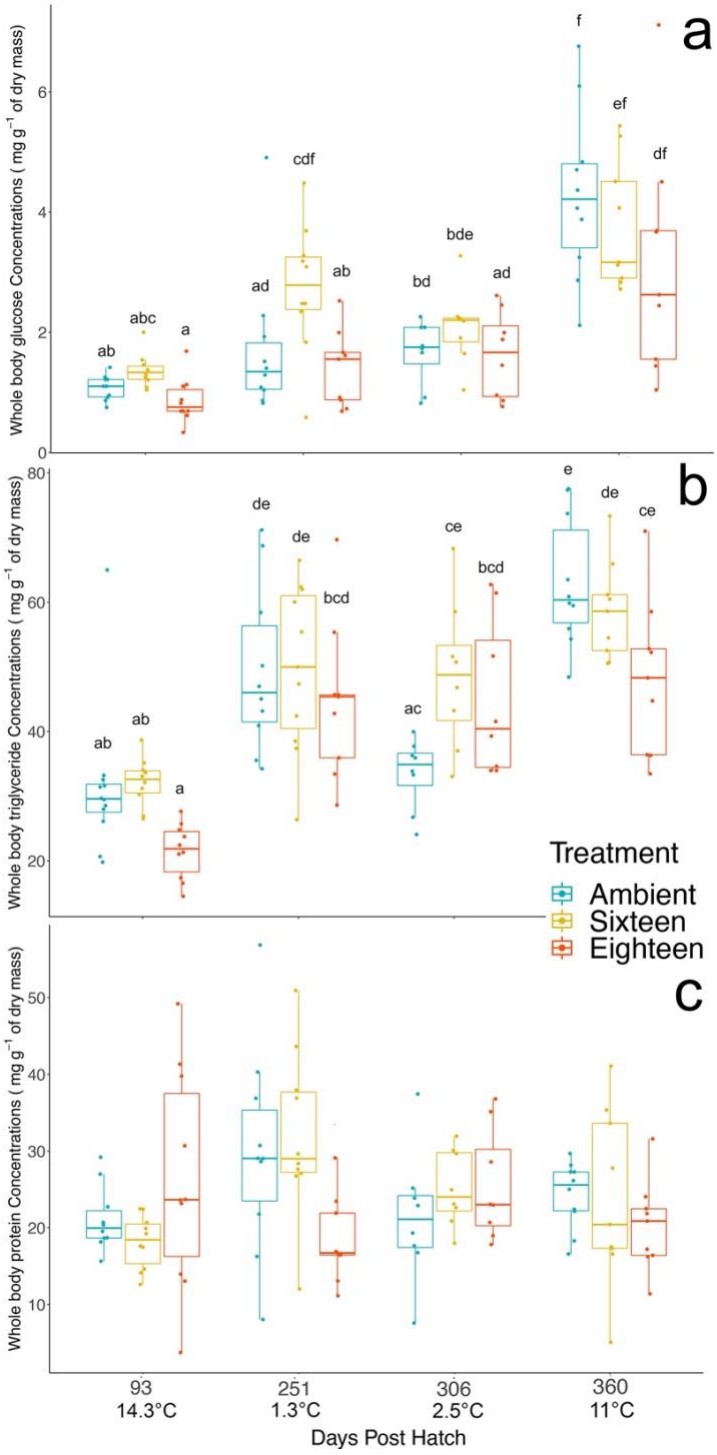

Figure 4.4. Changes in whole body glucose (a), protein (b) and triglyceride (c) concentrations of age-0 Lake Sturgeon (*Acipenser fulvescens*) raised in different rearing temperatures. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots represent individual data points. Letters denote significant differences between Temperatures and sampling points (Two-way ANOVA; $p<0.01$). Temperatures on the X-axis are measured water temperatures at each sampling point.

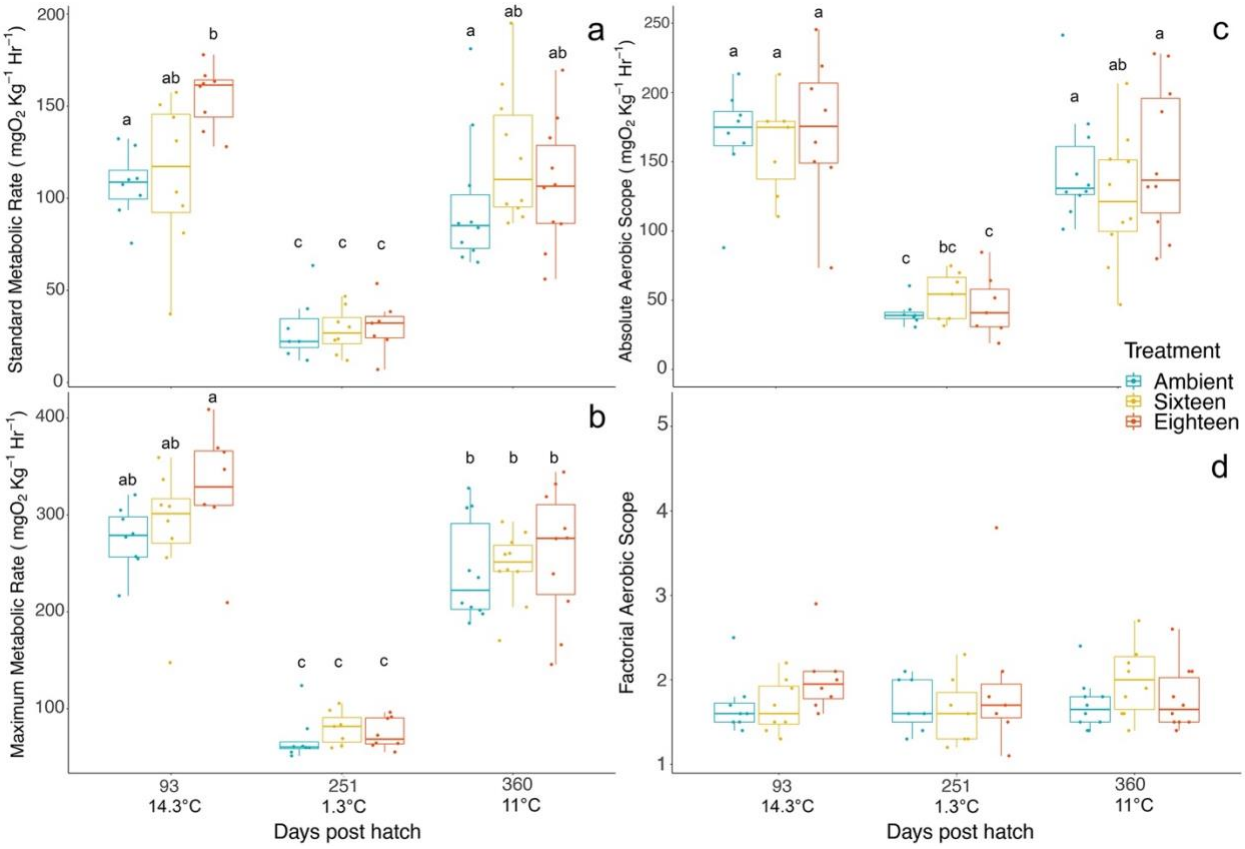

Figure 4.5. Changes in standard (a), maximum metabolic rate (b), absolute aerobic scope (c) and factorial aerobic scope (d) of age-0 Lake Sturgeon (*Acipenser fulvescens*) raised in different rearing temperature. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots represent individual data points. Letters denote significant differences between Temperatures and sampling points (Two-way ANOVA; $p<0.001$). Temperatures on the X-axis are measured water temperatures at each sampling point.

Chapter 4 demonstrated that temperature manipulation during early life could result in transient effects on growth and energy metabolism in age-0 lake sturgeon. Chapter 5 investigated how changes in temperature during early life could influence growth and fatty acid metabolism, both of which could affect overwintering survival in age-0 lake sturgeon.

5 Chapter 5: Long-term effects of temperature during early life on growth and fatty acid metabolism in age-0 lake sturgeon

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5.1 Abstract

Environmental temperature during early life may have prolonged effects on growth and fatty acid metabolism, which could strongly influence overwintering survival in the first year of life for temperate-zone fish. In the present study, we examined how temperature during early life history might influence growth performance and fatty acid metabolism in age-0 Lake Sturgeon (*Acipenser fulvescens*) when exposed to cold temperatures at later stages. Fish were initially at 16 ◦C and subsequently held at 16 ◦C or 20 ◦C for 60 days beginning at 34 days post fertilization (dpf). Then, all fish were subsequently raised at the same temperature of 16 ◦C until the onset of cold conditioning at 158 dpf where temperature was gradually decreased to 3.5 ◦C and remained there for two weeks. Samples were collected before (151 dpf) and after cold conditioning (199 dpf) to measure total length, body mass, whole body metabolic rate, fatty acid profile in phospholipids and triglycerides and mRNA expression of genes associated with fatty acid desaturation, elongation and β-oxidation. Results revealed that before cold conditioning, total length and body mass did not differ between temperature groups, but fish raised at 20 ◦C showed a lower condition factor. During the cold conditioning, only fish raised at 16 ◦C grew significantly longer and heavier. There was no difference in metabolic rates between treatments. Significant increases in total monounsaturated fatty acids with decreases in total saturated fatty acids were identified in phospholipids and triglycerides in both temperature groups after the cold conditioning; however, the 20 ◦C group did not signifi- cantly increase levels of gene expression associated with fatty acid desaturation (*SCD* and *FADS1*) whereas the 16 ◦C group did. Our results suggest that thermal experience during early life may influence overwintering survival of age-0 Lake Sturgeon.

5.2 Introduction

In ectotherms, environmental changes during early life may have prolonged effects on growth and metabolism (Brandt et al., 2022; Burggren, 2020; Vagner et al., 2019). For example, changes in temperature during embryogenesis in fish have been shown to have prolonged effects on enzymatic activities for oxidative phosphorylation (Schnurr et al., 2014) and/or muscle cellularity (Johnston, 2006; Martell and Kieffer, 2007), which may give rise to persistent effects on growth phenotypes (Burggren, 2020; Donelson et al., 2018; Mueller et al., 2019; Ryu et al., 2020). However, any prolonged effects of temperature during early life on metabolic phenotypes may become maladaptive where later environments become mismatched (Donaldson et al., 2008; Hazel, 1995; Vagner et al., 2019). For example, enhanced growth performance may result in an elevated metabolic cost for basal maintenance, which can lead to more rapid loss in energy storage when food becomes limited (Burton et al., 2011). Increased temperature during early life may also impair peroxisomal fatty acid metabolism (He et al., 2021), compounding to negatively influence cellular adaptation at cold temperatures (Hazel, 1995).

Maintenance of membrane fluidity within the range of biologically relevant temperatures for a given organism is crucial to sustain cellular functionality (Hazel, 1997). At colder temperatures, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) increase in the cellular membrane of ectotherms with a concomitant decrease in saturated fatty acids (SATs) in phospholipids (PLs). These changes in fatty acid composition have a net effect aiding to maintain cell membrane fluidity in cold environments, a process known as homeoviscous adaptation (Ernst et al., 2016). Arachidonic acid (AA, C20:4n6), eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) are the main omega-6 and omega-3 long-chain PUFAs (LC-PUFAs) found in PLs and are known to play key roles in growth and homeoviscous adaptation in fish (Monroig et al., 2018; Tocher, 2010). The metabolic pathway of these omega-3 and 6 LC-PUFAs are not interchangeable (Park 2018), and it is hypothesized that fish cannot synthesize C18:2n6 or C18:3n3 de novo due to the lack of Δ12 and 15 desaturases (Monroig et al., 2018). Although some fish may synthesize AA and DHA from dietary C18:2n6 and C18:3n3 (Tocher, 2010), the process of conversion may not be efficient because of the limited physiological and molecular capacity of fatty acid desaturation and elongation (Park, 2018). It is generally accepted that a majority of fish cannot synthesize sufficient amounts of LC-PUFAs de novo to meet their physiological needs so this deficit must

be obtained from their diet (Monroig and Kabeya, 2018). However, dietary intake of these fatty acids could change with seasonal variation of prey availability especially during cold winter months in northern habitats (McKinley et al., 1993; Paulsen et al., 2014). Thus, studies suggest that successful homeoviscous adaptation to cold environments in temperate aquatic species is dependent upon pre-existing substrates of fatty acids prior to over- wintering and/or fatty acid metabolism (Ernst et al., 2016; Snyder et al., 2012).

The significance of growth and fatty acid metabolism is particularly relevant to age-0, or young of the year northern temperate aquatic species because the growing season is typically short prior to overwintering conditions when food resources are limited and reduced temperatures are anticipated for months every year (Hurst, 2007; Rozsypal et al., 2014). Lake sturgeon (*Acipenser fulvescens*) are native to North America and currently endangered or at risk across their natural range (Bruch et al., 2016). Conservation aquaculture of Lake Sturgeon has been in operation for decades to release fall fingerlings or spring yearlings supporting populations in Manitoba, Canada. However, an 18-fold lower recruitment rate has been reported for fall fingerlings compared to those released as spring yearlings (McDougall et al., 2014). Despite smaller body size that may be vulnerable to predation and overwintering survival, release of fall fingerlings is often required due to logistical and financial constraints at the hatchery (McDougall et al., 2014). Increasing rearing temperature to enhance growth rate during early development has been proposed to improve overwintering survival of fall fingerlings. However, a recent study in our lab demonstrated that fish reared at 20°C during early life showed consistently lower body condition (Bugg et al., 2020) as well as altered transcriptional responses associated with cold adaptation compared to those raised at 16°C (Bugg et al., 2021), which could potentially influence overwintering survival. As body mass and homeoviscous adaptation play an important role in overwintering survival of age-0 lake sturgeon (Deslauriers et al., 2018; Yoon et al., 2021a), understanding the longer-term effects of early rearing temperature on growth and fatty acid metabolism at cold temperatures could inform current conservation practices for lake sturgeon.

In the present study, we examined how increased temperature during early life may influence growth and fatty acid metabolism based on the previous results reported in age-0 Lake Sturgeon (Bugg et al., 2021). We measured total length, body mass and energy density as a metric for growth performance as well as aerobic scope as a proxy for energy metabolism. In

addition to measurement of fatty acid profiles, we examined changes in expression of a number of candidate genes associated with fatty acid metabolism: Stearoyl-CoA desaturase (SCD) for its role in the synthesis of MUFAs; Δ5 (FADS1; fatty acid desaturase 1) and Δ6 desaturases (*FADS2*; fatty acid desaturase 2) for their role in the desaturation of fatty acids; elongation of very long-chain fatty acids protein 2 (*ELOVL2*) and elongation of very long-chain fatty acids protein 5 (*ELOVL5*) for their role in the elongation of fatty acids; and phospholipase A2 (*PLA2*) and carnitine palmitoyltransferase 1 (*CPT1*) for their role in fatty acid cleavage as well as oxidation. We tested the prediction that fish reared at high temperature during early life would show reduced growth performance and fatty acid metabolism of LC-PUFAs when experiencing cold temperatures later in life.

5.3 Materials and methods

5.3.1 Gamete collection and animal rearing

When the water temperature was at ~12 °C, spawning fish were caught by gill netting near the confluence of the Landing and Nelson Rivers in northern Manitoba (55º19′36’’N, 96º54′16’’W). Sperm was immediately collected in 50 mL Falcon tubes and stored at 4 °C while free flowing from two males upon capture or by gently massaging the ventral surface of the abdomen (Boase et al., 2011). Eggs were collected from one female followed by administration of gonadotrophin releasing hormone to facilitate the maturation and ovulation of eggs (Genz et al., 2014). Animal rearing was performed as previously described (Bugg et al., 2021). In brief, fertilized eggs were incubated in a McDonald tumbling jar at 12 °C, and following hatch fish were transferred to one of triplicate flow-through 12 L aquaria per treatment. Rearing density was equalized across tanks by visual counting of larvae (150 larvae per tank). At 13 days post fertilization (dpf), rearing temperature was increased from 12°C to 16°C by a rate of 1°C per day. Then, at 19 dpf, fish were fed freshly hatched *Artemia* (Artemia International LLC; Texas, USA) to promote recognition of prey, with all fish actively feeding by 21 dpf. At 50 dpf, diet was gradually transitioned from *Artemia* to bloodworm (Hikari, California, USA), with complete transition by 68 dpf. At 78 dpf, fish were transferred to one of two duplicate 250L flow-through tanks per treatment at an approximate density of 140 fish per tank, and fed bloodworm *ab libitum* two times a day until the end of the experiment.

5.3.2 Temperature manipulation and cold conditioning

Temperature manipulation was performed as previously described (Bugg et al., 2021). Rearing sturgeon at 16 ◦C was chosen based on current practices where fish are maintained for the first year of life in the fish hatchery, and the increased temperature exposure of 20 ◦C was chosen as the upper limit that the source population from the Nelson River would experience during the first three months of life (Bugg et al., 2021). Briefly, at 30 dpf, temperature in three of the tanks was increased to 20 °C at a rate of 1 °C per day (Fig. 1); the other three tanks were held at 16 ◦C. Photoperiod was held on a 12:12 h light:dark for the entire experiment. Fish were maintained at these two temperatures for the following 60 days when the temperature in the 20 °C group was reduced to 16 °C at 1 ◦C per day, and temperature of both treatments remained at 16 ◦C until cold conditioning was initiated at 158 dpf.

At 158 dpf, the protocol of cold conditioning was performed by decreasing temperature from 16 °C by 1°C per day until it reached 8 °C (Fig. 1). Then, fish remained at 8 ◦C for one week after which point all fish were transferred to one of two 250 L flow-through tanks per treat- ment situated in a controlled environment chamber (CMP 6050, Con- viron, Manitoba, Canada). We chose to transfer fish at 8 °C because this is the temperature when fall fingerlings are released in the wild in Manitoba. Then, temperature of both groups was further decreased to 3.5 °C at the same rate described above and maintained at 3.5 °C for 2 weeks to mimic a stocking event in late fall of Manitoba, Canada.

5.3.3 Total length and body mass

Total length and body mass were measured at two time points in our study before cold conditioning at 16 °C (151 dpf) and following cold conditioning at 3.5 °C (199 dpf). At each sampling point, fish were haphazardly captured from the tanks and sacrificed by immersion in an overdose of MS-222 (250mg·L^{-1}; Syndel Laboratory, British Columbia, Canada) buffered with equal amounts of sodium bicarbonate. Total length and body mass were measured to the nearest 1 mm and 0.001 g. Then fish were dissected and liver tissue was carefully removed. Liver tissue was frozen with liquid nitrogen and further stored in liquid nitrogen at -80°C for fatty acid analysis (n=10 per group), and another subset of samples (n=10 per group) were stored in RNAlater (Thermo Fisher, Massachusetts, USA) overnight at 4 °C followed by longer term

storage at -80°C for subsequent gene expression studies. Condition factor was calculated with the following equation: K = g · 100/ cm^3 : where K is the Fulton's condition factor, g is the body mass of fish, cm is the total length of fish (Nash et al. 2006).

5.3.4 Whole body metabolic rates and energy density

Whole body metabolic rates and energy density were measured at two time points similar to those described above. At 151-152 dpf (16 °C) and 197-198 dpf (3.5 °C), whole body oxygen consumption ($\dot{M}O_2$) was measured as a proxy for whole body metabolic rate using intermittent flow respirometry (Loligo Systems, Viborg, Denmark). The respirometry setup was comprised of 8 acrylic metabolic chambers (642.58 ± 16.42mL; mean ± S.D.) in a 250 L water bath with two Witrox 4 Oxygen meters connected to oxygen dipping probes situated in flow-through vessels for each respirometer (PreSens, Regensburg, Germany). Oxygen probes were calibrated at 0 and 100% with 2% sodium sulfite solution and fully air-saturated water. Tubing was non-oxygen permeable Tygon™ (91.34 ± 15.18 mL; mean ± S.D) and both flush and recirculation pumps were Eheim Universal 300. At both time points, fish were haphazardly sampled from the rearing tanks; total length and body mass were measured nearest to 1mm and 0.001g, respectively. Then, fish were placed into respirometry chambers. Average body mass of fish before and after cold conditioning was 7.73 ± 1.37 g and 12.30 ± 1.99 g respectively, and was not significantly different between temperature groups at each time point ($p>0.05$). Flow rate was 5L· min^{-1} across all pumps and observations confirmed that fish were not physically disturbed by the flow rate. A black curtain was hung around the experimental area to minimize visual disturbance and oxygen consumption ($\dot{M}O_2$) was measured for the next 24 hours to assess standard metabolic rate (SMR) after which fish were removed and a standardized chase protocol was performed for 15 minutes to elicit maximum metabolic rate (MMR) (Yoon et al., 2021b). Fish were immediately returned to the chambers and measured for an additional three measurement cycles. Before and after each $\dot{M}O_2$experiment, background respiration (BR) was measured without fish in the chambers for 15 minutes. Fish were then sacrificed by immersion in an overdose of MS-222 and carcasses were dried at 60 °C for 48 hours or until a constant weight was recorded to the nearest 0.001g. The dry-to-wet mass ratio was then used to estimate whole body energy density using the previously established model (Yoon et al. 2019).

Before and after each $\dot{M}O_2$ session, $\dot{M}O_2$ was measured for 15 minutes without fish to estimate background respiration. Assuming a linear increase, background respiration data were used to interpolate over the experiment period and all $\dot{M}O_2$ data were corrected by each corresponding background respiration data point (Rodgers et al., 2016). The relative ratio between background respiration to $\dot{M}O_2$ pre-cold-conditioning was 0.27 ± 0.13 (mean ± S.D.) and post-cold-conditioning was 0.45 ± 0.10 (mean ± S.D.). $\dot{M}O_2$ slopes with $R^2 \geqq 0.9$ were chosen while ensuring linearity. SMR was estimated using the 20th quantile method from 18 hours of $\dot{M}O_2$ data following the first 6 hours of acclimation period (Chabot et al., 2016). MMR was chosen as the highest $\dot{M}O_2$ value recorded following the standardized chase protocol. Absolute aerobic scope (AAS) was calculated by subtracting SMR from MMR, whereas factorial aerobic scope (FAS) was calculated by dividing MMR by SMR.

5.3.5 Lipid extraction and fatty acid analysis

We chose to measure fatty acid and mRNA expression in the liver tissue as it is the central organ to fatty acid metabolism (Monroig et al. 2018). Total lipids were extracted from liver tissue (n=10 per treatment) as previously described (Folch et al., 1957) with butylated hydroxytoluene as an antioxidant. In brief, liver tissue was placed in an ice cold 0.025% $CaCl_2$ buffer with equal dilution ratio across samples (i.e. 0.1 g of liver tissue in 0.4 mL of $CaCl_2$ buffer). Samples were then homogenized with a 50 Sonic Dismembrator (Fisher Scientific, Massachusetts, USA) until the tissue was fully emulsified. Then, 200 µl of whole homogenate was mixed with 1 mL of chloroform:methanol (2:1 by vol) to extract total lipid.

Analysis of fatty acids from PL and TG were performed as previously described (Feltham et al., 2019). In brief, PL and TG were separated from the lipid extract on silica gel G plates developed in a solvent system consisting of ether:diethylether:acetic acid in a ratio of 80:20:1. Each PL and TG band was visualized with 0.1% 8-anilino-1-naphthalene-sulfonic acid under UV light, followed by saponification and methylation (Kang and Wang, 2005). Fatty acid methyl esters were prepared by adding 2 mL hexane and 1.5 mL of 14% boron trifluoride in methanol at 110°C for 1 hr (Suh et al., 2009). The fatty acid methyl esters were separated using gas chromatography (Vista 6010 GLC and Vista 402 data system, Varian Instruments, Mississauga, Ontario, Canada) with a microcapillary column (15 m × 0.1 mm inner diameter; BPX70; SGE

Analytical Science, Carrboro, North Carolina, USA). Hydrogen gas was used as a carrier at a flow rate of 0.5 mL·min^{-1}. The initial oven temperature was maintained at 130 °C and was increased to 175 °C at 20 °C·min^{-1} held for 1 min then increased to 200 °C at 6 °C·min^{-1} with no hold, and finally increased to 280°C at 30°C·min^{-1}. Peaks were identified by using standard fatty acids mixture 461 (Nucheck Prep Inc., Waterville, Minnesota, USA). Fatty acids were presented as a mol% using molecular weight of fatty acid methyl esters.

5.3.6 RNA extraction, cDNA synthesis and RT-qPCR

Liver samples were homogenized with 600 µL of lysis buffer in a TissueLyser II (Qiagen, Hilden, Germany) at 30 Hz for 2 min. Total RNA was extracted from the homogenized samples using PureLink RNA Mini Kit (Invitrogen; Ambion Life Technologies, Waltham, Massachusetts, USA) following the manufacturer's instructions. Total RNA concentration was determined using a NanoDrop 2000c (Thermo Fisher Scientific; Waltham, Massachusetts, USA), and RNA integrity was visually assessed by gel electrophoresis. Complementary DNA (cDNA) was reverse transcribed from 1 µg of DNase (DNase I, Invitrogen) treated total RNA using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, California, USA) following the manufacturer's instructions. The quality of the generated cDNAs from all samples was visually assessed by running the PCR products of *RPS6* on an electrophoresis gel. Real-time quantitative polymerase chain reaction (RT-qPCR) for each gene of interest (*SCD, FADS1, FADS2, ELOVL2, ELOVL5, PLA2, CPT1*) was conducted using a Bio-Rad CFX Connect Real-Time System thermocycler with SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, California, USA). Each sample contained 6 µl SsoAdvanced Universal SYBR Green Supermix, 400nM forward and reverse primer, 2 µl diluted cDNA and nuclease-free water adjusted to 12 µl per well. The cDNA of all samples was diluted 1:16 with nuclease-free water for all RT-qPCR assays. Primers were designed and optimized for the following PCR reaction conditions: an initial denaturing at 98 °C for 3 min, followed by 40 cycles of 10 s at 95 °C, 30 s at 58 °C (*SCD, CPT1, PLA2, RPS6*), at 56 °C (*FADS2, ELOVL2*), at 54 °C (*FADS1, ELOVL5, RPL7*), and 15 s at 60 °C. Following the PCR reactions, a melting curve from 55–95°C was examined to check for the presence of clean single target product. Normalization by multiple housekeeping genes has been previously used to increase precision of gene expression data (Vandesompele et al. 2002). We found that gene expression levels of RPL7 and RPS6 were not significantly different between

groups (one-way ANOVA; p>0.1). The expression levels of the genes of interest were normalized to the relative expression of the reference genes of *RPL7* and *RPS6* and then analyzed after applying the $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). Also, expression levels of all genes were normalized to the mean expression of the 16°C before the cold conditioning (control) to facilitate the interpretation of relative changes in gene expression.

All primers used in the current study were designed based on conserved regions generated by aligning unpublished lake sturgeon transcripts with publicly available sequences, of similar species in the order Acipenseriformes (the sturgeons and paddlefishes), using NCBI blast (Johnson et al., 2008). Primer sequences for *SCD* (Δ9 desaturase) were the same as used in a previous study investigating responses to cold temperature in lake sturgeon (Bugg et al., 2021). Sequences for *FADS1* (Δ5 desaturase), *FADS2* (Δ6 desaturase), *ELOVL5, PLA2* and *CPT1* were designed using unpublished lake sturgeon tissue-specific transcriptomes (*FADS1, FADS2, ELOVL5*) and showed high sequence homology with other Acipenseriformes. *FADS1*: 98 and 94% identity with Sterlet sturgeon (*Acipenser ruthenus;* XM_034906830.1*)* and American paddlefish (*Polyodon spathul*a; XM_041276799.1) respectively; *FADS2*: 98, 98, and 97% identity with Sterlet sturgeon (XM_034057643.2), Russian sturgeon (*Acipenser gueldenstaedtii;* KT032068.1), and American paddlefish *(*XM_041276798.1) respectively; *ELOVL2* was designed based on the white sturgeon liver transcriptome (Doering et al., 2016): 98 and 91% identity with Sterlet sturgeon (*Acipenser rutehnus*; XM_034059048.2) and American paddlefish (*Polydon spathula*; XM_041247912.1) respectively. *ELOVL5:* 98 and 96% identity with Sterlet sturgeon (XM_034005500.2) and American paddlefish (XM_034018989.2) transcripts respectively; *PLA2*: over 99% with and the Chinese sturgeon (*Acipenser sinensis*; XM_034925014.1) and over 90% with the American paddlefish (XM_041222908.1). *CPT1*: over 96% with American paddlefish (XM_041218229.1), Dabrys sturgeon (MN685789.1), Sterlet sturgeon (XM_034058073.2), and the Chinese sturgeon (MK474630.1), respectively.

5.3.7 Statistical analysis

Initial analysis indicated that tank effect was not significant thus we removed tanks from subsequent analysis (p>0.1). We employed two-way ANOVA to assess effects of early thermal history (either 16 or 20°C) and cold conditioning on physiological measurements. When significance was indicated with $p < 0.05$, *post hoc* Tukey's HSD was performed to compare

individual treatments. Data were transformed by log, square root or Tukey Power transformations where necessary (Mangiafico, 2021). Assumptions of normality and homoskedasticity was visually assessed as previously described (Zuur et al., 2010). Specifically, we used *ggqqplot* from the package 'ggpubr' to plot dependent variables and residuals of ANOVA against normal quantile-quantile to ensure that assumptions of the parametric test were met. All data analysis was conducted on R 4.1.0 (R Core Team, 2021).

All experiments were approved by the University of Manitoba Animal Care Committee under the requirements established by the Canadian Council for Animal Care under protocol number F015–007.

5.4 Results

5.4.1 Body size and energy density

Length, mass and condition factor were not significantly different between pre- and post-cold conditioning in the 20 °C group ($p>0.05$; Table 5.2). However, fish raised at 16 °C were significantly longer and heavier post-cold conditioning compared to pre-cold conditioning ($p<0.05$; Figure 5.2a,b). Fish raised at 20 °C had a significantly lower condition factor pre-cold conditioning compared to those raised at 16 °C ($p<0.01$; Figure 5.2c). In addition, fish raised at both 16 and 20 °C had significantly higher energy density at the post-cold conditioning sampling point compared to the pre-cold conditioning sampling point ($p<0.01$; Figure 5.2d).

5.4.2 Whole body metabolic rate

SMR, MMR, AAS and FAS did not differ between groups at both sampling points (Table 5.2, $p>0.1$). However, while SMR, MMR and AAS significantly decreased post-cold conditioning (Figure 5.3a-c, $p<0.001$), FAS did not (Figure 5.3d).

5.4.3 Fatty acid in phospholipids

In hepatic phospholipids, we observed that total SATs and MUFAs were only significantly influenced by the cold conditioning ($p<0.001$). Within the groups, total SATs significantly decreased while total MUFAs increased after the cold conditioning ($p<0.01$; Table 5.3). Although total PUFAs were not significantly influenced by temperature treatment or cold

conditioning, after the cold conditioning there were significant changes in individual PUFAs such as EPA and DHA ($p<0.01$; Table 5.3). Total omega-3 fatty acids significantly decreased after the cold conditioning ($p<0.01$; Table 5.3) whereas total omega-6 fatty acids did not change. The ratio between total omega-3 and -6 fatty acids did not differ between treatments at both sampling point, but significantly decreased after the cold conditioning ($p<0.001$; Table 5.3). Unsaturated fatty acids to saturated fatty acids ratio significantly increased while the unsaturation index did not significantly change after the cold conditioning ($p<0.01$; Table 5.3).

5.4.4 Fatty acid in triglycerides

In hepatic triglycerides, total SATs were significantly influenced by both temperature and cold conditioning ($p<0.05$). Prior to cold-conditioning, fish raised at 16 °C showed significantly higher total SATs than fish raised at 20 °C ($p<0.05$; Table 5.4), but after the cold conditioning, total SATs of both groups significantly decreased ($p<0.05$, Table 5.4) with no final difference between groups. Total MUFAs were not different between groups at both sampling points, but significantly increased in each after the cold conditioning ($p<0.01$; Table 5.4). Total PUFAs did not differ by groups, and significantly decreased after the cold conditioning ($p<0.01$; Table 5.3). The ratio between total omega- 3 and -6 fatty acids also was not different between temperature groups at both sampling points, but significantly decreased in each group after the cold conditioning ($p<0.001$; Table 5.4).

5.4.5 mRNA expression

There was no difference in gene expression of any target genes between the 16 and 20 °C groups; however, cold conditioning did result in differential changes in target gene expression depending on the rearing temperature of the fish (Figure 5.4). Expression levels of *SCD* and *FADS1* significantly increased post-cold-conditioning in fish raised at 16 °C (Figure 5.4a, b); however, *ELOVL5* significantly decreased post-cold-conditioning in fish raised at 20 °C (Figure 5.4e). All other genes examined did not significantly change between pre- and post-cold conditioning regardless of thermal history.

5.5 Discussion

Our research demonstrated that early thermal experience strongly influenced growth and fatty acid metabolism at cold temperatures in age-0 Lake Sturgeon. Pre-cold-conditioning, we observed a trend of the 20 °C group being longer and heavier than the 16 °C group, but fish raised in 20 °C had a lower condition factor than those raised at 16 °C pre-cold-conditioning, which agrees with a parallel study (Bugg et al. 2021). The lower condition factor in the 20 °C group could be a result of different development rates/patterns at higher temperatures, or the feeding frequency of twice a day that may have limited growth owing to higher metabolic rates and ultimately led to a lower condition factor (Jutfelt et al., 2021). It is also possible that a temperature of 20 °C was not optimal for growth at this life stage. Based on the temperature profile where gametes were collected, temperatures that fish may experience in the wild during the period of experimentation would be in a range of 12 °C to 18 °C (Bugg et al. 2020). Furthermore, only the 16 °C group grew significantly during the cold conditioning while the 20 °C group did not. This may indicate a trade-off for fish in the 20 °C group where they may take advantage of accelerated growth during early life at a cost of reduced condition factor (Arendt, 1997; Dmitriew, 2011), but lead to metabolic deficit prior to cold conditioning (Angilletta et al. 2003; Monaghan and Ozanne 2018). While it remains unknown how growth performance of the 20 °C group was altered in cold temperatures, it may be partially due to the limited transcriptional responses of *SCD* and *FADS1* to cold temperatures in the 20 °C group. Post-cold-conditioning, we found that there was no difference in growth between groups. This could be due to the timing of the temperature manipulation that was already past the critical window for persistent effects on growth in Lake Sturgeon. Depending on timing, magnitude and duration of manipulation, temperature during early life could have irreversible or reversible effects on phenotypes (Burggren, 2015). For example, previous studies suggested the critical window for growth in Lake Sturgeon may be within the first month of life as a transient increase in environmental temperature during the first month of life had a long-term persistent effect on growth phenotypes (Brandt et al., 2022) whereas 35 days of temperature manipulation in fish at two months of age resulted in a short-term transient effect on growth (Yoon et al., 2022). It is worth noting that we did not adjust photoperiod as the cold conditioning progressed. Periodic changes of light may play an important role in regulating metabolism and growth in fish (Boeuf and Le Bail, 1999) and there is evidence that in age 1+ Lake Sturgeon, metabolic rate and

behaviours are influenced by photoperiod (Svendsen et al., 2014). Thus, lack of adjustment in photoperiod during the experimental period may have resulted in altered metabolic preparation and influenced growth of fish at colder temperatures.

It was no surprise that SMR, MMR and AAS significantly decreased in the post-cold-conditioning sampling point and is most likely due to the pervasive effect of environmental temperature on metabolic rates in ectotherms (Clarke and Fraser, 2004). We also observed that SMR, MMR, AAS and FAS were not different between temperature groups, which disagrees with previous studies where the effects of developmental plasticity on metabolic rate in fish have been documented (Hammond et al. 2002; Schaefer and Walters, 2010; Donelson et al. 2011). The discrepancy between our results and the literature is most likely because of the timing of temperature manipulation that was already past the critical window in the present study, or could also be due to phenotypic switching as fish were raised at the same temperature of 16 °C for 54 days following the temperature manipulation (Burggren, 2020). The plastic nature of SMR, MMR and AAS as physiological traits results in a rapid modulation dependent on environmental conditions such as temperature and food availability (Chabot et al., 2016; Clark et al., 2013; Norin and Clark, 2016; Salin et al., 2016). Thus, 54 days at the same environmental temperature would likely have negated any effects on whole body metabolic rates that would have manifested during temperature manipulation earlier in life. We also observed that AAS significantly decreased during cold conditioning, but FAS did not. A previous study in our lab showed a similar result; when age-0 Lake Sturgeon had access to food, AAS fluctuated throughout winter months while FAS remained unchanged (Yoon et al., 2022). Aerobic scope can be reported in AAS, which represents aerobic capacity to perform routine activities, or FAS, which represents oxygen transport capacity within the individual (Halsey et al., 2018). Thus, our results suggest that when provided food, age-0 Lake Sturgeon could retain oxygen transport capacity through a range of temperatures although aerobic capacity for activity may be constrained at cold temperatures.

In terms of fatty acid profile of phospholipids, we reported significant increases in total MUFAs with decreases in total SATs in post-cold conditioned fish, leading to a significant increase in unsaturated to saturated fatty acid ratio. These results support the notion of homeoviscous adaptation in age-0 Lake Sturgeon as reported previously (Yoon et al., 2021a).

We observed that changes in fatty acid levels did not agree with the gene expression patterns. For instance, changes of individual fatty acids that Δ 5 and Δ 9 desaturases would catalyze in phospholipids (e.g. C16:1n7, C18:1n9, C20:4n6, C20:5n3) did not match the transcriptional responses of *SCD* and *FADS1* following the cold conditioning. The discrepancy could be explained by three hypotheses. First, changes in fatty acids during the cold conditioning could be from dietary retention rather than *in vivo* synthesis (Coleman and Lee, 2004). Second, the discrepancy between the present study and literature could be due to tissues used for the analysis as fatty acid composition could significantly vary with tissues and organs that may reflect biological functionality (Shen et al., 2005). For example, when acclimated to cold temperatures, increases of total PUFAs was more pronounced in liver and muscle tissues than gill in freshwater alewife (*Alosa pseudoharengus*) (Snyder et al. 2012). Third, the discrepancy could be due to increased capacity of enzymatic kinetics in cold temperatures. Studies have shown that exposure to cold temperatures significantly increased gene expression levels of *SCD* in carp (*Cyprinus carpio*), milkfish (*Chanos chanos*) and Lake Sturgeon (Bugg et al., 2021; Hsieh et al., 2003; Trueman et al., 2000); however, increased gene expression may not always translate into increased enzymatic capacity. For example, it was reported that cold temperatures led to increased synthesis of MUFAs without a transcriptional response of *SCD* in liver tissue of the common carp, which is hypothesized to result from activation of latent Δ 9 desaturase (Tiku et al., 1996; Trueman et al., 2000). Future studies need to be conducted if this was the case, but limited transcriptional responses in desaturation and elongation in fatty acid metabolism may hinder adaptation to colder temperatures in fish (Tiku et al., 1996). In Manitoba, typical river water temperature during winter months could be near 0 °C (Hrenchuk et al., 2017). Therefore, our results may suggest that temperature during early life could influence homeoviscous adaptation at colder temperatures that would naturally occur for age-0 Lake Sturgeon in Manitoba (Yoon et al., 2021a).

We also found that total PUFAs in phospholipids did not significantly change, but the omega-3/omega-6 fatty acid ratio significantly decreased after cold conditioning, indicating greater relative increases in omega-6 fatty acids compared to omega-3 fatty acids. For example, C20:3n3 and C20:3n6 decreased following cold-conditioning and only C20:4n6 (AA) showed an increasing trend. Although AA, EPA and DHA could be elongated and desaturated *in vivo* from C18:2n6 or C18:3n3 (Park, 2018; Sprecher, 2000), it is hypothesized that substrate availability of

C18:2n6 or C18:3n3 could compete for a promiscuous desaturating enzyme Δ 6 desaturase, which significantly influences synthesis of omega-3 and 6 fatty acids (Berger and German, 1990; De Antueno et al., 2001; Emken et al., 1994). A previous study showed that a diet with higher omega 6 fatty acids (e.g. bloodworm) may promote downstream production of omega-6 fatty acids with relative decreases of omega-3 fatty acids in age-0 Lake Sturgeon (Yoon et al., 2021a). Although we did not quantify the dietary fatty acids of bloodworm (*Chironomus* spp), we used the same source of diet as was used in Yoon et al. (2021a). Thus, it is reasonable to speculate that a similar metabolic pathway, involving the conversion of C18:2n6 to AA (omega-6 fatty acids) rather than C18:3n3 to EPA or DHA (omega-3 fatty acids), would have occurred in the present study.

Following cold conditioning in the 16 °C group, the phospholipid C20:3n6 decreased while there was a trend of increased C20:4n6 which is likely linked with the increased mRNA expression of *FADS1* (a gene encoding Δ5 desaturase). It was reported that marine teleosts may have limited capacity for synthesizing EPA and AA due to low enzyme activity of Δ5 desaturase (Nakamura and Nara, 2004; Tocher, 2003; Tocher and Ghioni, 1999). For instance, Tocher and Ghioni (1999) used cultured cells derived of fin tissue in gilthead seabream (*Salmo auratus*) to investigate the capacity of Δ5 desaturase in a marine fish. Interpretation of this results may be limited due to the general inactivity of fin tissue in fatty acid synthesis because *FADS1* is known to be highly expressed in the liver tissue (Cho et al., 1999). However, it is known that in the marine environments, LC-PUFAs may be readily available from dietary sources such as algae or zooplankton (Monroig and Kabeya, 2018); thus, marine fish may have lost physiological and molecular capacity for synthesizing AA, EPA and DHA through evolution as they do not need to synthesize them *in vivo* (Castro et al., 2012). Conversely, it is hypothesized that freshwater fish may retain metabolic capacity to synthesize LC-PUFAs because these fatty acids may not be as abundant in the freshwater environment (Tocher, 2010). For example, the diadromous Japanese eel (*Anguilla japonica*) showed presence of *FADS1* and enzyme activity of Δ 5 desaturase (Lopes-Marques et al., 2018). As sturgeons have an anadromous origin, Lake Sturgeon may have evolved to cope with low dietary LC-PUFAs in their freshwater aquatic prey (Barth et al. 2013; Bell et al. 1994; Castro et al. 2012) and thus could synthesize AA and EPA *in vivo* as reported in other freshwater fishes.

In terms of fatty acids in triglycerides, we observed that the 20 °C group showed higher unsaturated to saturated fatty acid ratio and unsaturation index than the 16 °C group following the cold conditioning. These results are likely due to reduced total SATs in the 20 °C group as total MUFAs and PUFAs did not differ between temperature groups. It remains unknown why total SATs were lower in the 20 °C pre-cold-condition. Following cold conditioning, changes in fatty acid profiles of triglycerides were similar to that of phospholipids, mirroring results reported across taxa such as insects (Rozsypal et al., 2014), reptiles (Shen et al., 2005), fish (Yoon et al., 2021a) and hibernating heterothermic mammals (Geiser et al., 2013). Homeoviscous adaptation may not be particularly relevant in triglyceride metabolism as they are considered more as energy storage molecules where less PUFAs are present compared to phospholipids (Ernst et al., 2016). However, it is hypothesized that mobilization of fatty acids in triglycerides at cold temperatures may be influenced by a fluid state of molecules; thus, increased unsaturation in triglycerides may prevent solidification of a fat depot, which can be utilized as a metabolic substrate when required (Hazel, 1995; Munro and Thomas, 2004). Indeed, we saw that omega-3/omega-6 ratio significantly decreased in both lipid classes whereas total omega-6 fatty acids in phospholipids did not change after the cold conditioning, suggesting that homeoviscous adaptation may differ between phospholipids and triglycerides due to biological functionality.

5.6 Conclusion

Our research revealed that temperature during early life may affect growth and fatty acid metabolism when exposed to cold temperatures at later stages. Our study emphasizes that the temperature during early life may influence overwintering survival of age-0 fish. Based on the results, as the goal of conservation programs is to maximize post-release survival rate, we recommend that conservation fish hatcheries consider improving growth rate of sturgeon through diet enrichment and inclusion of substrate instead of temperature manipulation.

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5.8 References

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Table 5.1. Primer sequences used for age-0 lake sturgeon (*Acipenser fulvescens*) in the present study. Genes were chosen based on their roles of desaturation, elongation and β-oxidation in fatty acid metabolism. *RPS6* and *RPL7* were used as reference genes as they showed stable expression across temperature groups. *SCD*: stearoyl CoA desaturase. *FADS1*: fatty acid desaturase 1 (Δ5 desaturase). *FADS2*: fatty acid desaturase 2 (Δ6 desaturase). *ELOVL2*: elongation of very long chain fatty acid protein 2. *ELOVL5*: elongation of very long chain fatty acid protein 5. *PLA2*: phospholipase A2. *CPT1*: Carnitine palmitoyltransferase 1. *RPS6*: Ribosomal protein S6. *RPL7*: 60S ribosomal protein L7.

Gene of interest	Forward	Reverse	Efficiency (%)
SCD	AGCCAAGTTGCGTTGAGA	GTCCTCGTGGGTTGGTTACTT	103 at 58°C
FADS1	GGGCTACAGTACCAGAACAAA	CTTCAGAGAGAGCACGATGTC	101 at 54°C
FADS2	ACGGATTCCTGGGCTCCATA	ACTGCTGTGATTTAGTGGCTCT	104 at 56°C
ELOVL2	CTTGGACACTCATTAGGGAACT	CATCTTCCACTGGGATTGAATTG	107 at 56°C
ELOVL5	CCATGCCTAACATCTGGTGGT	GCCCCGAAATAGGAGTGTCC	109 at 54°C
PLA2	GCGGGCACAGTTAATACCCA	CCCTAACCCACAGTAGCAGC	112 at 58°C
CPT1	CAGAAGAAAGCTGGACAGAGAG	CATACGCTCATACTGGGAAGTG	101 at 58°C
RPS6	CTGGCTGGATTCTGATTTGGATG	ATCTGATTATGCCAAGCTGCTG	107 at 58°C
RPL7	TGCTTAGGATTGCTGAGCCG	GATCTTTCCGTGACCCCGTT	112 at 54°C

Table 5.2. Summary of two-way ANOVA for total length (TL), body mass (BM), condition factor (K), energy density (ED), standard metabolic rate (SMR), maximum metabolic rate (MMR), absolute aerobic scope (AAS) and factorial aerobic scope (FAS) of age-0 Lake sturgeon (*Acipenser fulvescens*) experienced different thermal histories. Data were transformed by log, square root, cube root or Tukey Power where appropriate to meet the assumptions of normality and homoskedasticity.

Measurement	Variable	Df	Sum Sq	Mean Sq	F value	p
TL	Temperature	1	1653	1653.40	7.658	0.007
	Cold	1	1023	1023	4.74	0.033
	Temperature: Cold	1	134	134.40	0.622	0.433
	Residuals	76	16409	215.90		
BM	Temperature	1	35.10	35.06	4.794	0.032
	Cold	1	42	41.96	5.738	0.019
	Temperature: Cold	1	0	0.01	0.001	0.972
	Residuals	76	555.80	7.31		
K	Temperature	1	1245	1245.10	7.459	0.008
	Cold	1	14	14.30	0.086	0.770
	Temperature: Cold	1	405	404.60	2.424	0.124
	Residuals	76	12688	166.90		
ED	Temperature	1	0.00366	0.00366	1.532	0.224
	Cold	1	0.22834	0.22834	95.468	<0.001
	Temperature: Cold	1	0.00063	0.00	0.265	0.610
	Residuals	36	0.09	0.00		
SMR	Temperature	1	3	3	0.004	0.951
	Cold	1	49100	49100	68.194	<0.001
	Temperature: Cold	1	2	2	0.003	0.959
	Residuals	28	20160	720		
MMR	Temperature	1	0	0	0.015	0.902
	Cold	1	7.71	7.71	267.643	<0.001
	Temperature: Cold	1	0.01	0.01	0.171	0.682
	Residuals	27	0.78	0.03		
AAS	Temperature	1	2	2	0.002	0.966
	Cold	1	44969	44969	47.146	<0.001
	Temperature: Cold	1	14	14	0.015	0.905
	Residuals	27	25753	954		
FAS	Temperature	1	0	0.00	0.001	0.978
	Cold	1	0.00	0.00	0.035	0.853
	Temperature: Cold	1	0.00	0.00	0.005	0.945
	Residuals	27	0.88	0.03		

Table 5.3. Changes in hepatic fatty acids of phospholipids in age-0 lake sturgeon (*Acipenser fulvescens*) experienced thermal histories before and after the cold conditioning. SAT: saturated fatty acid. MUFA: monounsaturated fatty acid. PUFA: polyunsaturated fatty acid. U/S Ratio: ratio between unsaturated and saturated fatty acid. UI: unsaturation index. * denote significant increase within the temperature group after the cold conditioning. Data are expressed in mol % (mean ± S.D.).

Phospholipid	Pre cold conditioning		Post cold conditioning	
	16°C	20°C	16°C	20°C
C14:0	0.64 ± 0.19	0.62 ± 0.25	0.49 ± 0.15	0.54 ± 0.23
C14:1	0.42 ± 0.13	0.43 ± 0.18	0.25 ± 0.11	0.3 ± 0.14
C15:0	0.62 ± 0.19	0.66 ± 0.11	0.47 ± 0.11	0.58 ± 0.19
C16:0	28.36 ± 3.29	28.95 ± 3.47	25.69 ± 3.02	26.11 ± 3.65
C16:1t	0.53 ± 0.09	0.53 ± 0.1	1.22 ± 0.16*	1.08 ± 0.24*
C16:1	1.58 ± 0.32	1.48 ± 0.46	6.35 ± 1.07*	5.3 ± 1.82*
C17:0	0.96 ± 0.35	1.05 ± 0.27	0.26 ± 0.05*	0.37 ± 0.23*
C18:0	9.06 ± 1.14	8.96 ± 1.09	3.21 ± 0.44*	4.1 ± 1.75*
C18:1	12.16 ± 1.11	11.53 ± 1.18*	13.8 ± 1.7	13.44 ± 1.08*
C18:1n7c	4.79 ± 0.52	5.19 ± 0.42	8.15 ± 0.47*	7.96 ± 1.47*
C18:2n6	6.62 ± 0.61	6.46 ± 0.65	5.1 ± 0.52*	5.45 ± 0.52*
C18:3n6	1.13 ± 0.15	1.19 ± 0.14	1.19 ± 0.1	1.34 ± 0.22
C18:3n3	0.79 ± 0.09	0.72 ± 0.13	0.51 ± 0.08*	0.56 ± 0.09*
C20:0	0.36 ± 0.05	0.33 ± 0.05	0.31 ± 0.03	0.34 ± 0.15
C20:1	1.96 ± 0.46	1.67 ± 0.25	3.19 ± 0.45*	2.98 ± 0.68*
C20:2n6	2.17 ± 0.52	1.68 ± 0.36	4.81 ± 1.01*	3.61 ± 1.57*
C20:3n6	3.26 ± 0.53	2.86 ± 0.5	2.63 ± 0.44*	3.13 ± 0.58
C20:3n3	0.48 ± 0.10	0.47 ± 0.11	0.2 ± 0.03*	0.24 ± 0.08*
C20:4n6	9.00 ± 1.20	9.41 ± 1.24	9.84 ± 0.68	9.76 ± 1.32
C20:5n3	1.02 ± 0.15	0.94 ± 0.19	0.88 ± 0.09	0.86 ± 0.11
C22:0	0.51 ± 0.16	0.47 ± 0.14	0.5 ± 0.06	0.43 ± 0.11
C22:1	0.06 ± 0.04	0.05 ± 0.01	0.13 ± 0.02*	0.12 ± 0.04*
C22:4n6	0.3 ± 0.04	0.33 ± 0.12	0.36 ± 0.03	0.34 ± 0.06
C22:5n3	0.58 ± 0.08	0.53 ± 0.1	0.64 ± 0.1	0.55 ± 0.11
C22:6n3	9.11 ± 2.2	9.71 ± 1.83	6.47 ± 0.76*	7.16 ± 2.21*
C24:0	3.06 ± 0.63	3.34 ± 0.59	2.87 ± 0.26	2.82 ± 0.64
C24:1	0.47 ± 0.18	0.44 ± 0.05	0.47 ± 0.08	0.53 ± 0.12
∑ SAT	43.56 ± 3.55	44.38 ± 3.10	33.8 ± 3.67*	35.29 ± 4.87*
∑ MUFA	21.97 ± 1.51	21.32 ± 1.64	33.56 ± 3.15*	31.7 ± 4.22*
∑ PUFA	34.47 ± 4.45	34.3 ± 4.23	32.64 ± 1.44	33.01 ± 4.32
∑ n-3	11.99 ± 2.37	12.37 ± 2.05	8.71 ± 0.82*	9.38 ± 2.33*
∑ n-6	22.48 ± 2.25	21.92 ± 2.28	23.93 ± 1.33	23.63 ± 2.83
n-3/ n-6	0.54 ± 0.07	0.56 ± 0.05	0.37 ± 0.04*	0.40 ± 0.09*
U/S Ratio	1.31 ± 0.17	1.26 ± 0.15	1.99 ± 0.28*	1.88 ± 0.35*
UI	156.41 ± 19.67	157.89 ± 17.67	154.26 ± 6.03	156.09 ± 17.18

Table 5.4. Changes in hepatic fatty acids of triglycerides in age-0 lake sturgeon (*Acipenser fulvescens*) experienced thermal histories before and after the cold conditioning. SAT: saturated fatty acid. MUFA: monounsaturated fatty acid. PUFA: polyunsaturated fatty acid. U/S Ratio: ratio between unsaturated and saturated fatty acid. UI: unsaturation index. Letters indicate significant difference between temperature groups and * denote significant increase within the temperature group after the cold conditioning. Data are expressed in mol % (mean ± S.D.).

Triglyceride	Pre cold conditioning		Post cold conditioning	
	16°C	20°C	16°C	20°C
C14:0	4.75 ± 2.31	5.29 ± 1.02	2.42 ± 0.3*	2.61 ± 0.4*
C14:1	2.92 ± 0.97	3.15 ± 0.67	1.49 ± 0.26*	1.66 ± 0.3*
C15:0	1.52 ± 0.50	1.68 ± 0.36	0.61 ± 0.12*	0.72 ± 0.15*
C16:0	29.48 ± 4.92	25.13 ± 4.14	25.2 ± 1.65	24.69 ± 2.27
C16:1t	1.72 ± 0.21	1.90 ± 0.24	1.29 ± 0.48	1.25 ± 0.39*
C16:1	7.83 ± 0.67	8.38 ± 1.09	16.09 ± 0.85*	15.53 ± 2.27*
C17:0	1.13 ± 0.52	1.22 ± 0.38	0.15 ± 0.02*	0.20 ± 0.06*
C18:0	4.43 ± 0.48	3.82 ± 0.50	1.22 ± 0.08*	1.36 ± 0.38*
C18:1	25.2 ± 4.09	24.95 ± 3.29	36.25 ± 2.84*	33.93 ± 4.13*
C18:1n7c	8.43 ± 2.02	9.80 ± 1.70	8.46 ± 2.89	10.51 ± 3.47
C18:2n6	6.16 ± 1.83	6.83 ± 1.97	2.32 ± 0.31*	2.69 ± 0.59*
C18:3n6	1.03 ± 0.34	1.40 ± 0.76	0.62 ± 0.09	0.66 ± 0.22*
C18:3n3	1.05 ± 0.35	1.21 ± 0.42	0.28 ± 0.09*	0.32 ± 0.09*
C20:0	0.27 ± 0.11	0.37 ± 0.32	0.17 ± 0.03	0.16 ± 0.06
C20:1	2.10 ± 0.43	2.17 ± 0.56	2.35 ± 0.34	2.37 ± 0.81
C20:2n6	0.66 ± 0.32	0.84 ± 0.30	0.45 ± 0.32	0.42 ± 0.18*
C20:3n6	0.51 ± 0.3	0.69 ± 0.29	0.22 ± 0.1	0.32 ± 0.14*
C20:3n3	0.22 ± 0.12	0.24 ± 0.09	0.03 ± 0.01*	0.04 ± 0.01*
C20:4n6	0.23 ± 0.16	0.38 ± 0.19	0.14 ± 0.09	0.21 ± 0.23
C20:5n3	0.04 ± 0.02	0.04 ± 0.03	0.06 ± 0.02	0.07 ± 0.03*
C22:0	0.05 ± 0.02	0.07 ± 0.04	0.02 ± 0.02	0.03 ± 0.02*
C22:1	0.05 ± 0.02	0.07 ± 0.03	0.10 ± 0.02*	0.11 ± 0.04
C22:4n6	0.01 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01*
C22:5n3	0.01 ± 0.01	0.02 ± 0.02	0.00 ± 0.00	0.01 ± 0.02
C22:6n3	0.10 ± 0.08	0.16 ± 0.15	0.02 ± 0.02	0.05 ± 0.06
C24:0	0.09 ± 0.06	0.15 ± 0.16	0.03 ± 0.02	0.04 ± 0.03
C24:1	0.01 ± 0.01	0.02 ± 0.02	0.03 ± 0.01	0.03 ± 0.02
∑ SAT	41.71 ± 2.64a	37.72 ± 3.07b	29.81 ± 1.28*	29.81 ± 2.16*
∑ MUFA	48.25 ± 2.1	50.45 ± 2.41	66.05 ± 0.84*	65.4 ± 1.61*
∑ PUFA	10.04 ± 3.02	11.83 ± 3.41	4.14 ± 0.86	4.79 ± 1.14
∑ n-3	1.43 ± 0.51	1.66 ± 0.59	0.4 ± 0.11*	0.48 ± 0.16*
∑ n-6	8.61 ± 2.61	10.17 ± 2.92	3.74 ± 0.8*	4.31 ± 1.04*
n-3/ n-6	0.17 ± 0.04	0.16 ± 0.04	0.11 ± 0.03*	0.11 ± 0.03*
U/S Ratio	1.41 ± 0.15a	1.67 ± 0.21b	2.36 ± 0.15*	2.37 ± 0.25*
UI	72.2 ± 6.58a	79.25 ± 7.74b	76.05 ± 2.43	77.16 ± 3.73

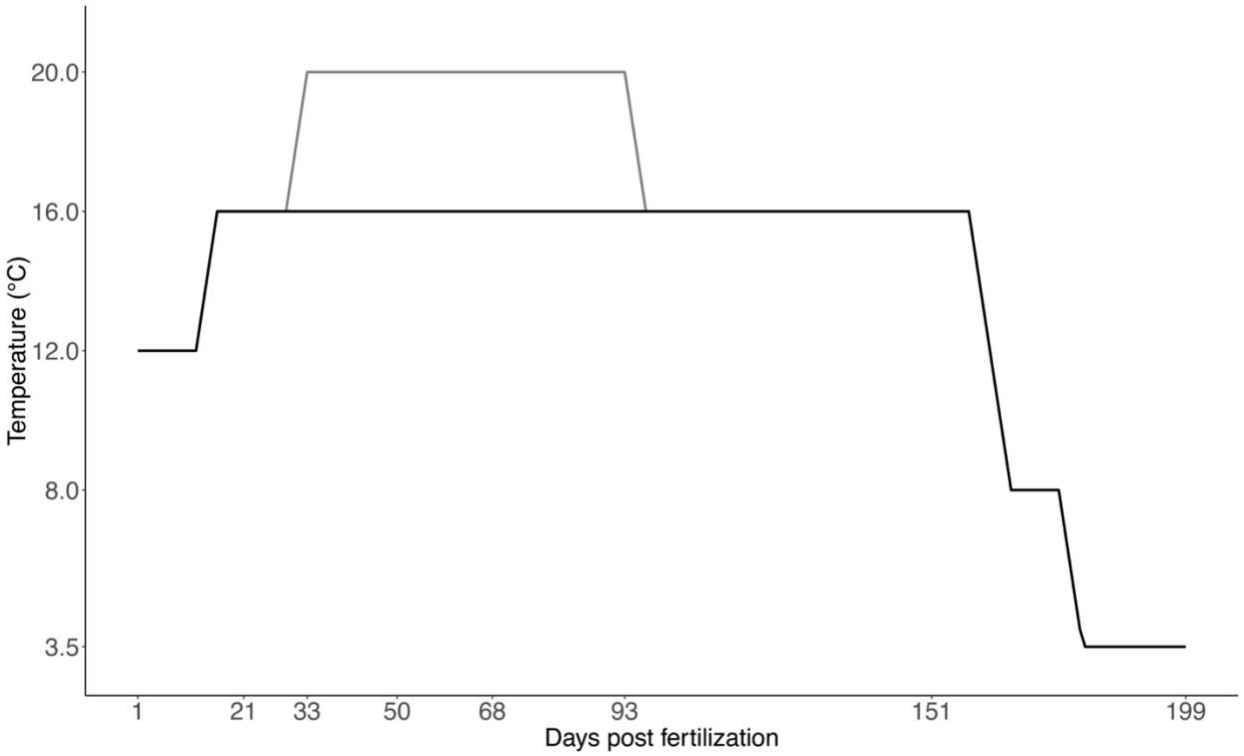

Figure 5.1. Temperature profile used in the present study. Black and grey lines represent 16°C and 20°C group, respectively. Following the temperature manipulation, all fish were raised in the temperature profile of 16°C until 158 days post fertilization at which a cold conditioning initiated.

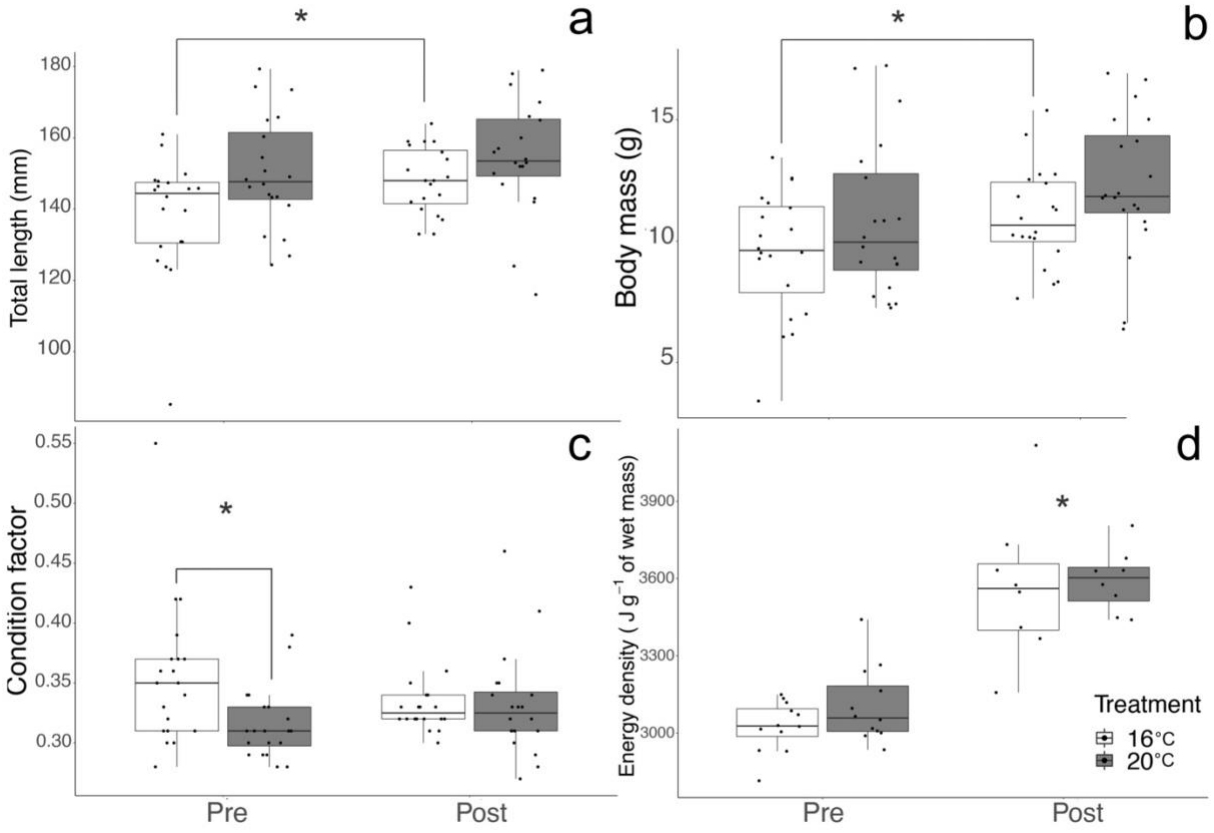

Figure 5.2. Changes in total length (a; n=20 per group), body mass (b; n=20 per group), condition factor (c; n=20 per group) and energy density (d; n=8-12 per group) of age-0 lake sturgeon *Acipenser fulvescens* experienced different thermal histories of either 16°C (open box) or 20°C (grey box). * indicate significant difference within temperature group or time points. Pre and Post denote pre and post cold conditioning event. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots represent individual data points.

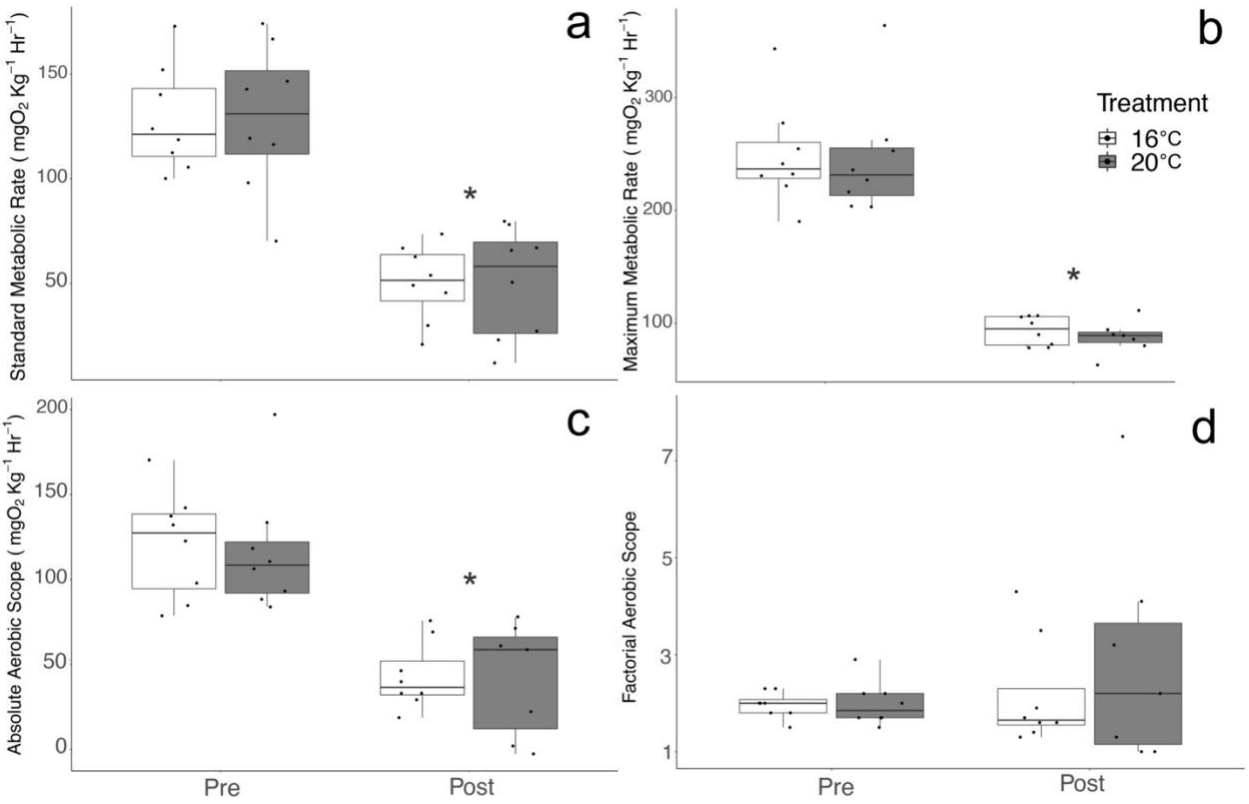

Figure 5.3. Changes in standard (a), maximum metabolic rate (b), absolute aerobic scope (c) and factorial aerobic scope (d) of age-0 lake sturgeon (*Acipenser fulvescens*) experienced different thermal histories of either 16°C (open box) or 20°C (grey box). * indicate significant difference within temperature groups. Pre and Post denote pre and post cold conditioning event. N=8. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots represent individual data points.

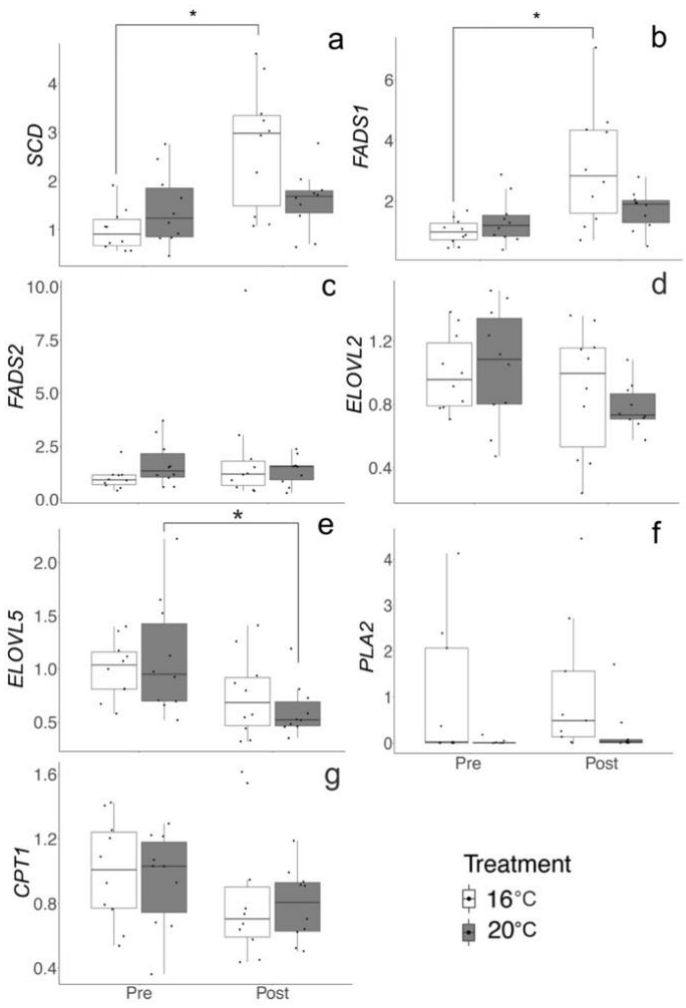

Figure 5.4. Changes in mRNA expression of age-0 lake sturgeon *Acipenser fulvescens* experienced different thermal histories of either 16°C (open box) or 20°C (grey box). *SCD*: stearoyl CoA desaturase. *FADS1*: fatty acid desaturase 1 (Δ5 desaturase). *FADS2*: fatty acid desaturase 2 (Δ6 desaturase). *ELOVL2*: elongation of very long chain fatty acid protein 2. *ELOVL5*: elongation of very long chain fatty acid protein 5. *PLA2*: phospholipase A2. *CPT1*: Carnitine palmitoyltransferase 1. Gene expression data were normalized to 16°C pre-cold conditioning. * indicate significant difference between time points. Pre and Post denote pre and post cold conditioning event. N=9-10. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots represent individual data points.

Chapters 4 and 5 demonstrated that depending on timing and duration, temperature during early life may have transient or prolonged effects on growth and metabolism within the first year of life. Chapter 6 was designed to investigate how diet (i.e. dietary fatty acids) at the onset of exogenous feeding could influence development of metabolic phenotypes in age-0 lake sturgeon.

6 Chapter 6: Long-term effects of EPA and DHA enriched diets on digestive enzyme activity, aerobic scope, growth and survival in age-0 Lake Sturgeon (*Acipenser fulvescens*)

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6.1 Abstract

Nutritional condition during early life could have long lasting effects on metabolism, growth trajectory and ultimately ecological fitness. We examined effects of *Artemia* nauplii enriched with eicosapentaenoic acid (EPA; C20:5n3) and docosahexaenoic acid (DHA; C22:6n3) on fatty acid profile, digestive enzyme activity, energy density, aerobic scope, growth and survival in age-0 Lake Sturgeon (*Acipenser fulvescens*). Four diets were used in the study: 1) control (absence of EPA and DHA): freshly hatched *Artemia* nauplii, 2) low DHA: *Artemia* enriched with *Chlorella vulgaris*, 3) high DHA: *Artemia* enriched with Red AlgaMac and 4) time-control: *Artemia* left for the time to match growth that would occur during the enrichment. At 20 days post hatch (dph), fish were fed one of four diets three times a day *ad libitum* for 29 days after which all fish were fed the same diet of bloodworm twice a day *ad libitum* until the end of the experiment, which included a simulated overwintering event for 45 days at 3°C. Fish were sampled after 24 days of feeding the aforementioned diets, as well as before and after the simulated overwintering event at 145 and 190 dph, respectively. Fish fed the high DHA diet did not change the measured parameters compared to fish fed the control diet after 24 days of feeding; however, before overwintering fish fed high DHA showed higher lipase activity ($p=0.01$) compared to the control group and significantly increased body mass when temperature decreased. Further, fish fed the high DHA diet showed a substantially lower mortality than fish fed the control diet following the diet transition from *Artemia* to bloodworm (44%; $p<0.05$). Our results suggest the importance of EPA and DHA during early life on growth and survival, which could be used to improve post-release survival rates in conservation aquaculture of Lake Sturgeon, a species at risk or endangered across its natural range.

6.2 Introduction

Environmental conditions at early life stages have a profound effect on phenotypic development, which strongly influences subsequent developmental trajectory and growth in fish (Álvarez and Metcalfe, 2005; Metcalfe and Monaghan, 2001; Mueller et al., 2019). Specifically, studies have shown that dietary conditions during a critical window could lead to nutritional programming (Hou and Fuiman, 2020), which may have long lasting effects on metabolism, growth trajectory and ultimately ecological fitness (Fuiman and Perez, 2015; Kemski et al., 2018; Vagner et al., 2007).

Omega-6 arachidonic acid (AA; C20:4n6) and omega-3 eicosapentaenoic acid (EPA; C20:5n3) and docosahexaenoic acid (DHA; C22:6n3) are long-chain polyunsaturated fatty acids (LC-PUFAs) that play crucial roles in ontogenesis, cellular structure, immune responses, gene regulation and homeoviscous adaptation in fish (Monroig et al., 2018; Tocher, 2010). These fatty acids are synthesized *in vivo* from either α-linolenic acid (ALA; C18:3n3) or linoleic acid (LA; C18:2n6) through a series of biochemical reactions (Park, 2018; Sprecher, 2000), but this conversion may not be sufficient due to limitations in enzymes responsible for desaturation and/or elongation (Gerster, 1998; Tocher and Ghioni, 1999). It is hypothesized that a majority of fish cannot synthesize ALA nor LA due to a lack of Δ 12 and 15 desaturases (Monroig et al., 2018); thus, these fatty acids must be obtained from the diet to meet the physiological needs of the organism as essential fatty acids (EFAs). Insufficient dietary intake of EFAs may result in developmental abnormality (Cahu et al., 2003; Roo et al., 2009; Salini et al., 2015), altered lipid metabolism (Ruyter et al., 2000), reduced resistance to infection (Bransden et al., 2003) and also decreased LC-PUFAs (AA, EPA and DHA) that are important for neural development (Twinings et al. 2021).

The importance of LC-PUFAs may be particularly evident in northern temperate aquatic species where age-0 fish may have a limited growing season and thus opportunity to acquire dietary fatty acids (Hurst, 2007; Post and Parkinson, 2001) for survival over winter (Ibarz et al., 2005; Rozsypal et al., 2014). Lake Sturgeon (*Acipenser fulvescens*) is a freshwater fish species endangered or at risk across its natural range in North America (Bruch et al., 2016). Fishery managers have been using conservation aquaculture to enhance populations with limited success mainly owing to low recruitment rates (Osborne et al., 2020). For instance, fall fingerlings and spring yearlings are released in Manitoba, Canada, but studies have suggested that age-0 stocked

fish showed lower recruitment rates than those stocked at age-1+ (McDougall et al., 2020, 2014). Despite low recruitment rates of age-0 fish, fall release is often required due to financial and logistical constraints in fish hatcheries (Brown and Day, 2002). One of the mechanisms for reduced survival rate of fall fingerlings could be overwintering where temperatures of ~0°C and month-long periods of starvation are anticipated (Hrenchuk et al., 2017). Thus, energy reserves and cellular adaptation to cold temperatures (i.e. homeoviscous adaptation) may influence the success of overwintering in age-0 Lake Sturgeon. Although it was suggested that some freshwater fish may synthesize LC-PUFAs *in vivo*, dietary fatty acids at the onset of exogenous feeding may have prolonged effects on growth and fatty acid metabolism (Kabaran and Besler, 2015), which may ultimately impact overwintering survival in age-0 fish. Thus, understanding how dietary fatty acids at the onset of exogenous feeding could influence digestive capacity for somatic growth (i.e. trypsin) and lipid reserves (i.e. lipase), aerobic metabolism and growth as well as metabolic performance during overwintering will inform current conservation aquaculture on possible ways to increase post-release survival rates of fall fingerlings.

Dietary enrichment is a useful tool to increase the post-release survival of hatchery-reared fish. In conservation fish hatcheries, many larval fish are reared using *Artemia* as a starting diet as it can be massively produced in a cost-effective manner (Conceição et al., 2010; Hamre et al., 2013). However, the nutritional composition of *Artemia* may not be adequate owing to low levels of EFAs such as ALA (Léger et al., 1987), which is also the precursor of EPA and DHA. This is further complicated by an unreliable composition of macronutrients as a result of harvest region and cultivation protocols (Bookhout and Costlow, 1970; Sorgeloos et al., 2001). A number of candidates have been suggested to replace *Artemia* (Klassen and Peake, 2007; Rasdi and Qin, 2016), but it has to be balanced with logistical and/or financial constraints at the fish hatchery. Therefore, dietary enrichment of *Artemia* nauplii with EPA and DHA may be useful to enhance dietary quality and improve growth performance. Importantly, diets enriched with omega-3 fatty acids (EPA and DHA) could significantly influence metabolic rates in fish (McKenzie, 2001), which could lead to altered metabolic capacity for routine activities such as locomotion, digestion and growth (Clark et al., 2013; Metcalfe et al., 2016).

In the present study, we examined how early dietary enrichment could influence metabolism and growth in age-0 Lake Sturgeon. Based on observations in age-0 Siberian Sturgeon (*Acipenser baerii*) (Luo et al. 2019), we used EPA and DHA enriched *Artemia* and

predicted that 1) fish fed high DHA would show enhanced digestive enzyme activity, growth rate and energy reserves prior to overwintering; and 2) fish fed high DHA would show higher survival rates than fish fed low DHA or without DHA.

6.3 Methods and Material

6.3.1 Diet preparation and manipulation

We used freshwater live algae (*Chlorella vulgaris*) or a commercial dried marine algae product (Red AlgaMac; Aquafauna Bio-Marine, Hawthorne, California) to differentiate the dietary DHA levels (see Table 6.1). We chose to use *Chlorella vulgaris* because it is one of the most common freshwater algae species that may be suitable for freshwater fish in terms of dietary fatty acids (Hamre et al. 2013). *Chlorella vulgaris* was cultured in a sterile reference medium (modified WC; Wright Chu medium) at room temperature as previously established (Guillard and Lorenzen, 1972; Huebner et al., 2009). Algal growth was assessed by measuring cell numbers everyday via hemocytometer on a light microscope. Once cell numbers indicated the end of logarithmic growth, *Chlorella vulgaris* was mixed with *Artemia* nauplii in a ratio of 6,000 to 6,500 cells·*Artemia*$^{-1}$ while salinity was maintained at 28 parts per thousands (Chakraborty et al., 2007; Rosowski, 1989).

There were four diets in our study: 1) control: freshly hatched *Artemia* nauplii following 24 hours of incubating cysts using conditions described in the manufacture's instruction (Brine Shrimp Direct, Odgen, Utah); 2) low DHA: *Artemia* nauplii enriched by *Chlorella vulgaris* with moderate aeration for 6 hours to allow *Artemia* to ingest algae as previously recommended (Ritar et al., 2004); 3) high DHA: *Artemia* nauplii enriched by Red AlgaMac following the manufacture's instruction and 4) time-control: *Artemia* grown for 6 hr without any enrichment to match growth that would occur during the enrichment. *Artemia* nauplii were cultivated at 30°C for 24 hrs and harvested three times a day (6 am, 12 pm and 6 pm). Those harvested at 6 am and 12pm were enriched or grown for 6 hours whereas those harvested at 6pm were kept at 4°C until enrichment was performed at 12 am.

It is important to note that we used 6 hr of enrichment because a preliminary experiment revealed that 1) 24 hours of enrichment may lead to growth of *Artemia* nauplii making it challenging for larvae to capture and ingest *Artemia* at the onset of exogenous feeding leading to increased mortality rate in age-0 Lake Sturgeon; 2) we found higher levels of DHA in *Artemia*

enriched with Red AlgaMac after a 6 hrs compared to those enriched for 24 hrs (Table 6.6.; Figueiredo et al. 2009); and 3) there was no significant difference in size between non-enriched *Artemia* nauplii and 6 hr enriched *Artemia* nauplii.

6.3.2 Animal husbandry and environmental manipulation

Gamete collection and animal rearing were performed as previously outlined (Bugg et al. 2020). At 7 days post hatch (dph), 300 larvae per each treatment were situated in three 3L flow-through aquaria (100 larvae per tank) at 16°C on a 12:12h light:dark photoperiod. Each tank was supplied with dechlorinated Winnipeg tap water with moderate aeration. At 17 days post hatch (dph), fish were fed control diet for 3 days, and then one of the four experimental diets *ad libitum* three times a day for the following 29 days (see Figure 6.1). At 49 dph, all fish were provided ground bloodworm (Hikari, California, USA) at a ratio of 1:9 and gradually transitioned (increasing bloodworm content by 10% every two days) to a full bloodworm diet over the course of 18 days. It is important to note that during the diet transition fish were continuously fed *Artemia* according to their treatments, and at 67 dph all fish were fed the same diet of bloodworm for the remainder of the experiment. At 75 dph, 38 fish per tank were randomly captured and transferred to one of two 9L flow-through aquaria (57 fish per tank). At 127 dph, a simulated overwintering event was performed by decreasing temperature of tanks by a rate of 1°C per day. At 135 dph, temperature reached 8°C and 48 fish per treatment were haphazardly captured and transferred to one of four 227L flow-through tanks with decreasing temperature from 8°C to 3°C at a rate of 1°C per day. We chose to transfer fish at 8°C because this is the stocking temperature of age-0 Lake Sturgeon in Manitoba. It is worth noting that typical overwintering temperature in Manitoba is near 0°C (Hrenchuk et al., 2017), but mechanical constraints did not allow us to reduce the temperature below 3°C. At 145 dph, fish were deprived of food at 3°C for 45 days to simulate an overwintering event. Any mortality was removed and recorded twice a day.

6.3.3 Sampling

For fatty acid composition and digestive enzyme activity, there were three sampling points in this study: (1) after the 24 days of experimental diet (44 dph), (2) before overwintering

(127 dph) and (3) at the end of overwintering (190 dph). Prior to sampling, fish were fasted overnight. Total length and body mass were measured every month to monitor patterns of growth (n=8). At each sampling point, fish were haphazardly captured from tanks and sacrificed with an overdose of anesthetic (tricane methanesulfonate; MS-222, Syndel Laboratory, British Columbia, Canada), and total length and body mass were measured using a conventional stainless-steel ruler (Westcott; New York, USA) and electronic scale (PI-214, Denver Instrument; New York, USA) to the nearest mm and mg, respectively. Fulton's condition factor (K) was calculated by the following equation: $K = g \times 100 / cm^3$. It is important to note that at 44, 127 and 190 dph, growth data was taken from fish used for fatty acid composition, digestive enzyme and energy density (see sections 6.3.4 to 6.3.6; n=24).

6.3.4 Fatty acid analysis

The fatty acid profiles of Red AlgaMac (n=6), enriched *Artemia* (n=6 per group) and fish (n=8 per treatment) were assessed using direct methylation and saponification as previously outlined with some modifications (Kang and Wang, 2005). It is worth noting that due to technical issues, we could not reliably assess one sample for Red AlgaMac and thus this was removed from further analysis. In brief, enriched *Artemia* were taken from three different batches (three different days), rinsed with dechlorinated tap water and gently dried on a paper towel, and stored at -80°C until analysis of fatty acids was performed. With regards to the fatty acid profile of fish at 44 dph, we chose to measure the truncated body without organs due to a smaller body size whereas at 127 and 190 dph hepatic fatty acid profile was assessed for before and after the overwintering event. Samples were homogenized with a 50 Sonic Dismembrator (Fisher Scientific, Massachusetts, USA) until fully emulsified. 170 µL of homogenate was added to an 8 mL glass tube filled with 2 mL hexane and 1.5 mL of 14% boron trifluoride in methanol. Once tubes were capped and lined with Teflon tape, samples were vortexed and heated at 110°C in a sand bath for 1 hr (Suh et al., 2009). Samples were allowed to cool down for at least 20 minutes, and the hexane phase was reconstituted by adding 1 mL of H_2O and centrifuging at 13,000 rpm. The hexane phase was then transferred to gas chromatography vials, and fatty acid methyl esters were analyzed by gas chromatography (Vista 6010 GLC and Vista 402 data system, Varian Instruments, Mississauga, Ontario, Canada) with a microcapillary column (15 m × 0.1 mm inner diameter; BPX70; SGE analytical science, North Carolina, USA). Analysis

conditions were set as previously established (Feltham et al., 2019). Then, peaks were identified by using standard fatty acid methyl esters mixture 461 (Nucheck Prep Inc., Minnesota, USA). Fatty acids were calculated and presented as mol %.

6.3.5 Digestive enzyme assays

We measured lipase and trypsin activities in the gastrointestinal tract (GIT) to assess effects of enriched diets on digestion and assimilation capacity in fish (Kolkovski, 2001). At each sampling point, fish (n=8 per treatment) were dissected to reveal the GIT, which was removed in whole, from esophagus to anus due to small size of tissue. Any remaining food was removed by gently pushing the tissue and the empty tract was then stored at -80°C until analysis. GITs were homogenized in 20 µL (44 dph), 50 µL (127 dph) and 100 µL (190 dph) of buffer (100 mM Tris-HCl, 0.1 mM EDTA, 0.1% Triton X-100, pH 7.8) on ice using a motorized homogenizer. Homogenates were then centrifuged at 13,000 *g* for 20 min at 4°C. Protein content was measured by the Bradford assay as per the manufacturer's instructions (Bio-Rad Laboratories, California, USA). In brief, standard curves were generated with readings at A595 against known bovine serum albumin concentrations at 0.125, 0.25, 0.5, 0.75 and 1 $mg \cdot mL^{-1}$. Then, readings of samples were linearly regressed to calculate the concentration of protein in each sample. Lipase activity was measured using a previous protocol using the end point method (Iijima et al., 1998). Briefly, 5 µL of homogenate was incubated at room temperature for 15 min in 71.5 µL of sodium deoxycholate and 2.5 µL of 10 mM ethanol. Then 21 µL of substrate at 5.2mM (0.55 mM *p*-nitrophenyl myristate) was added to the homogenate mixture and incubated for another 40 min at room temperature. Samples were read at A405 nm against a *p*-nitrophenol standard curve with activity expressed as nmol *p*-nitrophenol released·mg $protein^{-1} \cdot min^{-1}$. Trypsin activity was measured using a previously established method (Preiser et al., 1975). Homogenate (28 µL) was incubated with 0.125U/sample recombinant enterokinase for 15 min at room temperature to activate trypsinogen. Substrate (200 µL of 2 mM Na-benzoyl-l-arginine-p-nitroanilide hydrochloride; BAPNA) was then added to the homogenate and samples were incubated at room temperature for 40 min. Samples were centrifuged at 13,000 *g* for 2 min and 200 µL was plated prior to reading at A405 nm against a *p*-nitroaniline standard curve. Trypsin activity is expressed as mmol *p*-nitroaniline released·mg $protein^{-1} \cdot min^{-1}$. All assays were run in

duplicate on clear, flat-bottom 96-well microplates using a spectrophotometer (BioTek Powerwave; BioTek, Vermont, USA) with Gen5 software (v. 1.11).

Preliminary experiments were conducted to determine the appropriate working concentration of homogenate. We noted linear increases in product with more homogenate as well as over time. The amount of homogenate and the time required to obtain a reliable and consistent measurement of activity (i.e. colour development) was selected as the final endpoint for each of the assays. Wells containing no substrate (negative control) were run for each assay and demonstrated negligible colour development. All assays were carried out in duplicate using clear, flat-bottom 96-well microplates and a microplate spectrophotometer (BioTek Powerwave; BioTek, VT, USA).

6.3.6 Whole body metabolic rate

At 117-122 (pre-overwintering) and 188-193 (post-overwintering) dph, we measured whole body oxygen consumption rate ($\dot{M}O_2$) as a proxy of metabolic rate using intermittent flow respirometry. The respirometry setup consisted of metabolic chambers (chamber volume of 42.32 ± 1.06 mL; tubing volume of 3.07 ± 0.11 mL; mean ± S.D) and Witrox 4 Oxygen meter to which oxygen probes were connected. Oxygen sensor spots were placed on the surface of borosilicate chambers to read oxygen concentration every second (PreSens, Regensburg, Germany). Oxygen probes were calibrated at 0 and 100% with 2% sodium sulfite solution and fully saturated water. Fully saturated water was ensured by reading dissolved oxygen at 100% followed by vigorous aeration of water for at least 15 minutes. Tubing was non oxygen permeable Tygon. At each sampling point, fish (n=8) were haphazardly captured from tanks, and total length and body mass were measured to the nearest mm and mg, respectively. Then, fish were placed in the metabolic chambers. Flow rate was set to sufficiently replenish water between measurement cycles but not cause physical disturbance for fish. Fish were left in the chambers for 24 hours to estimate standard metabolic rate (SMR). Following the measurement of SMR, fish were removed from chambers and chased by gentle prodding in the tail for 15 minutes to elicit maximum metabolic rate (MMR). After the chasing protocol, fish were returned to the chambers, and $\dot{M}O_2$ was assessed for three measurement cycles. Before and after each

experiment, $\dot{M}O_2$ was measured for 15 minutes without fish to assess background respiration, which was used to interpolate over time and correct all $\dot{M}O_2$ data (Rodgers et al., 2016).

Only $\dot{M}O_2$ slopes with $r^2 \geqq 0.9$ were used for data analysis. SMR was calculated with 20th quantile method from 18 hours of $\dot{M}O_2$ slopes excluding the first 6 hours of $\dot{M}O_2$ slopes (Chabot et al., 2016). MMR was chosen as the highest $\dot{M}O_2$ following the chase protocol (Norin and Clark, 2016). Absolute aerobic scope (AAS) was calculated from subtracting SMR from MMR whereas factorial aerobic scope (FAS) was calculated from dividing MMR by SMR.

6.3.7 Whole body energy density

At 44, 83, 127 and 190 dph, fish (n=8 per treatment) were haphazardly captured from tanks and sacrificed with an overdose of MS-222. Then, carcasses were dried at 60°C or constant weight was recorded. Dried carcasses were used to calculated dry to wet mass ratio (DWR), which was used to estimate whole body energy density (ED) using the previously established model (Yoon et al., 2019): $ED = 23{,}965.7 \times DWR - 433.1$; $p < 0.001$, $r^2 = 0.96$.

6.3.8 Ethical statement

All experiments were approved by the University of Manitoba Animal Care Committee under the requirements established by the Canadian Council for Animal Care under protocol number F015–007.

6.3.9 Statistical Analysis

Our initial analysis did not indicate strong evidence for significant effects of rearing tanks on the data and thus were removed from the analysis ($p>0.1$). In terms of dietary fatty acid, we used a Student's T-test to compare fatty acid profiles between two enrichments and one-way ANOVA for fatty acid profiles of four experimental diets. Because we used different tissues for measuring fatty acid in fish across time points (see section 6.3.4.), we used one-way ANOVA for body fatty acid profiles at 45 dph and two-way ANOVA to examine effects of diet and time on fatty acid profiles in the liver tissue before and after the overwintering event. We also performed two-way ANOVA to examine effects of diet and time on digestive enzyme activity, energy density, metabolic rates, and growth. Assumptions of normality and homoskedasticity were

visually assessed as previously outlined (Zuur et al., 2010). Specifically, we used *ggqqplot* from the package 'ggpubr' to plot dependent variables and residuals of ANOVA against the normal quantile-quantile to meet the assumptions for parametric test. Data were transformed by log, cube root or Tukey Power transformation from the package 'rcompanion' where necessary (Mangiafico, 2021). We analyzed cumulative mortality by Cox proportional hazard regression using the package 'survival' (Therneau, 2020). Specifically, we analyzed cumulative mortality before and after 67 dph when provision of enriched artemia ceased. When the analysis indicated significance, a *post hoc* log-rank test was conducted to examine the difference between individual groups with p-values adjusted by Bonferroni correction. Independence of proportional hazards for each variable to time was tested using the 'cox.zph' function in the package and no significance was observed ($p > 0.1$). All data analysis was conducted in R 4.1.0 (R Core Team, 2021).

6.4 Results

6.4.1 Enrichment and dietary fatty acid profile

Our results indicated that fatty acid profile was significantly different between enrichments of *Chlorella vulgaris* and Red AlgaMac (Table 6.1). Specifically, total saturated fatty acids (SATs) were higher in Red AlgaMac than *Chlorella vulgaris* (55.8 ± 1.86% vs 24.55 ± 2.38%; $p<0.001$). Conversely, total monounsaturated fatty acids (MUFAs) were substantially lower in Red AlgaMac than *Chlorella vulgaris* (3.71 ± 3.27 vs 36.16 ± 1.06 %; $p<0.001$) whereas there was no difference in polyunsaturated fatty acids (PUFAs) between enrichments ($p>0.1$). DHA (C22:6n3) was significantly higher in Red AlgaMac compared to *Chlorella vulgaris* (32.44 ± 1.51 vs 0.22 ± 0.29%; $p<0.001$), which agrees with the product description from the manufacturer.

Our dietary fatty acid profile varied by enrichment protocols (Table 6.1). Specifically, *Artemia* enriched with *Chlorella vulgaris* (low DHA) and Red AlgaMac (high DHA) had lower total SAT than the control (23.68 ± 2.57 and 28.26 ± 2.5 vs 48.38 ± 5.36 %; $p<0.001$) and almost three fold higher total MUFAs than the control diet. Total PUFAs did not differ among diets, but low DHA and high DHA diets showed higher total omega-3 and 6 fatty acids than the control. We found neither EPA nor DHA in the control diet. Also, *Artemia* enriched with Red AlgaMac showed a significantly higher DHA than the other diets ($p<0.001$, Table 6.1).

6.4.2 Body fatty acid profile

At 44 dph, fish fed high DHA diet had higher SAT (38.17 ± 4.56% vs 32.14 ± 3.53 %; $p<0.05$, Table 2) but lower MUFA (26.71 ± 2.56 % vs 32.56 ± 2.61%; $p<0.001$, Table 6.2) than fish fed the control diet. While there was no difference in total PUFAs among diet groups ($p>0.1$), fish fed high DHA diet showed significantly higher DHA than the other diet groups ($p<0.001$).

6.4.3 Hepatic fatty acid profile by overwintering

Overall, our results indicated that total SAT, MUFA, PUFA, omega 3 and 6 fatty acids did not significantly change within treatments during the overwintering event ($p>0.1$, Table 6.3), but there were changes in individual fatty acids within diet groups after the overwintering event. Specifically, we saw a trend of decreasing SATs of C14:0, C17:0 and C18:0, except increasing C16:0, but increases in MUFAs of C18:1n7c, C20:1, C22:1 and C24:1 across treatment groups. After winter we saw decreases in C18:2n6, C18:3n3, C20:5n3 with an increasing trend in major PUFA of omega-6 and omega-3 fatty acids, C20:4n6 and C22:6n3, respectively, across all treatments. However, statistical significance of changes in C20:4n6, C20:5n3 and C22:6n3 was only found in fish fed the low DHA diet ($p<0.001$; Table 6.3).

6.4.4 Digestive enzyme activity

Our results indicated that lipase activity was significantly influenced by dietary treatment and time ($p \leqq 0.01$; Table 6.4). At 44 dph, there was no difference in lipase activity among diet groups. However, fish fed high DHA showed a significantly higher lipase activity than the control group at 127 dph ($p<0.05$; Figure 6.2a). At the end of overwintering, lipase activity of all groups decreased but statistical significance was only found in fish fed low DHA and high DHA diets ($p<0.05$; Figure 6.2a). Trypsin activity was only significantly influenced by time ($p<0.001$, Table 6.2). Specifically, there was no difference in the activity between diet groups at both 44 and 127 dph, but trypsin activity of all groups significantly decreased at the end of the overwintering event ($p<0.05$; Figure 6.2b)

6.4.5 Whole body metabolic rate

Our analysis indicated that SMR, MMR and AAS were only significantly influenced by time ($p<0.001$) where these significantly decreased at the end of overwintering event ($p<0.05$; Figure 6.3). Conversely, FAS did not differ by diet treatments or time ($p>0.1$).

6.4.6 Growth

Overall total length and body mass increased until the initiation of the overwintering event at 127 dph after which total length did not change while body mass decreased. Our two-way ANOVA results indicated that total length was significantly influenced by diet and time ($p \leqq 0.001$; Table 6.4) but not their interaction ($p>0.1$; Table 6.4). However, our *post hoc* analysis indicated that total length was only influenced by time. Specifically, there was no difference in total length at any sampling points, but total length increased within treatments from 44 to 83 dph (Figure 6.4a). Although total length of all groups increased until 127 dph, significant increases were only found in the high DHA group between 83 to 112 dph ($p<0.05$, Figure 6.4a) and time-control group between 112 and 127 dph ($p<0.01$, Figure 6.4a) with no further change until the end of experiment ($p>0.05$; Figure 6.4a).

Body mass was significantly influenced by diet and time ($p<0.001$; Table 6.4) but not their interaction ($p>0.1$; Table 6.4). Body mass was significantly different among groups at 44 dph where control group was significantly heavier than low DHA and time-control groups ($p<0.01$, Figure 6.4b) but not than high DHA ($p>0.1$). Body mass of all groups increased until 127 dph. Body mass of control, low DHA and time-control groups did not significantly change until the end of the experiment; however, the high DHA group showed a significant increase in body mass between 127 to 142 dph when temperature decreased from 16 to 3°C ($p<0.05$, Figure 6.4b).

K was significantly influenced by time and interaction between diet and time ($p<0.001$; Table 6.4). Specifically, K of control and time-control groups fluctuated until 142 dph after which K significantly decreased in all groups ($p \leqq 0.01$; Figure 6.4c).

6.4.7 Whole body energy density

Overall, ED did not differ by dietary treatments, but increased until the onset of overwintering and declined afterwards. Our results of two-way ANOVA indicated that ED was

only influenced by time ($p<0.001$; Table 6.4). Specifically, fish fed low DHA showed a significant decrease in ED during the overwintering ($p<0.05$) whereas the other groups did not (Figure 6.5).

6.4.8 Cumulative mortality

Our analysis indicated that cumulative mortality did not differ by diets from 20 to 67 dph ($p>0.1$; Table 6.5). However, fish fed the high DHA diet had significantly decreased cumulative mortality with a hazard ratio of 0.4393 (0.2336 - 0.8262; 95% CI) compared to the control diet between 67 to 190 dph ($p=0.01$; Table 6.5), and our *post hoc* log-rank test supported that cumulative mortality was significantly different between control and fish fed high DHA diet ($p<0.05$; Figure 6.6).

6.5 Discussion

Our research demonstrated that DHA enrichment at the onset of exogenous feeding may have effects on growth and survival prior to overwintering in age-0 Lake Sturgeon. The fatty acid profiles of the four diets demonstrated successful enrichment of EPA and DHA. In the present study, freshly hatched Artemia nauplii (control diet) were deficient in omega 3 PUFAs such as EPA and DHA, which agrees with a previous study (Watanabe et al., 1983). We also noted the absence of DHA in Artemia nauplii grown without enrichment for 6 hrs (the time-control diet), which is in agreement with what has been reported in the literature (Conceição et al., 2010; Léger et al., 1987; Sorgeloos et al., 2001). Deficient EPA and DHA in Artemia nauplii could be less than ideal as a food source for fish larvae (Hamre et al., 2013), but could be in part solved by nutritional enrichment (Sorgeloos et al., 2001). In Artemia, it is known that the mouth of newly hatched nauplius (Instar I) usually is not fully opened until approximately two hours post hatch for them to be able to start feeding (Instar II). Thus, Artemia enrichment protocols should consider incubation conditions (i.e. temperature, salinity and pH) that influence growth of Artemia as well as morphological constraints of fish (i.e. gape size). In the present study, we found that the DHA content of Artemia enriched with AlgaMac was lower than expected (1.6%). A previous study demonstrated that the retention of DHA in Artemia during enrichment was strongly influenced by temperature (Figueiredo et al. 2009). In the present study, we performed Artemia enrichment at 20 °C (i.e. room temperature), which may have resulted in lower DHA in

the Artemia enriched with Red AlgaMac than what could be expected at high temperatures (Figueiredo et al. 2009).

Our results of high DHA in the body of fish fed high DHA diet at 44 dph indicate that DHA was incorporated by the fish. While high DHA diet had 7 or 10 times higher DHA than low DHA or time-control diets, this trend was not found in the body fatty acid profile. This may be explained by a number of factors. First, these data may indicate that dietary retention of DHA in Lake Sturgeon is limited; alternatively at the life stage investigated there may be a greater proportion of other fatty acids used by the fish during development; finally the reduced amounts of whole body DHA may be due to the type of tissue (muscle and skin) analysed. It is important to note that we chose to use the body without organs (skeletal muscle and skin) at 44 dph due to the small size of liver. Fatty acids in muscle have been shown to reflect dietary fatty acids, so our results of low DHA levels may be related to DHA utilization by the fish (Enjoji et al., 2018).

Interestingly, we saw the presence of EPA and DHA in the control group fish at 44 dph, which had been fed *Artemia* deprived of EPA and DHA. It remains unclear how the control group fish have EPA and DHA, but two hypotheses may explain this. First, the results may be due to the pre-existing fatty acids derived from endogenous yolk (Jobling, 2003; Kamler, 2008). Secondly, like other freshwater fish, age-0 Lake Sturgeon may synthesize EPA and DHA *in vivo*, resulting in gradual increases in these fatty acids during development. As EPA and DHA are essential for neural development in vertebrates, it is hypothesized that EPA and DHA must be *de novo* synthesized when EPA and DHA are not available from the diet (Twining et al. 2021). Studies have suggested that freshwater fish may have the physiological and molecular capacity to synthesize LC-PUFAs because dietary PUFAs are not readily available from algae or zooplankton compared to the marine environment (Castro et al., 2012; Monroig and Kabeya, 2018; Tocher and Ghioni, 1999). For example, in freshwater salmonids such as brown trout (*Salmo trutta*) and rainbow trout (*Onchorynchus mykiss*), a similar level of LC-PUFAs was documented between liver and muscle tissue suggesting that liver tissue may utilize alpha linoleic acid and linoleic acid to endogenously synthesize and allocate LC-PUFAs to other tissues (Ebm et al. 2021). In line with this finding, a recent study in our lab demonstrated that expression of *FADS1* (a gene encoding Δ 5 desaturase) increased with decreasing temperature in age-0 Lake Sturgeon, suggesting the presence of the capacity to synthesize AA and EPA in Lake Sturgeon similar to other freshwater fish species (Yoon et al. 2022).

We also found that during overwintering total SATs, total MUFAs and PUFAs in the liver tissue did not significantly change, but there were changes in some individual fatty acids. This could be likely due to fasting conditions during overwintering. A previous study using a similar age group showed that age-0 Lake Sturgeon significantly increased both MUFAs and PUFAs in phospholipids and triglycerides when temperature decreased from 16°C to 1°C (Yoon et al., 2021). However, a fasting condition in the present study may have triggered lipolysis (Cabodevilla et al., 2013), leading to no difference in fatty acids of phospholipids or triglycerides before and after overwintering (see below discussion of lipase activity).

Lipase activity in the GIT was higher in fish fed high DHA than in the control group at 127 dph, and we also saw a similar trend in trypsin activity. These results are interesting because at 127 dph fish were feeding on the same diet of bloodworm rather than distinct diets. It is well recognized that early nutritional environment could result in reversible or irreversible effects on nutrient metabolism (Gluckman and Hanson, 2004; Hales and Barker, 1992). It is hypothesized that critical window for gut development in fish is at the onset of exogenous feeding (Hou and Fuiman, 2020; Pittman et al., 2013). For example, in rainbow trout (*Oncorhynchus mykiss*), when fish were fed a high-glucidic diet during early life and later challenged with a high carbohydrate diet at the juvenile stage, fish showed upregulated gene expression of pancreatic α-amylase and intestinal maltase (Geurden et al., 2007). Thus, our results may suggest that fish fed the high DHA diet may have had enhanced nutrient uptake in the intestine, which also in part explains how fish fed the high DHA diet showed significant increases in body mass when temperature was decreasing between 127 to 142 dph (see below discussion of growth). It remains unknown why this trend was not evident in the earlier sampling timepoint of 44 dph, but it could be due to the smaller sample size and tissue size of GIT leading to a larger amount of variation in the data or due to the elimination of food from the digestive tract that may have removed a part of the digestive enzymes associated with alimentary chyme.

Following overwintering, we observed substantially reduced trypsin, but a marginal activity in lipase. In the present study, we measured trypsin, which is a pancreatic enzyme that is directly related to digestion of proteins (Cara et al., 2007); however, lipase could be involved in dietary uptake of lipids and lipolysis for β-oxidation (Babaei et al., 2017; Hayes and Volkoff, 2014; Weinrauch et al., 2019). It is well known that food availability could strongly influence activity of digestive enzymes in fish (Furne and Sanz, 2017). Thus, our results may indicate that

reduced trypsin activity could be due to the prolonged deprivation of food intake whereas marginal activity of lipase represents an increased lipolysis activity in the lipid reserves of the GIT such as mesenteric tissue (Furné et al., 2008; Sheridan, 1994), which in part may explain the lack of changes in PUFAs after the overwintering event (see above).

We found no dietary effects on metabolic rates in age-0 Lake Sturgeon. Previous studies have shown dietary enrichment of omega-3 fatty acids significantly decreased both SMR and routine metabolic rate (RMR) in European Eel (*Anguilla Anguilla*) and Adriatic Sturgeon (*Acipenser naccarii*) as well as RMR in Dover Sole (*Solea solea*) (McKenzie, 2001; McKenzie et al., 2008). It could be due to a ratio between DHA and EPA (DHA/EPA) in the diets as DHA/EPA was 0.6 ± 0.1 (mean ± S.E.) in McKenzie et al. (2008) whereas 0.84 ± 0.01 (mean ± S.E.) was in the present study. The discrepancy could be most likely because fish were fed the same diet of bloodworm following the diet manipulation. The plastic nature of SMR, MMR and AAS as physiological traits results in a rapid modulation dependent on environmental conditions such as temperature and diet (Chabot et al., 2016; Clark et al., 2013; Norin and Clark, 2016). Thus, 60 days of the same diet likely have negated any effects on whole body metabolic rates that would have manifested during earlier in life. Also, it is important to note that species used in the studies above were diadromous species whereas Lake Sturgeon is a freshwater species. As dietary requirements of fatty acids are known to be different between freshwater and marine fishes (Tocher, 2010), it is hypothesized that metabolic effects of omega-3 fatty acids on aerobic metabolism in fish may differ as well.

We found that omega-3 fatty acids, especially DHA enrichment did not immediately improve growth rate in age-0 Lake Sturgeon. Although the interpretation of results is limited owing to small and variable sample sizes across time points, it may be possible that the prey size in the control diet gave rise to improved growth at 44 dph despite the fact that we did not find statistically significant difference in size between the control and enriched diets ($p>0.1$). Also, it is possible that Artemia enrichment may have altered dietary proximal contents (i.e. protein and lipid) and influenced growth at earlier life stages. The effects of dietary omega-3 fatty acids on growth in fish have been reported to be equivocal. For example, in Atlantic sturgeon (Acipenser oxyrhyncus), Artemia enriched with omega-3 fatty acids resulted in significantly improved growth rate (Kolman et al., 2018) but not in larval Senegalese sole (Solea senegalensis) (Morais et al., 2004). The heterogeneity of these results in the literature may reflect differing fatty acid

requirements between marine and freshwater species or across developmental stages (Cerezuela et al., 2012; Kestemont et al., 2007; Tocher, 2010). Despite no remarkable difference in growth at early stages, fish fed a high DHA diet showed a significant increase in body mass when environmental temperature decreased from 16 °C to 3 °C (127 to 142 dph). It is well known that the digestive system is regulated by both the nervous and endocrine system. As DHA is essential in the development of the nervous system (e.g. cell membrane and myelin formation), dietary intake of DHA may have promoted development of the autonomous and enteric nervous system that may influence the function of the digestive system at early life stages (Heuckeroth and Schäfer, 2016).

We observed that mortality rate increased with dietary transition progressing and soared two weeks after the diet transition was completed. Our result agrees with a previous study in age-0 Lake Sturgeon showing increasing mortality rate after diet transition was completed in a hatchery setting (Yoon et al., 2020). Diet transition involves changes in quantity and/or quality of diet that will strongly influence capture and digestion of prey for fish, which is particularly challenging in age-0 fish leading to increased mortality rate (Sánchez-Hernández et al., 2019). In the present study, we also observed that after the diet transition, fish fed the high DHA diet showed a significantly lower cumulative mortality by 44% than those fed the control diet. Studies have shown that dietary supplementation of DHA increased survival rate in milkfish and red porgy during early life (Gapasin and Duray, 2001; Watanabe et al., 2016). The potential mechanism behind how dietary DHA could reduce mortality rate in the present study needs to be determined, but it is possible that higher dietary intake of DHA during early life may have facilitated gut development, leading to reduced mortality rate afterwards (Heuckeroth and Schäfer, 2016).

6.6 Conclusion

The present study demonstrated that high DHA *Artemia* did not show immediate effects but later showed increased digestive enzyme activities of lipase and trypsin. However, we found a prolonged effect of dietary DHA where fish fed high DHA *Artemia* significantly increased body mass by 37% while fish fed other diets did not when environmental temperature decreased. Also, feeding high DHA *Artemia* reduced mortality by 44% compared to the fish fed control *Artemia* after the diet transition from *Artemia* to bloodworm. Our research suggests a potential role of dietary DHA on growth and survival in age-0 Lake Sturgeon, which has significant

implications for development of rearing protocols in conservation aquaculture of Lake Sturgeon, an endangered species across its natural range.

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6.8 References

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Table 6.1.Fatty acid profile of experimental diets. Red AlgaMac: commercial product of dry-frozen algae. Diets were enriched by incubating Control with one of enrichments for 6 hrs. Control: Freshly hatched artemia. Low DHA: Artemia enriched with Chlorella vulgaris. High DHA: Artemia enriched with Red AlgaMac. Time-control: just incubation for 6 hrs to match the growth that could occur during the enrichment. U/S ratio: unsaturated to saturated fatty acid ratio, Data are expressed in mol % (mean ± S.D.). n.d.: not detected. * represents significant difference between enrichments (T-test). Letters indicate significant difference between enriched diets (One-way ANOVA; $p<0.05$).

	Source of Enrichment		Diets			
	Chlorella vulgaris	Red AlgaMac	Control	Low DHA	High DHA	Time-control
C14:0	1.07 ± 0.26	14.11 ± 2.13*	1.81 ± 0.56^{a}	1.07 ± 0.13^{b}	2.35 ± 0.39^{a}	0.86 ± 0.15^{b}
C14:1	1.22 ± 0.27	0.13 ± 0.04*	0.15 ± 0.17^{a}	1.25 ± 0.10^{b}	1.24 ± 0.22^{b}	0.86 ± 0.13^{c}
C15:0	0.22 ± 0.04	0.5 ± 0.05*	0.67 ± 0.12^{a}	0.21 ± 0.02^{b}	0.24 ± 0.03^{b}	0.20 ± 0.03^{b}
C16:0	14.12 ± 0.46	28.47 ± 1.70*	39.8 ± 4.16^{a}	14.47 ± 0.76^{b}	17.12 ± 1.82^{c}	14.58 ± 0.51^{b}
C16:1t	0.82 ± 0.02	0.01 ± 0.02*	2.21 ± 1.3	0.82 ± 0.05	0.73 ± 0.08	0.66 ± 0.07
C16:1	3.40 ± 0.14	0.33 ± 0.06*	0.91 ± 0.26^{a}	3.45 ± 0.13^{b}	3.20 ± 0.14^{bc}	2.77 ± 0.24^{c}
C17:0	1.21 ± 0.43	0.28 ± 0.19*	1.40 ± 0.50	1.37 ± 0.13	1.65 ± 0.75	1.14 ± 0.18
C18:0	7.67 ± 0.63	1.27 ± 0.13*	4.13 ± 0.48^{a}	6.33 ± 3.14^{a}	6.36 ± 3.15^{a}	10.54 ± 1.08^{b}
C18:1	21.67 ± 0.65	2.84 ± 0.33*	9.44 ± 1.10^{a}	21.85 ± 1.3^{b}	20.54 ± 1.56^{b}	21.27 ± 0.54^{b}
C18:1n7c	9.04 ± 0.61	0.36 ± 0.13*	0.06 ± 0.10^{a}	9.25 ± 0.75^{b}	8.93 ± 1.44^{b}	10.69 ± 1.28^{b}
C18:2n6	6.38 ± 0.05	4.02 ± 0.49*	18.21 ± 2.73^{a}	6.57 ± 0.23^{b}	5.96 ± 0.35^{bc}	5.64 ± 0.34^{c}
C18:3n6	0.89 ± 0.06	0.29 ± 0.03*	n.d.	0.93 ± 0.08	1.64 ± 1.28	0.80 ± 0.08
C18:3n3	27.64 ± 1.52	0.47 ± 0.17*	20.21 ± 9.49^{a}	27.85 ± 1.15^{b}	23.8 ± 3.44^{a}	23.93 ± 2.16^{a}
C20:0	0.16 ± 0.04	0.17 ± 0.03	0.37 ± 0.04^{a}	0.15 ± 0.02^{b}	0.14 ± 0.07^{b}	0.15 ± 0.08^{b}
C20:2n6	0.22 ± 0.05	n.d.	n.d.	0.20 ± 0.04	0.23 ± 0.09	0.27 ± 0.06
C20:3n6	0.09 ± 0.05	0.35 ± 0.04*	0.01 ± 0.02	0.10 ± 0.02	0.09 ± 0.05	0.07 ± 0.06
C20:3n3	0.87 ± 0.07	0.01 ± 0.02*	n.d.	0.82 ± 0.1^{ab}	0.74 ± 0.08^{a}	0.95 ± 0.07^{b}
C20:4n6	0.89 ± 0.08	0.64 ± 0.06*	0.23 ± 0.15^{a}	0.83 ± 0.12^{b}	0.86 ± 0.14^{b}	1.31 ± 0.25^{b}
C20:5n3	1.94 ± 0.06	1.44 ± 0.16*	n.d.	1.99 ± 0.18^{ab}	1.94 ± 0.37^{a}	2.53 ± 0.34^{b}
C22:0	0.14 ± 0.03	0.23 ± 0.04*	0.18 ± 0.05^{ab}	0.13 ± 0.01^{a}	0.19 ± 0.07^{ab}	0.22 ± 0.06^{b}
C22:1	0.08 ± 0.05	0.03 ± 0.05	0.07 ± 0.06^{a}	0.06 ± 0.03^{a}	0.11 ± 0.09^{a}	0.32 ± 0.07^{b}
C22:4n6	n.d.	0.14 ± 0.00	n.d.	n.d.	n.d.	n.d.
C22:5n3	0.00 ± 0.00	0.47 ± 0.05*	n.d.	n.d.	n.d.	n.d.
C22:6n3	0.22 ± 0.29	32.44 ± 1.51*	n.d.	0.27 ± 0.41^{a}	1.64 ± 0.61^{b}	0.19 ± 0.17^{a}
C24:0	0.02 ± 0.03	10.97 ± 0.55*	0.12 ± 0.06	0.02 ± 0.03	0.28 ± 0.30	0.04 ± 0.07

C24:1	n.d.	0.04 ± 0.01*	n.d.	n.d.	n.d.	n.d.
∑ SAT	24.55 ± 2.38	55.8 ± 1.86*	48.38 ± 5.36[a]	23.68 ± 2.57[b]	28.26 ± 2.5[c]	27.84 ± 12.63[c]
∑ MUFA	36.16 ± 1.06	3.71 ± 3.27*	12.78 ± 2.34[a]	36.62 ± 0.65[b]	34.64 ± 0.92[b]	36.25 ± 12.32[b]
∑ PUFA	39.28 ± 3.03	40.49 ± 2.94	38.84 ± 7.35	39.70 ± 2.7	37.10 ± 2.55	35.91 ± 6.05
∑ n-3 FA	30.81 ± 2.84	35.05 ± 4.07*	20.40 ± 9.45[a]	31.06 ± 2.52[b]	28.32 ± 2.32[ab]	27.82 ± 9.82[ab]
∑ n-6 FA	8.47 ± 0.22	5.44 ± 1.23*	18.45 ± 2.71[a]	8.64 ± 0.26[b]	8.78 ± 0.33[b]	8.09 ± 6.24[b]
U/S Ratio	3.07 ± 0.34	0.79 ± 0.22*	1.09 ± 0.20[a]	3.28 ± 0.28[b]	2.55 ± 0.36[c]	2.60 ± 0.78[c]

Table 6.2.Whole body fatty acid profile of age-0 lake sturgeon (*Acipenser fulvescens*) at 44 days post hatch after feeding for the last 24 days. U/S ratio: unsaturated to saturated fatty acid ratio, Data are expressed in mol % (mean ± S.D.). Values with a different letter in each fatty acid are significantly different (One-way ANOVA; $p<0.05$)

	Control	Low DHA	High DHA	Time-control
C14:0	0.61 ± 0.21^{a}	0.35 ± 0.06^{b}	0.49 ± 0.19^{ab}	0.39 ± 0.11^{b}
C14:1	0.69 ± 0.23^{a}	0.42 ± 0.07^{b}	0.37 ± 0.15^{b}	0.44 ± 0.16^{b}
C15:0	0.30 ± 0.09	0.27 ± 0.04	0.30 ± 0.10	0.26 ± 0.04
C16:0	20.02 ± 2.39	20.61 ± 1.81	21.85 ± 1.56	20.78 ± 2.79
C16:1t	1.09 ± 0.17^{a}	0.91 ± 0.14^{ab}	0.8 ± 0.09^{b}	0.88 ± 0.12^{b}
C16:1	2.56 ± 0.44^{a}	1.96 ± 0.19^{b}	1.63 ± 0.2^{c}	1.92 ± 0.31^{bc}
C17:0	1.18 ± 0.11	1.16 ± 0.04	1.15 ± 0.14	1.19 ± 0.09
C18:0	9.46 ± 0.94^{a}	11.13 ± 0.81^{b}	12.24 ± 2.31^{b}	11.08 ± 0.88^{b}
C18:1	19.38 ± 1.52^{a}	17.44 ± 0.71^{ab}	15.95 ± 1.45^{ab}	15.89 ± 4.54^{b}
C18:1n7c	7.99 ± 0.35	7.97 ± 0.52	7.27 ± 0.77	10.25 ± 5.99
C18:2n6	4.95 ± 0.38^{a}	4.53 ± 0.26^{ab}	4.24 ± 0.36^{b}	4.52 ± 0.63^{ab}
C18:3n6	0.78 ± 0.26^{a}	0.56 ± 0.12^{ab}	0.55 ± 0.08^{b}	0.65 ± 0.14^{ab}
C18:3n3	13.67 ± 2.58^{a}	11.62 ± 1.78^{ab}	8.98 ± 1.9^{b}	10.99 ± 3.62^{ab}
C20:0	0.23 ± 0.07	0.27 ± 0.05	0.52 ± 0.77	0.24 ± 0.05
C20:1	0.64 ± 0.05^{a}	0.60 ± 0.06^{ab}	0.51 ± 0.07^{b}	0.59 ± 0.09^{ab}
C20:2n6	0.35 ± 0.06	0.38 ± 0.06	0.60 ± 0.69	0.36 ± 0.06
C20:3n6	0.73 ± 0.1	0.66 ± 0.12	0.60 ± 0.11	0.69 ± 0.07
C20:4n6	2.95 ± 0.68^{a}	4.19 ± 0.47^{b}	4.26 ± 0.70^{b}	4.14 ± 0.99^{b}
C20:3n3	1.47 ± 0.29	1.21 ± 0.18	1.16 ± 0.24	1.29 ± 0.33
C20:5n3	4.52 ± 1.30	5.19 ± 0.57	5.05 ± 0.8	5.28 ± 1.03
C22:0	0.18 ± 0.12	0.17 ± 0.06	0.35 ± 0.64	0.20 ± 0.12
C22:1	0.09 ± 0.12	0.08 ± 0.07	0.09 ± 0.06	0.13 ± 0.17
C22:5n6	0.14 ± 0.34	0.03 ± 0.01	0.08 ± 0.11	0.18 ± 0.44
C22:5n3	1.50 ± 0.63	1.87 ± 0.33	1.59 ± 0.25	1.72 ± 0.67
C22:6n3	4.24 ± 1.63^{a}	5.96 ± 0.55^{a}	8.01 ± 1.42^{b}	5.59 ± 2.00^{a}
C24:0	0.16 ± 0.06^{a}	0.30 ± 0.13^{b}	1.26 ± 0.14^{c}	0.29 ± 0.10^{b}
C24:1	0.12 ± 0.06	0.14 ± 0.09	0.09 ± 0.03	0.11 ± 0.03
$\sum$ SAT	32.14 ± 3.53^{a}	34.26 ± 2.50^{ab}	38.17 ± 4.65^{b}	34.42 ± 3.68^{ab}
$\sum$ MUFA	32.56 ± 2.61^{a}	29.52 ± 0.65^{ab}	26.71 ± 2.56^{b}	30.18 ± 3.03^{a}
$\sum$ PUFA	35.30 ± 4.06	36.21 ± 2.31	35.12 ± 2.75	35.39 ± 4.30
$\sum$ n-3	25.40 ± 3.93	25.85 ± 2.30	24.79 ± 3.06	24.85 ± 4.05
$\sum$ n-6	9.90 ± 0.34	10.36 ± 0.36	10.33 ± 0.63	10.54 ± 0.61
U/S Ratio	2.14 ± 0.33^{a}	1.93 ± 0.21^{ab}	1.65 ± 0.28^{b}	1.94 ± 0.32^{ab}

Table 6.3.Hepatic fatty acid profile of age-0 lake sturgeon (*Acipenser fulvescens*) pre and post a simulated overwintering event. The simulated overwintering event was 45 days of food deprivation at 3°C. Data are expressed in mol % (mean ± S.D.). * indicates significantly changes within each diet group after the overwintering (Two-way ANOVA, $p<0.05$)

	Pre-overwintering				Post-overwintering			
	Control	Low DHA	High DHA	Time-control	Control	Low DHA	High DHA	Time-control
C14:0	1.87 ± 0.88	1.87 ± 0.54	1.56 ± 0.55	1.43 ± 0.96	0.55 ± 0.12*	0.54 ± 0.17*	0.85 ± 0.46*	0.61 ± 0.16
C14:1	1.18 ± 0.49	1.18 ± 0.28	1.04 ± 0.34	0.96 ± 0.58	0.35 ± 0.12*	0.36 ± 0.14*	0.50 ± 0.32*	0.45 ± 0.13
C15:0	1.38 ± 0.17	1.38 ± 0.21	1.36 ± 0.15	1.27 ± 0.26	1.11 ± 0.30	0.90 ± 0.13*	0.94 ± 0.10*	0.92 ± 0.11*
C16:0	26.69 ± 3.05	26.69 ± 2.45	25.85 ± 0.98	27.06 ± 1.75	31.89 ± 1.64*	30.53 ± 1.73	29.32 ± 2.46*	30.35 ± 4.04a*
C16:1t	1.38 ± 1.75	1.38 ± 0.18	0.72 ± 0.17	0.67 ± 0.26	0.82 ± 0.09	0.80 ± 0.05	0.86 ± 0.19	0.81 ± 0.10
C16:1	3.32 ± 1.38	3.32 ± 0.81	3.45 ± 0.83	3.06 ± 1.52	2.48 ± 0.34	2.06 ± 0.24	3.26 ± 1.73	2.56 ± 0.67
C17:0	1.55 ± 0.18	1.55 ± 0.04	1.54 ± 0.11	1.5 ± 0.10	0.74 ± 0.12*	0.74 ± 0.10*	0.73 ± 0.11*	0.72 ± 0.08*
C18:0	11.3 ± 1.89	11.3 ± 1.79	11.81 ± 1.53	11.75 ± 1.83	10.01 ± 1.87	8.68 ± 1.19*	8.46 ± 2.36*	8.79 ± 2.15
C18:1	9.63 ± 1.39	9.63 ± 1.24	9.51 ± 0.80	9.20 ± 1.35	8.28 ± 0.66	7.72 ± 1.02	10.59 ± 4.35	8.84 ± 2.13
C18:1n7c	5.81 ± 0.37	5.81 ± 0.58	6.02 ± 0.50	5.89 ± 0.53	7.13 ± 0.61*	7.05 ± 0.22*	7.54 ± 0.64*	7.37 ± 0.94*
C18:2n6	8.09 ± 1.37	8.09 ± 0.66	8.16 ± 0.48	7.35 ± 1.06	4.41 ± 0.35*	4.12 ± 0.33*	4.75 ± 0.78*	4.65 ± 1.07*
C18:3n6	1.27 ± 0.23	1.27 ± 0.14	1.17 ± 0.40	1.17 ± 0.15	1.27 ± 0.14	1.36 ± 0.07	1.34 ± 0.18	1.33 ± 0.12
C18:3n3	1.29 ± 0.35	1.29 ± 0.23	1.31 ± 0.20	1.09 ± 0.4	0.31 ± 0.08*	0.29 ± 0.06*	0.40 ± 0.19*	0.36 ± 0.15*
C20:0	0.41 ± 0.06	0.41 ± 0.06	0.35 ± 0.08	0.47 ± 0.14	0.30 ± 0.09	0.28 ± 0.08	0.30 ± 0.07	0.28 ± 0.06*
C20:1	0.66 ± 0.13	0.66 ± 0.25	0.64 ± 0.16	0.81 ± 0.14	1.26 ± 0.29*	1.54 ± 0.35*	1.53 ± 0.49*	1.46 ± 0.31*
C20:2n6	1.47 ± 0.25	1.47 ± 0.36	1.40 ± 0.22	1.66 ± 0.17	1.27 ± 0.29	1.30 ± 0.18	1.17 ± 0.24	1.25 ± 0.27
C20:3n6	2.41 ± 0.45	2.41 ± 0.50	2.5 ± 0.38	2.78 ± 0.42	2.53 ± 0.59	2.58 ± 0.53	2.36 ± 0.58	2.50 ± 0.42
C20:4n6	8.28 ± 1.65	8.28 ± 1.4b	9.18 ± 0.98	9.26 ± 1.85	11.8 ± 0.83*	13.08 ± 0.54*	11.02 ± 2.10	12.66 ± 1.79*
C20:3n3	1.29 ± 1.07	1.29 ± 0.47	0.72 ± 0.18	0.93 ± 0.76	0.41 ± 0.06*	0.49 ± 0.17*	0.59 ± 0.56*	0.41 ± 0.07*
C20:5n3	1.16 ± 0.32	1.16 ± 0.28	1.34 ± 0.26	1.26 ± 0.31	0.64 ± 0.10*	0.71 ± 0.16*	0.69 ± 0.14*	0.76 ± 0.18*
C22:0	0.11 ± 0.09	0.11 ± 0.11	0.08 ± 0.08	0.16 ± 0.08	0.09 ± 0.07	0.07 ± 0.05	0.12 ± 0.14	0.12 ± 0.12
C22:1	0.09 ± 0.02	0.09 ± 0.01	0.08 ± 0.03	0.10 ± 0.03	0.17 ± 0.04*	0.16 ± 0.04*	0.17 ± 0.05*	0.16 ± 0.04*
C22:5n6	0.27 ± 0.37	0.27 ± 0.18	0.09 ± 0.08	0.15 ± 0.25	0.11 ± 0.05	0.11 ± 0.03	0.12 ± 0.13	0.08 ± 0.04
C22:5n3	0.66 ± 0.18	0.66 ± 0.17	0.72 ± 0.11	0.73 ± 0.14	0.42 ± 0.15	0.51 ± 0.13	0.46 ± 0.15	0.48 ± 0.12
C22:6n3	7.10 ± 2.24	7.10 ± 1.74	7.90 ± 0.93	7.66 ± 1.49	9.69 ± 2.33	11.44 ± 1.73*	9.94 ± 2.67	10.21 ± 2.50
C24:0	1.20 ± 0.48	1.20 ± 0.30	1.39 ± 0.19	1.51 ± 0.35	1.64 ± 0.24	2.25 ± 0.48	1.65 ± 0.41	1.59 ± 0.77
C24:1	0.14 ± 0.07	0.14 ± 0.03	0.11 ± 0.01	0.13 ± 0.06	0.29 ± 0.09*	0.31 ± 0.1*	0.35 ± 0.15*	0.27 ± 0.07*
∑ SAT	44.52 ± 4.42	44.52 ± 3.58	43.94 ± 1.85	45.15 ± 2.47	46.34 ± 3.28	43.99 ± 1.62	42.35 ± 4.20	43.39 ± 6.24

∑ MUFA	22.20 ± 3.84	22.20 ± 3.04	21.57 ± 2.09	20.82 ± 4.11	20.79 ± 1.27	20.02 ± 1.50	24.8 ± 6.97	21.91 ± 3.86
∑ PUFA	33.28 ± 3.68	33.28 ± 3.59	34.48 ± 1.78	34.04 ± 2.92	32.87 ± 3.04	35.98 ± 0.97	32.85 ± 4.15	34.71 ± 5.08
∑ n-3	11.50 ± 1.92	11.50 ± 1.98	11.99 ± 1.11	11.66 ± 1.70	11.48 ± 2.43	13.44 ± 1.69	12.09 ± 2.57	12.23 ± 2.70
∑ n-6	21.79 ± 2.05	21.79 ± 1.86	22.49 ± 0.84	22.37 ± 1.52	21.4 ± 0.92	22.54 ± 1.05	20.76 ± 2.15	22.47 ± 2.83
UI	146.72 ± 17.82	146.72 ± 16.15	152.71 ± 8.58	150.85 ± 13.64	158.28 ± 16.23	174.61 ± 7.49	161.57 ± 18.2	167.51 ± 23.56

Table 6.4.Results of two-way ANOVA for digestive enzyme activities, growth and energy density of age-0 lake sturgeon (*Acipenser fulvescens*). Letters in Note indicate a type of transformation performed on the data to meet the assumptions for parametric test. L: log. C: cube root. S: square root.

Measurement	Variables	Sum Sq	df	F	p	Note
Lipase	Diet	4.86	3	3.944	0.011	L
	Time	42.172	2	51.326	<0.001	
	Diet×Time	2.266	6	0.919	0.485	
Trypsin	Diet	2.077	3	1.607	0.194	L
	Time	310.651	2	360.43	<0.001	
	Diet×Time	0.743	6	0.288	0.941	
Total length	Diet	1184	3	3.936	0.001	
	Time	134228	5	267.855	<0.001	
	Diet×Time	1199	15	0.798	0.681	
Body mass	Diet	3.59	3	6.945	<0.001	L
	Time	373.01	5	432.454	<0.001	
	Diet×Time	3.33	15	1.286	0.207	
Condition factor	Diet	0.000315	3	1.174	0.319	C
	Time	0.022941	5	51.204	<0.001	
	Diet×Time	0.003825	15	2.846	<0.001	
Energy density	Diet	0.13769	3	1.722	0.167	L
	Time	0.89519	3	11.195	<0.001	
	Diet×Time	0.39074	9	1.629	0.115	

Table 6.5. Summary of Cox proportional hazards regression for the cumulative mortality of age-0 lake sturgeon (*Acipenser fulvescens*) raised with different diets. DPH denotes days post hatch and p represents probability for the significance. Dataset for 20-67 dph was n= 39, number of events= 39 whereas dataset for 67-190 dph was n= 1161, number of events= 92.

DPH	Treatment	Hazard ratio	95 % CI	Z value	p
20 – 67	Low DHA	0.984	0.394 - 2.246	-0.035	0.972
	High DHA	0.589	0.239 - 1.45	-1.148	0.251
	Time-control	0.661	0.235 - 1.86	-0.786	0.432
67 -190	Low DHA	0.762	0.441 - 1.317	-0.973	0.331
	High DHA	0.439	0.234 - 0.826	-2.553	0.011
	Time-control	0.948	0.559 - 1.606	-0.199	0.842

Table 6.6. Fatty acid profile of *Artemia* enriched with either *Chlorella vulgaris* or Red AlgaMac for 6 or 24 hours. n.d.: not detected. Data are expressed in mol % (mean ± S.D.).

Enrichment	*Chlorella vulgaris*		Red AlgaMac	
Duration	6 hrs	24 hrs	6 hrs	24 hrs
C14:0	1.07 ± 0.13	1.16 ± 0.58	2.35 ± 0.39	2.09 ± 0.32
C14:1	1.25 ± 0.1	1.17 ± 0.41	1.24 ± 0.22	1.24 ± 0.39
C15:0	0.21 ± 0.02	0.28 ± 0.04	0.24 ± 0.03	0.21 ± 0.12
C16:0	14.47 ± 0.76	15.27 ± 0.89	17.12 ± 1.82	15.56 ± 0.93
C16:1t	0.82 ± 0.05	0.59 ± 0.11	0.73 ± 0.08	0.67 ± 0.08
C16:1	3.45 ± 0.13	2.88 ± 0.37	3.2 ± 0.14	2.8 ± 0.33
C17:0	1.37 ± 0.13	1.59 ± 0.32	1.65 ± 0.75	1.38 ± 0.16
C18:0	6.33 ± 3.14	10.65 ± 1.34	6.36 ± 3.15	10.11 ± 1.87
C18:1	21.85 ± 1.3	21.23 ± 0.94	20.54 ± 1.56	20.23 ± 0.45
C18:1n7c	9.25 ± 0.75	11.33 ± 0.85	8.93 ± 1.44	10.45 ± 0.97
C18:2	6.57 ± 0.23	4.5 ± 2.22	5.96 ± 0.35	5.43 ± 0.4
C18:3n6	0.93 ± 0.08	1.86 ± 2.49	1.64 ± 1.28	0.92 ± 0.13
C18:3n3	27.85 ± 1.15	20.79 ± 1.79	23.8 ± 3.44	22.18 ± 2.86
C20:0	0.15 ± 0.02	0.27 ± 0.09	0.14 ± 0.07	0.18 ± 0.09
C20:2	0.2 ± 0.04	0.33 ± 0.11	0.23 ± 0.09	0.19 ± 0.09
C20:3n6	0.1 ± 0.02	0.14 ± 0.13	0.09 ± 0.05	0.05 ± 0.06
C20:4	0.83 ± 0.12	1.15 ± 0.31	0.86 ± 0.14	1.48 ± 0.38
C20:3n3	0.82 ± 0.1	0.9 ± 0.21	0.74 ± 0.08	0.87 ± 0.1
C22:0	0.13 ± 0.01	0.21 ± 0.12	0.19 ± 0.07	0.25 ± 0.09
C22:1	0.06 ± 0.03	0.36 ± 0.21	0.11 ± 0.09	0.48 ± 0.31
C20:5	1.99 ± 0.18	2.24 ± 0.69	1.94 ± 0.37	2.57 ± 0.35
C24:0	0.02 ± 0.03	0.12 ± 0.1	0.28 ± 0.3	0.02 ± 0.04
C22:6n3	0.27 ± 0.41	0.98 ± 1.3	1.64 ± 0.61	0.62 ± 0.47
∑ SAT	23.68 ± 2.64	29.71 ± 1.69	28.26 ± 1.67	30.04 ± 2.46
∑ MUFA	36.62 ± 1.92	37.2 ± 1.25	34.64 ± 2.81	35.39 ± 0.69
∑ PUFA	39.7 ± 1.18	33.09 ± 2.52	37.1 ± 3.08	34.57 ± 2.72
∑ ω3	31.06 ± 0.99	25.11 ± 2.63	28.32 ± 4.01	26.5 ± 2.54
∑ ω6	8.64 ± 0.37	7.98 ± 0.19	8.78 ± 1.03	8.07 ± 0.24
U/S Ratio	3.28 ± 0.57	2.38 ± 0.19	2.55 ± 0.2	2.35 ± 0.27



Figure 6.1. Schematic figure of experiment flow. Fish were fed one of four enriched *Artemia* (blue) from 20 to 49 days post hatch (dph). Then, dietary transition from *Artemia* to bloodworm (AB Transition; purple) was gradually performed from 49 to 67 dph while enriched *Artemia* was provided in accordance with its treatment. After 67 dph, fish were fed the same diet of bloodworm (red) until the end of experiment. At 127 dph, water temperature decreased from 16 to 3.5°C at the rate of 1°C per day, and at 145 dph where fish were deprived of food at 3°C for 45 days.

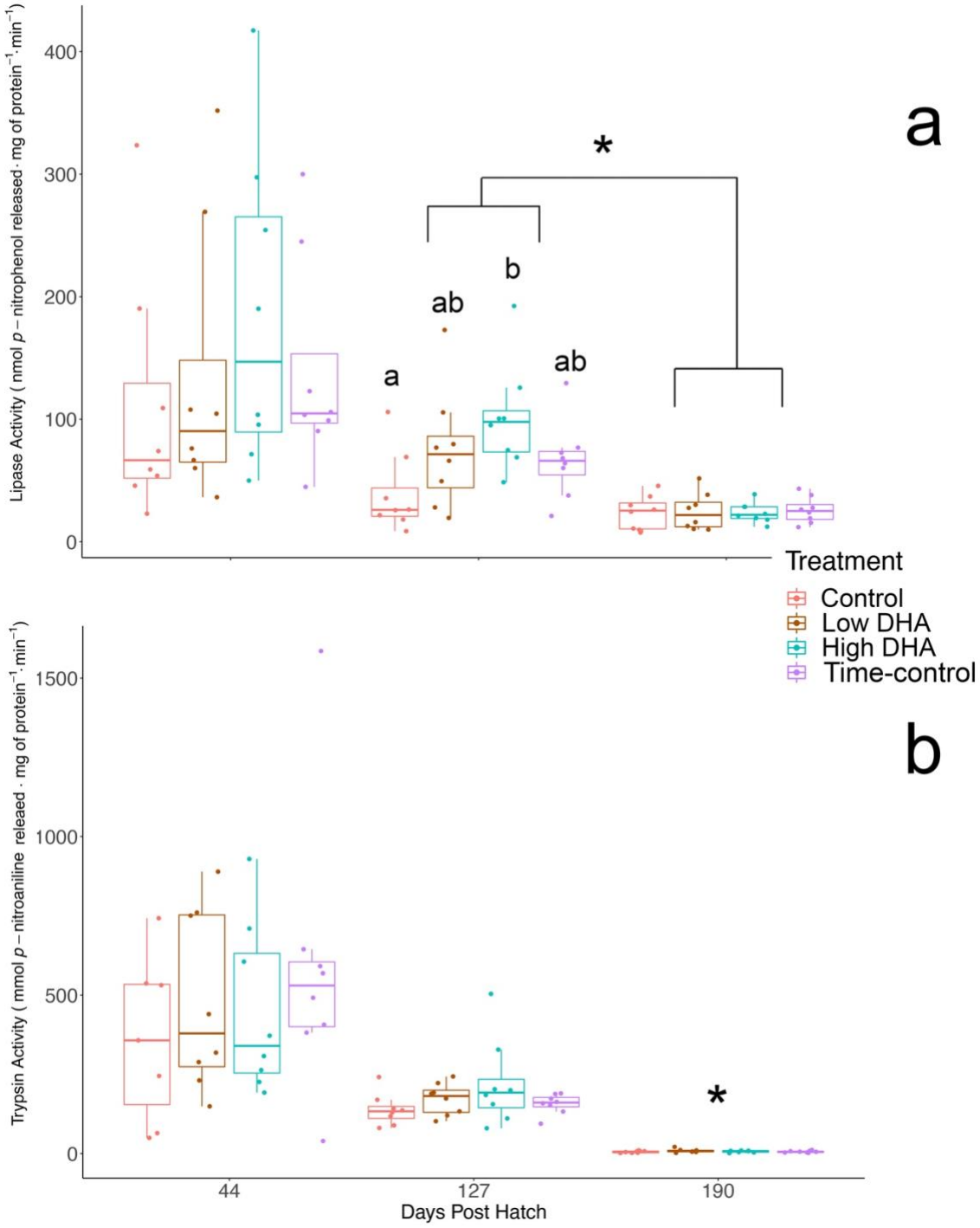

Figure 6.2.Changes in lipase (a) and trypsin activity (b) of gastrointestinal tract in age-0 lake sturgeon (*Acipenser fulvescens*) raised in different diets. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots are individual data plotted over boxplots. Letters indicate significant difference among treatments and * denote significant changes within each treatment across time points.

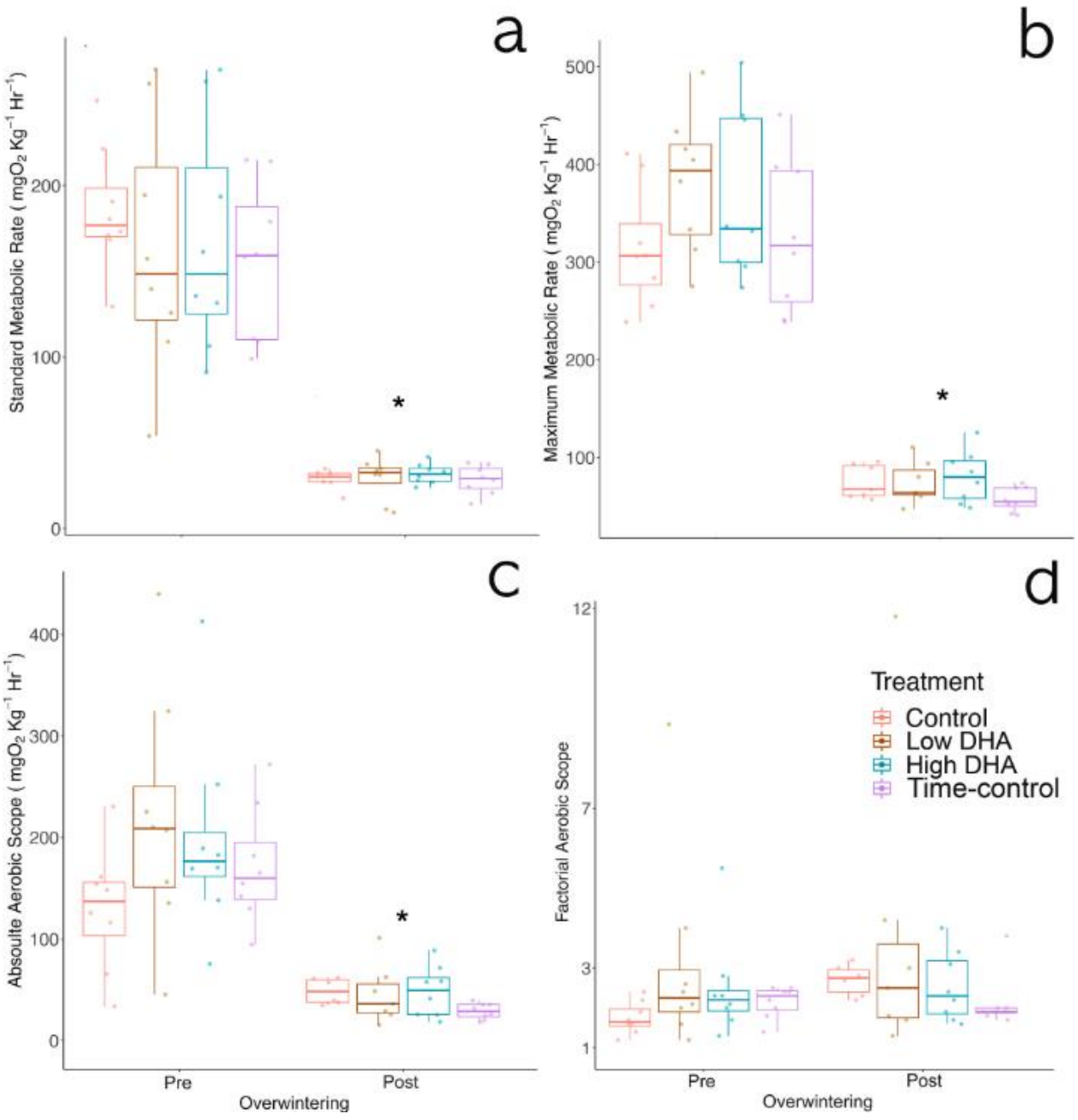

Figure 6.3. Changes in standard (a), maximum (b) metabolic rate, absolute aerobic scope (c) and factorial aerobic scope (d) in age-0 lake sturgeon (*Acipenser fulvescens*) raised in different diets. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Data are not transformed for graphical purpose. Dots are individual plotted over boxplots. * denote significant changes within each treatment.

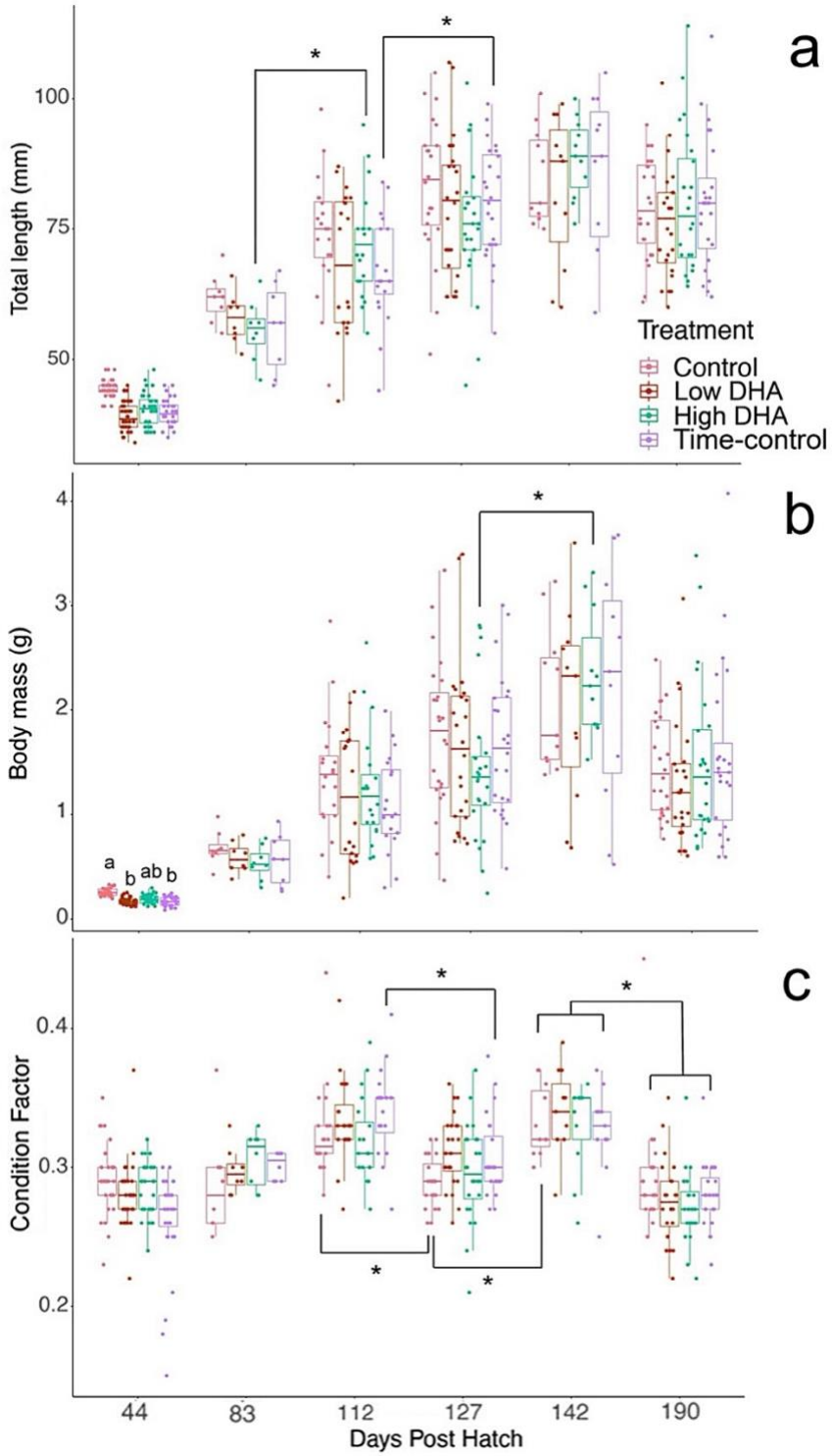

Figure 6.4. Changes in total length (a) and body mass (b) of age-0 lake sturgeon (*Acipenser fulvescens*) fed different diets from 20 to 44 days post hatch. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Dots are individual data plotted over boxplots. * denote significant changes within each treatment.

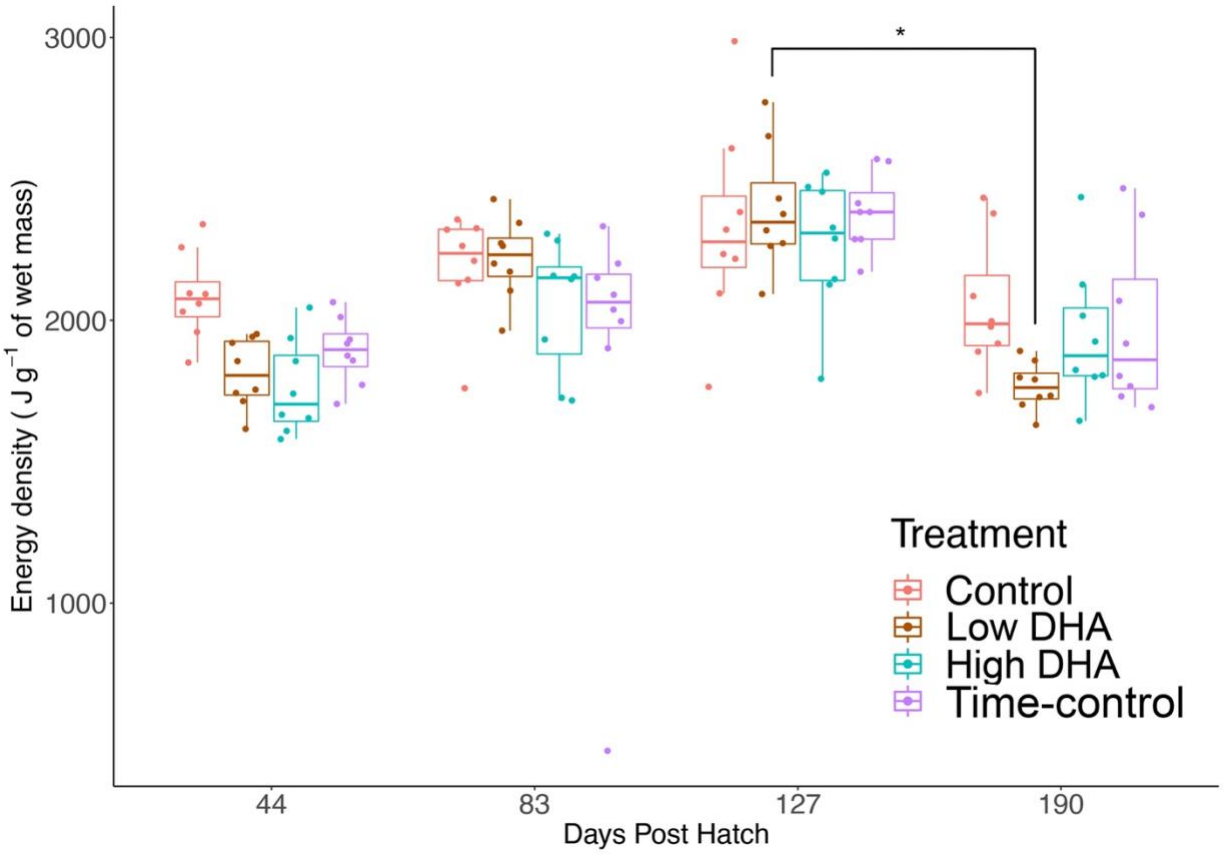

Figure 6.5. Changes in energy density of age-0 lake sturgeon (*Acipenser fulvescens*) raised in different diets. The middle horizontal line represents median value; lower and upper lines represent the 25 and 75 percentiles. The line parelle with boxplot (i.e. whisker) represent data distribution. Data are not transformed for graphical purpose. Dots are individual data plotted over boxplots. * denote significant changes within each treatment.

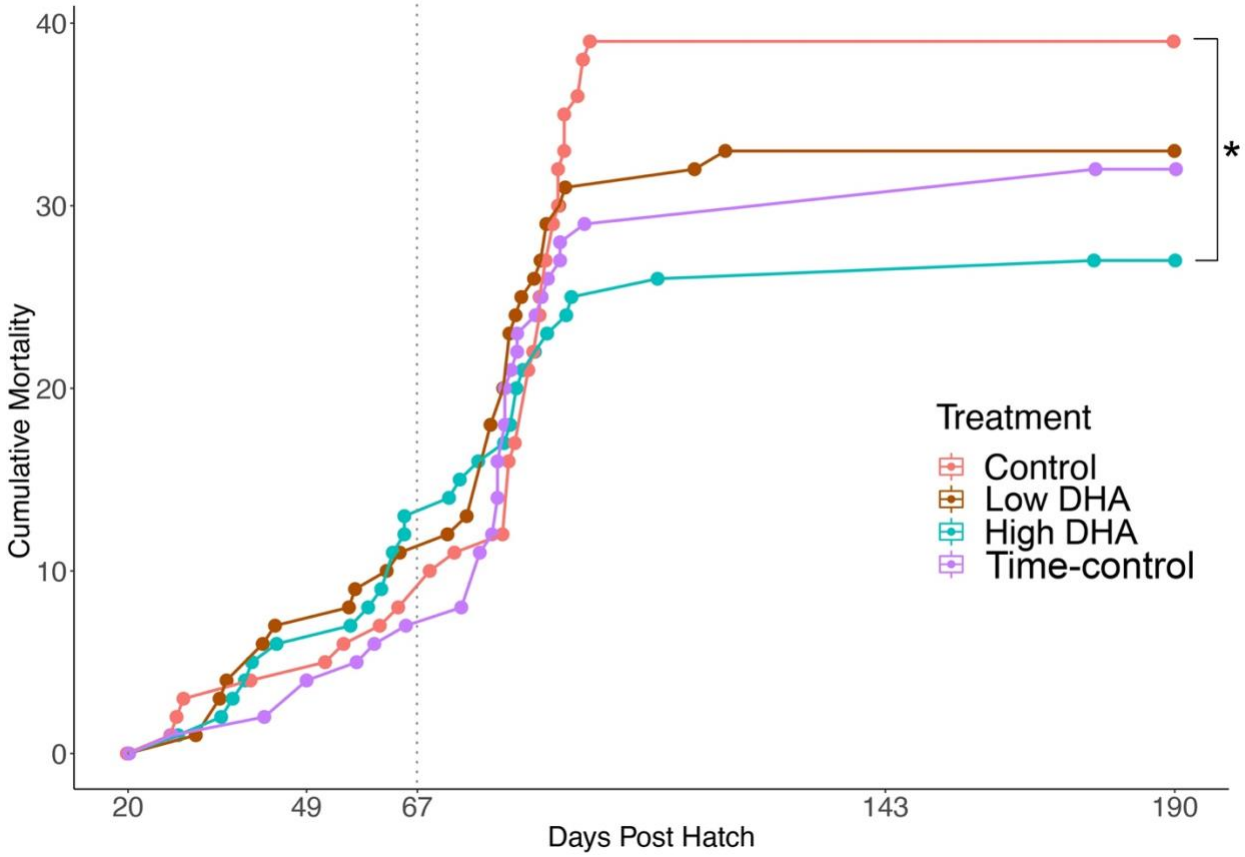

Figure 6.6. Cumulative mortality of age-0 lake sturgeon (*Acipenser fulvescens*) raised with different diets. Fish were fed one of four diets (enriched *Artemia*) from 20 to 49 days post hatch (dph), and diet was gradually transitioned from *Artemia* to bloodworm from 49 to 67 dph after which all fish were fed the same diet of bloodworm until the end of experiment. * denote significant difference in mortality between treatments from 67 to 190 dph assessed by Cox proportional hazard regression and *post hoc* pairwise comparison using Log- Rank test with Bonferroni correction ($p<0.05$).

7 Chapter 7. General discussion and future perspectives

This doctoral thesis examined how temperature and diet could result in short-term and long-term changes in phenotypes of age-0 lake sturgeon. The experimental chapters have focused on understanding effects of dietary shifts on development of metabolic rate during early life (Chapter 2); effects of temperature and food availability on fatty acid profile and plasma cortisol concentration (Chapter 3); long-term effects of temperature or diet during early life on development of metabolic phenotypes within age-0 lake sturgeon (Chapters 4, 5, 6). This chapter will summarize and connect findings of each experiment chapter and describe potential avenues for future research.

7.1 Plastic nature of metabolic phenotypes in age-0 lake sturgeon

The first two experimental chapters (Chapters 2 and 3) of this PhD thesis examined how metabolic phenotypes change by environment. Results from these two chapters demonstrated that all measured phenotypes such as fatty acid profile, energy density, metabolic rate and growth were strongly influenced by temperature and diet, supporting the prediction that these metabolic phenotypes are plastic.

Chapter 2 provided some insight into how metabolic phenotypes may change during early development in age-0 lake sturgeon and documented ontogenetic changes in metabolic rate, energy density and growth during early life, which may reflect developmental processes that are genetically programmed (Osse et al., 1997). The results agree with previous studies showing that metabolic rate and growth significantly vary during early development in fish (Post and Lee, 1996; Yagi and Oikawa, 2014). Specifically, changes in metabolic rate during early ontogenetic development may be driven by allometric growth of the gas exchange system (i.e. gill) (Post and Lee, 1996) and/or relative growth of metabolically active organs such as the kidney, liver and heart (Rosenfeld et al., 2015). Results of Chapter 2 illustrated that growth arrest during the second dietary transition coincided with increased routine metabolic rate, which may indicate metabolic regulation by the gastrointestinal tract in response to a change in diet (Gisbert et al., 2001; Sánchez-Hernández et al., 2019). Chapter 3 demonstrated that fatty acid profiles in the liver were strongly influenced by temperature, reporting that decreasing temperatures led to increases in both mono- (MUFA) and polyunsaturated fatty acids (PUFA) in phospholipids and triglycerides. It is well known that temperature changes result in rapid regulation of fatty acid composition, a process which is termed homeoviscous adaptation (Hazel, 1997). For example,

cold temperatures may result in increased desaturation of fatty acids through increased mRNA expression levels of Δ 5, 6 and 9 desaturases and increased activity of these desaturases (Ernst et al., 2016; Schünke and Wodtke, 1983; Trueman et al., 2000).

7.2 Effects of the environment on phenotypic development in age-0 lake sturgeon

Chapters 4, 5 and 6 were designed to examine effects of environment during early life on phenotypic development within age-0 lake sturgeon. It is worth noting that lake sturgeon is known to have a long lifespan potentially living up to 150 years (Bruch et al., 2016), which is a less than ideal model to examine plasticity of phenotypes throughout the life history in this species. However, as a northern temperate aquatic species that needs to survive overwintering conditions, any prolonged effects of environment during early life significantly influence growth and metabolism that may directly impact survival during the first winter of life (Hurst, 2007; Radchuk et al., 2013).

With the importance of overwintering survival in age-0 fish, Chapters 4, 5 and 6 highlight how timing and duration of environmental changes during early life may result in transient or prolonged effects on metabolic phenotypes in age-0 lake sturgeon. Chapters 4 and 5 used a similar approach where temperature was manipulated during early life and then subsequently maintained at the same temperature regime whereas in Chapter 6, enriched diets (*Artemia*) were provided at the onset of exogenous feeding and then subsequently the same diet of bloodworm was fed for the remainder of experiment.

Chapter 4 demonstrated that temperature manipulation for 35 days at 58 days post hatch (i.e. 16°C, 18°C and ambient temperatures) resulted in transient effects on growth and energy metabolism and that these effects diminished during the overwintering period. Chapter 5 demonstrated that when temperature was manipulated between 33 and 93 dpf (i.e. 16 and 20°C), there was a prolonged effect on growth performance. Although in Chapter 5 differences in growth between temperature groups diminished during the cold conditioning, fish reared at higher temperature (20°C) showed lack of transcriptional responses in terms of fatty acid desaturation compared to those reared at the control temperature (16°C) when exposed to a cold temperature later in life, which suggests that fatty acid metabolism may have been altered due to thermal history during early life. Interestingly, a previous study in our lab demonstrated that transient increases in rearing temperature during the first month of life had persistent effects on growth phenotypes in lake sturgeon almost a year later (Brandt et al., 2022). Thus, results of

Chapters 4 and 5 highlighted that timing and duration of environmental changes during early life may result in reversible or irreversible effects on phenotypic development (Burggren, 2015), and phenotypic switching later in life (Burggren, 2020). For example, growth rate does not stay at an enhanced level probably due to ecological and physiological trade-offs (Ali et al., 2003; Dmitriew, 2011). Accelerated growth phenotypes may be beneficial when resources are abundant, but it can lead to more rapid loss in body mass when resources becomes limited (Auer et al., 2016). Thus, it is possible that our observation of differences in growth trajectory diminishing during the winter months in Chapter 4 or cold conditioning in Chapter 5 could be adaptive responses to regulate metabolism and switch back to a slow growth phenotype (Burggren, 2020). Results of Chapters 4 and 5 suggest that a critical window for growth in lake sturgeon may be the first month of life, but temperature changes a month later in life may still have longer-term effects on other metabolic processes such as fatty acid metabolism in age-0 lake sturgeon.

While a majority of literature reported short-term effects of enriched diet on growth and metabolism in fish (Hamre et al., 2013; Kolman et al., 2018; Lund et al., 2012; McKenzie et al., 2008), Chapter 6 of the present thesis illustrated that dietary condition during early life may have longer-term effects on growth and metabolism, which could influence overwintering survival in age-0 fish. In Chapter 6, results showed that DHA enrichment may have prolonged effects on digestive physiology, and growth and survival prior to overwintering, indicating the importance of dietary conditions at the first exogenous feeding. Indeed in oviparous fish, a critical window for growth and development is hypothesized to be the onset of exogenous feeding (Hou and Fuiman, 2020). As fatty acids are involved in ontogenesis, growth, metabolism and cell signalling (Calder, 2006; Tocher et al., 2008), fatty acids during early life may have profound effects on phenotypic development (Hamre et al., 2013; Innis, 2011). While the mechanism involved in our findings have yet to be examined, it is hypothesized that DHA at the first feed may be involved in gut development in age-0 lake sturgeon.

7.3 Future perspectives

7.3.1 Sources that drive variation of metabolic phenotypes

Results from the experimental chapters in this thesis indicated that some metabolic traits may be correlated. For example, Chapter 2 illustrated growth arrest during dietary transition may be correlated with increased routine metabolic rate. In fishes, it has been previously hypothesized

that growth trajectory and metabolic rate may be correlated with each other (Metcalfe et al., 2016). Indeed, there is evidence from previous work in our lab, demonstrating that local adaptation may be a source for variation of metabolic rate in age-0 lake sturgeon. When raised at the same temperature in a common garden setup, individuals from a northern population showed lower routine metabolic rate and reduced growth rates compared to individuals from a southern population (Bugg et al., 2020). While these results suggest counter-gradient variation of growth and metabolic rate in age-0 lake sturgeon, which may be due to physiological adaptation to geographically distinct temperature profiles (Bugg et al., 2021), additional research in our lab has demonstrated a strong maternal influence on standard metabolic rate of age-0 lake sturgeon (Deslauriers et al., *unpublished*) that may explain fine-scale variation of growth across populations. In the present thesis, experiments were conducted through multiple years, and gametes were sourced from two geographically distinct populations. Chapters 3 and 6 used gametes from a southern population (Winnipeg River) whereas Chapters 4 and 5 used gametes collected from a northern population (Nelson River). Thus, it is reasonable to speculate that variation of metabolic phenotypes in this thesis may also be correlated with populations.

7.3.2 Covariation of phenotypes

Phenotypes do not vary independently, rather they are integrated through developmental, morphological, behavioural, physiological and functional processes (Armbruster et al., 2014). Therefore, understanding variation in phenotype provides insight into how biological traits are intertwined through environment and genotype interactions (Hallgrímsson and Hall, 2005). For example, growth rate during early life is typically very high as body size plays an important role in avoiding predation and survival (Dmitriew, 2011). Indeed, elevated growth rate during early life history may be an evolutionary adaptation (Arendt, 1997), and is hypothesized that physiological traits related to growth rate including metabolic rate may be also strongly subjected to co-selection (Pettersen et al., 2018; Ricklefs and Wikelski, 2002). Further, higher growth rate can be achieved by increased foraging activity and aggressive behaviours (Biro and Post, 2008), which may be possible through increased metabolic costs for basal maintenance (i.e. basal metabolic rate) (Rosenfeld et al., 2015). Thus, understanding sources of intraspecific variation in metabolic rate during early life will facilitate interpretation of adaptive phenotypic

responses at a most vulnerable life stage (Dmitriew, 2011; Killen et al., 2007; Pettersen et al., 2018; Speakman, 2005).

7.3.3 What drives variation and plasticity of metabolic rate during early life?

Metabolic rate is a rate of energy turnover that reflects biochemical processes in an organism (Nelson, 2016). Since oxygen is the final electron receptor in cellular aerobic respiration that produces the majority of ATP in animals, metabolic rate can be indirectly assessed by measuring whole body oxygen consumption rate (Rolfe and Brown, 1997). The minimum energetic costs required to maintain metabolic machinery is termed basal metabolic rate (BMR). Therefore, BMR represents the minimum energy expenditure to support vital processes such as ion regulation, substrate cycling and organismal integrity (Rolfe and Brown, 1997). BMR is measured when an animal is at post-prandial status and rest in a thermoneutral environment where a subject does not use excessive energy expenditure toward thermogenesis (Hulbert and Else, 2004). In contrast to mammals, metabolic rate of ectotherms changes with ambient temperature, so standard metabolic rate (SMR; BMR at particular temperature) is commonly used to represent the minimum energy expenditure to sustain life. Hereafter, SMR will be used to represent the minimum energy expenditure to support vital processes. The maximum oxygen consumption rate that can be achieved is termed maximum metabolic rate (MMR) (Norin and Clark, 2016). MMR represents the maximal capacity of an animal that could take up oxygen from the ambient environment, which sets the theoretical ceiling of aerobic metabolism. The difference between SMR and MMR is termed aerobic scope (AS), which represents the metabolic capacity that can support routine activities beyond basal maintenance (Clark et al., 2013). In this chapter, SMR, MMR and AS will be referred to metabolic rate phenotypes.

As SMR is the indispensable energetic cost to maintain life, studies have examined the relationship between SMR and growth, showing that a positive relationship exists when resources are abundant, such as in salmonids fed *ad libitum* (Cutts et al., 1998; Yamamoto et al., 1998). Increased foraging activity and basal metabolic costs for metabolic machinery such as the gastrointestinal tract could give rise to increased SMR, which can be associated with enhanced growth performance in fish (Zeng et al., 2017). It has been suggested that individuals with a relatively high SMR may be more efficient at digestion and assimilation as specific dynamic

action (i.e. energetic cost for digestion and assimilation of food) of these individuals was faster compared to those with a low SMR (Fu et al., 2005; Millidine et al., 2009). In addition, variability of SMR becomes important where resources change as those with greater variability in SMR demonstrated faster growth rate when food availability fluctuated (Auer et al., 2015). Interestingly, in the common minnow (*Phoxinus phoxinus*), those with accelerated growth rates following food deprivation and feeding *ad libitum* (i.e. compensatory growth) showed increased SMR with no change in MMR (Killen, 2014), suggesting that growth trajectory may be closely related to variation of energetic cost for maintenance of metabolic machinery (i.e. SMR) (Allen et al., 2016).

In contrast to SMR, MMR is the peak of aerobic metabolism that is not required for everyday life, variation in MMR may be associated with biophysical properties of the cardiorespiratory system (Hillman et al., 2013), which may be the result of physiological adaptations to foraging strategies and habitats. For example, acipenseriforms (i.e. sturgeon and paddlefish) are known to have a lower MMR than salmonids (Baker et al., 2005; Brown and Kieffer, 2019), which may reflect the lifestyle of the species (sedentary vs active). In addition, in rainbow trout (*Onchorynchus mykiss*), swimming performance was positively correlated with MMR and cardiovascular function (Claireaux et al., 2005). The difference between MMR and SMR can be regarded as the metabolic potential within an organism; thus, AS may represent the energetic capacity to perform aerobic activities such as locomotion, foraging and growth (Clark et al., 2013). Indeed, a previous study showed that AS in Atlantic cod (*Gadus morhua*) was positively correlated with critical swimming speed (Reidy et al., 2000), and those with greater AS showed a more rapid recovery from an exhaustive exercise protocol in European sea bass (*Dicentrarchus labrax*) (Marras et al., 2010). Despite theoretical suggestion of AS, the recent literature highlights that the correlation between AS and growth is equivocal and may be context specific (Grans et al., 2014; Leal et al., 2021; Messmer et al., 2017; Norin et al., 2014).

As metabolic rate may underpin physiological phenotypes, studying intraspecific variation of metabolic rate may help understand how organisms adjust phenotypic responses to changing environments (Norin and Metcalfe, 2019b). The concept of intraspecific variation in metabolic rate may be particularly important during early life history where energetic demands are high to support rapid growth rate. Therefore regulation of metabolic machinery may directly influence energetic allocation toward growth in fish (Rosenfeld et al., 2015). To date, most

studies have examined how variation of metabolic rate phenotypes are influenced by environmental conditions such as temperature, dissolved oxygen, diet and pH (Bouyoucos et al., 2020; Durhack et al., 2021; McKenzie, 2001; Pörtner, 2010). However, intraspecific variation of metabolic rate may be related to variability in the architecture of the gas exchange system and preference of respiration substrate (see Figure 7.1.) that may be the result of interactions between the genotype and environment (Dhillon and Schulte, 2011; Hillman et al., 2013).

Studying intraspecific variation of metabolic rate is challenging because interpretation of results can often be dependent upon experimental artifacts. For example, subtle changes in respirometry methodology can result in different estimations of metabolic rate (Chabot et al., 2021; Killen et al., 2021; Raby et al., 2020). Therefore, metabolic rate data should ideally be collected using the same methodology. The data in this thesis represents an excellent opportunity to compare within species across space and time as we have used the same method to record metabolic rate of lake sturgeon over six generations and two populations.

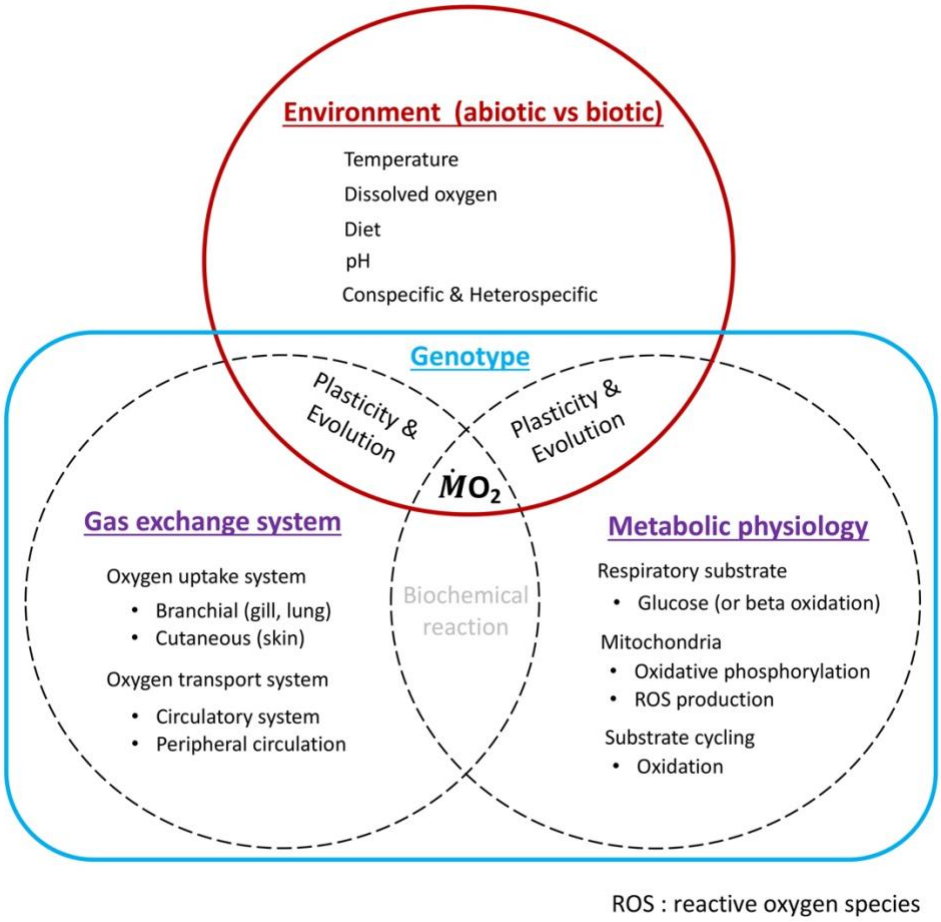

Figure 7.1. Schematic figure to illustrate factors to influence intraspecific variation of metabolic rate phenotype in animals. Environment strongly influence biochemical reactions between gas exchange system and metabolic physiology, which directly influences metabolic rate ($\dot{M}O_2$; oxygen consumption rate). Also, environment interacts with genotypes of gas exchange system and metabolic physiology, which leads to plasticity and evolution of metabolic rate phenotypes that may be ultimately involved in physiological and behavioural phenotypes.

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