The effects of flow on growth, fin, and otolith development in young of year lake sturgeon, *Acipenser fulvescens*

By

Kassandra Merks

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Department of Biological Sciences

University of Manitoba

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Abstract

Early rearing environment is critical for development of phenotypes, which will ultimately influence the fitness of the individual. In conservation aquaculture it is even more important to ensure the development of appropriate phenotypes upon release from the hatchery. A natural part of the Lake sturgeon (*Acipenser fulvescens*) habitat is water flow; therefore understanding its role in development is important for our conservation aquaculture programs. This thesis set out to examine the effects of flow on growth, fin, and otolith development in two cohorts of larval lake sturgeon. Larvae were reared in three treatments designed to increase the complexity of flow. A simple treatment with no flow, a moderate treatment with the addition of flow, and a complex treatment with both flow and substrate. Measurements of mass, total length, body depth, fin length, and finspan were taken for chapter 2 to investigate growth and fin differences across rearing environments. In chapter 3, measurements of otolith surface area, perimeter, and maximum diameter were taken from otolith imagery for differences in size across rearing environments. Additionally, otolith polymorph composition was quantified and difference between left and right otoliths were taken. This research demonstrated that growth was affected by flow and substrate use as fish were largest in the simple treatment. This was likely the result of a combination between poor foraging in substrate and above optimum flow. Further, the differences in growth were no longer seen by the last sampling point indicating fish grew larger and could forage efficiently or they perished. Fin metrics did show some differences with treatment; however, it was determined to be highly correlated with fish length. Otolith polymorph composition was not influenced by the rearing environment, and the ratio of aragonite and vaterite appeared similar in both years and across studies suggesting otolith development may be a fixed phenotype. However, otolith size was influenced by rearing

environment where the simple treatment had the largest otoliths in the 2019 while the moderate treatment had the largest otoliths in the 2020 cohort. Further research into differences in swimming abilities and balance related to otolith size, as well as behavioural benefits such as foraging efficiency and predator evasion in this environment is indicated before hatcheries should widely adopt rearing with flow.

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Chapter 1: General Introduction

1.1 General biology of Lake Sturgeon (*Acipenser fulvescens)*

Sturgeon are considered a prehistoric fish that evolved approximately 200 million years ago and are sometimes referred to as 'living fossils' (Bemis et al., 1997). They are large bodied, cartilaginous, benthic fish (Peterson et al., 2007) and the sturgeon family (Acipenseridae) is comprised of 27 species in four genera, the largest of which is *Acipenser* containing 17 species (Auer, 1996a; IUCN 2015). Sturgeon populations globally have been deemed the most threatened group of animals on the IUCN Red List, with 85% of sturgeon at risk of extinction (IUCN 2015). Since the early 1900s, sturgeon populations have declined largely due to overharvest for their prized caviar, this combined with pollution, habitat fragmentation and loss, and their life history traits such as late age to sexual maturity, intermittent spawning, and slow growth have led to slow population recovery (Peterson et al., 2007).

The lake sturgeon (*Acipenser fulvescens)* Rafinesque 1817, is native in North America occupying the Laurentian Great Lakes, Hudson Bay, and Mississippi River drainage basins (Peterson et al., 2007). Lake sturgeon are thought to sexually mature at 12-15 years for males and 18-27 years for females and spawn only every 1-3 years and 4-9 years respectively (Bruch and Binkowski, 2002). Females carry an approximate average of 400,000 eggs (Bruch et al., 2006) and spawning typically occurs in early spring at water temperatures 11.5 - 16°C over gravel or cobble substrate with water flows from 0.5-1.3 $m \cdot s^{-1}$ (Bruch and Binkowski, 2002). The fertilized eggs fall within the crevasses of the substrate where they develop and hatch as yolk sac larvae between 7-12 days post fertilisation (Eckes et al., 2015).

They are unique in that among the *Acipenser* species they are the only species that complete their entire life cycle in freshwater (potandromous). Like other sturgeons, they have three rows of scutes, a heterocercal tail, a white ventral surface, and sensory barbels. Sturgeon use their pectoral fins to actively swim in the current as well as to perform station holding. In the Winnipeg river the diet of juvenile Lake Sturgeon consisted of a variety of benthic invertebrates from the orders, Trichoptera, Diptera, and Ephemeroptera (Barth et al., 2013). Total length and body mass range within adults from 100-200cm and 11-100Kg respectively, with females typically being larger in both mass and length than males at an equivalent age (Peterson et al., 2007).

Despite moratoriums on lake sturgeon harvest across North America and continued stocking programs within Canada the species remains threatened or endangered in most of its native range (COSEWIC, 2017). For decades, hatcheries have been raising lake sturgeon for stock enhancement of natural populations (Pollock et al., 2015) and will produce fish typically in one of three ways: gametes are collected directly from spawning males and females and are then fertilized and on-grown for subsequent stocking (Ceskleba et al., 1985; Crossman et al., 2011); recently naturally fertilized eggs are collected and allowed to develop and on-grown in a hatchery environment (Crossman, 2008; Crossman et al., 2011); recently hatched larval sturgeon are collected in drift traps and on-grown in a hatchery environment prior to release at site of capture (Auer and Baker, 2002; Crossman et al., 2011). These programs have been ongoing without fully considering the effects of the rearing environment on phenotypic development. This is of the utmost importance during early rearing where development is critical to promote wild-like phenotypes.

Several studies have examined the effects of different rearing environments on a variety of phenotypes. For example, lake sturgeon incubated in tumbling jars compared to egg mats showed reduced growth and a delayed cortisol response (Earhart et al., 2020). Lake sturgeon reared in different calcium concentrations demonstrated that calcium limitation increased growth rate and rate of yolk absorption (Genz and Anderson, 2020). In white sturgeon (*Acipenser transmontanus*) yolk sac larvae reared in elevated temperatures with gravel substrate grew larger than the fish reared at lower temperatures without substrate (Boucher et al., 2014). Further, survival was increased by 20% in larval fish reared with substrate and the fish contained more lipid vacuoles in their liver (Boucher et al., 2014). Some phenotypic changes have been seen to persist up to a year later and theoretically could persist their entire life. For example, lake sturgeon reared in an elevated temperature treatment in the first three weeks of life continued to have an increased growth rate up to one-year post-manipulation compared to fish reared at a lower temperature in the first three weeks of life (Brandt et al., 2022). All of these examples provide evidence of the importance of rearing environment on development and how this could impact the fish beyond their time within the hatchery.

1.2 Otoliths

Otoliths are paired calcified structures in the inner ear of fish that are responsible for hearing and balance (Campana, 1999). There are three pairs of otoliths in fishes: the sagittal, asterisci and lapilli. They are located in the saccule, lagena, and utricle respectively within the three connected semi-circular canals of the inner ear (Oxman et al., 2007). The sagittal otolith has been used to determine the ages of fish for over a century due to the daily and annular growth increments (Campana, 1999). These increments create rings called circuli, similar to tree rings that are defined as increased growth producing lighter or translucent growth bands and

darker more opaque bands defining slower growth periods. The pattern of light and dark bands represents a year of growth called an annulus. The otolith is also metabolically inert, and retains any element accreted onto its growing surface (Campana, 1999). Elements are retained in the otolith by either taking the place of calcium (i.e. strontium) or elements bind within the lattice structure of the calcium carbonate (Veinott et al., 1999). All of these properties make them ideal for use in fisheries science. Not only are otoliths used for age identification, but they can also be used for taxon identification, stock discrimination, growth estimates, evolutionary relationships, and tracing habitat movement (Nolf, 1993; Elder et al., 1996; Jackson, 2007; Chalupnicki and Dittman, 2016).

The primary biomineral in otoliths is calcium carbonate ($CaCO_3$) that crystalizes in the form of aragonite, calcite, and/or vaterite (Campana, 1999). Each polymorph of calcium carbonate has different structural and chemical properties with calcite and aragonite being more thermodynamically stable than vaterite and found abundantly within nature (Ni and Ratner, 2008). Calcite is the densest followed by aragonite, then vaterite (Ni and Ratner, 2008). The polymorphs have different densities due to the crystal shape, where calcite is rhombic, aragonite needle like, and vaterite spherical (Ni and Ratner, 2008). A typical teleost sagittal otolith is comprised of aragonite (Oxman, et al., 2007). However, the polymorph composition in fishes has been found to be influenced by development and rearing environment (Bowen et al., 1999; Reimer et al., 2017; Loeppky et al., 2019). Specifically, differences have been reported in hatchery reared fish likely due to environmental difference between the rearing environment in the wild compared to the hatchery. For example, hatchery reared Atlantic salmon (*Salmo salar)* were found to have vaterite inclusions ten times higher than in wild Atlantic salmon (Reimer et al., 2016). In lake trout (*Salvelinus namaycush*) hatchery fish transitioned to vaterite from

aragonite within the first 3-4 months in early life at a higher proportion than the wild counterparts (Bowen et al., 1999). These differences are not limited to just polymorph composition but also size and shape. In coho salmon (*Oncorhynchus kisutch*) wild fish otoliths were found to be smaller, more dense, white and regularly shaped compared to the hatchery otoliths, which were larger, less dense, translucent and irregularly shaped (Chittenden et al., 2010). There has also been reported differences between the left and right otolith, where vaterite inclusions were greater in the left sagittal otolith compared to the right in Atlantic salmon (Reimer et al., 2016).

There are notable differences between teleost and sturgeon otoliths. As previously mentioned, teleost otoliths are described to be primarily comprised of aragonite (Oxman et al., 2007). Lake sturgeon otoliths were previously believed to be completely comprised of vaterite (Carlstrom, 1963). However, it was recently demonstrated that adults contained a large proportion of calcite within the sagittal otolith (Pracheil et al., 2017). It was also shown that larval and juvenile life stages, have a substantial proportion of aragonite within their sagittal otolith (Loeppky et al., 2019). Thus, providing evidence that sturgeons have the ability to produce all three polymorphs (Loeppky et al., 2019). However, the polymorph composition in lake sturgeon has been shown to differ with temperature, where higher temperatures produced otoliths containing a small proportion of calcite (Leoppky et al., 2021). The difference in polymorph composition is not the only difference between teleost and sturgeon otoliths. The shape of sturgeon otoliths also tends to be more irregular, and the otoliths tend to be very granular in texture (Arai and Miyazaki, 2002). This granularity makes otoliths difficult to bisect for ageing (Secor et al., 1992). So alternatively, fin rays are used as a nonlethal sampling technique for aging in sturgeon (Bruch et al., 2009).

1.3 Pectoral fin ray

Another structure that holds similar properties to the otolith is the fin ray. Fin rays are composed of hydroxyapatite, a calcium phosphate-based structure (Outridge et al., 1995), which like otoliths incorporate different elements within their lattice structure. The fin ray also contain annuli and similar methods comparable to otoliths are used to estimate age in numerous species of fish (Rude et al., 2013) and as such a non-lethal sampling of a fin ray for age and life history estimations is a useful tool, particularly for endangered species like the lake sturgeon. There are a few sampling techniques for obtaining fin rays; either to remove the entire leading fin ray or dissecting a notch out of the leading fin ray (Nguyen et al., 2015). Age estimates from pectoral fin rays are accurate from younger fish, however, they begin to lose accuracy as the fish gets older (Bruch et al., 2009). Indeed, age estimates from using pectoral fin rays from older white sturgeon (*Acipenser transmontanus),* shovelnose (*Scaphirhynchus platorynchus*), and pallid sturgeon (*Scaphirhynchus albus*) were determined unreliable (Rien and Beamesderfer, 1994; Hurley et al., 2004; Whiteman et al., 2004).

Fin rays provide a structural framework that supports the fins but allows them to be flexible to support the variety of movements in fishes, including propulsion, braking, and maneuvering (Taft, 2011; Deslauriers et al., 2016). Sturgeon pectoral fins are extensions of the basal and radial bones of the fin from its body and are non-collapsible; a morphological feature of their place in the evolution of ray-finned fish (Wilga and Lauder, 1999). They play an important role in regulating swimming performance by generating lift and facilitate station holding behaviour (Wilga and Lauder, 1999; Adams et al., 2011; Deslauriers et al., 2016). For example, wider fin area can enhance the ability of the fish to navigate habitats with higher currents (Wainwright et al., 2002). In sand darters occupying similar habitats, the species

occupying swifter currents demonstrated a difference in pectoral fin shape and length, which resulted in observed differences in their station holding behaviour (Carlson and Lauder, 2009). Therefore, it is reasonable to presume that fish reared in a hatchery setting may demonstrate differences in fin ray development that are dependent on the early rearing environment.

1.4 Environment phenotype interactions

A phenotype is an observable physical trait of an organism that is determined by its genotype as well as its interaction with the environment. Phenotypes can be heritable, where the events the parents experience can directly translate to offspring having phenotypes who never experience the original events themselves (Best et al., 2018). For example, maternal cortisol levels can alter physiological and behavioural phenotypes in their offspring (Wassink et al., 2020). This has been seen in cod (*Gadus morhua*), where stressed females having higher cortisol levels, deposited higher levels of cortisol into developing eggs, which resulted in elevated egg cortisol post spawn (Kleppe et al., 2013). Phenotypes can also be plastic, meaning the organisms are able to respond to changes in the environment without genetic changes. For example, adults can respond to different environments during spawning by behaviourally selecting breeding locations to optimize egg survival (Dammerman et al., 2015). For sturgeon one example is adjusting the location of spawning to find an appropriate flow rate because excessive flows can dislodge fish eggs from substrate and flows too low can decrease oxygen levels in the interstitial spaces in which eggs reside causing increased mortality (Auer, 1996b; Dammerman et al., 2015). These interactions between the environment and phenotypes happen throughout an organism's lifetime, but are especially significant during early development (Monaghan, 2008).

During early development, fish are extremely vulnerable, growing rapidly, and the conditions they are exposed to directly affect the phenotypes both during residence and later in life which ultimately influence the fitness of the individual (Dammerman et al., 2015). Therefore, the habitat during embryonic and larval stages are critical in developing appropriate phenotypes. Understanding this is perhaps of particular importance when rearing fish for conservation aquaculture, as a major goal in conservation aquaculture is to create fish with wild like phenotypes. This is difficult to achieve, as most hatchery systems are sufficiently dissimilar to the wild environment during that period of life, and therefore phenotypic differences between wild and hatchery reared fishes are unavoidable (Chittenden et al., 2010). However, changes to the hatchery environment might facilitate phenotypic development that is more similar to their wild counterparts and therefore might allow avoidance of maladaptive traits. One environment phenotype interaction that has been studied frequently is the effect of temperature on development. It is well known that temperature within a fish's natural range is typically positively correlated with growth (Cardinale et al., 2004). The manipulation of temperature during early development has been shown to influence the number of fin rays and vertebrae formed in a variety of fishes (Barlow, 1961). Further, temperature has been shown to alter otolith size and polymorph composition (Loeppky et al., 2021). Temperature is not the only abiotic variable we manipulate in the hatchery that can affect phenotypes. Another environmental variable that is manipulated is water flow. In aquaculture, the addition of flow has been found to have a profound impact on metabolism, growth, behaviour, and welfare of fish (Palstra & Planas, 2011; Nilsen et al., 2019). In Atlantic salmon parr, the parr reared in complex flow environments of the stream had significantly longer fin lengths than hatchery reared parr (Pelis and McCormick, 2003). In a study on lake sturgeon reared under different flow conditions that mimic

the natural environment fish were shown to have greater variation in growth under higher flow conditions (Dammerman et al., 2015). Another study on lake sturgeon demonstrated difference in growth by incubation method, where eggs incubated on egg matts with flow had greater mass and length than eggs incubated in tumbling jars (Earhart et al., 2020). There have been fewer studies conducted with regards to water flow and sturgeon, despite it being part of their natural early rearing environment. There is a need to further investigate how flow affects larval sturgeon development to not only understand environment phenotype interactions, but how it could relate to potential maladaptive traits in fish reared in aquaculture environments for conservation purposes.

1.5 Thesis hypotheses and objectives

In this study, the effects of flow and substrate on growth, fin and otolith development in larval lake sturgeon were explored to test the following hypotheses:

(1) Different rearing environments will result in differences in growth rate during development.
(2) Different rearing environments will result in differences in fin length during development.
(3) Different rearing environments will result in differences in otolith size during development.
(4) Different rearing environments will result in differences in otolith polymorph composition during development.
(5) Difference in left and right otoliths will be seen in size and polymorph composition

I predict as environment complexity increases there will be a negative relationship with growth and development, but otolith composition will be relatively stable as rearing environments alter. In testing these hypotheses, I hope to improve our understanding of how different environmental conditions effect fish growth and otolith development. This research if applied, could potentially lead to different hatchery rearing protocols that promote the development of alternative phenotypes that may aid in survival once released.

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Chapter 2: Effect of water flow and tank substrate on growth and fin development in hatchery reared larval lake sturgeon, *Acipenser fulvescens*

*Kassandra M. Merks and W. Gary Anderson

Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada, R3T 2N2

KMM – study conception and design; data collection, curation and analysis; drafting of manuscript; WGA study conception and design; manuscript and data review and editing; funding acquisition

*Corresponding author (Merksk1@myumanitoba.ca)

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2.1 Abstract

Environmental enrichment can influence phenotypic development during early rearing of fish in a hatchery setting bridging the gap between wild and hatchery environments. In the present study, we examined the effects of water flow and tank substrate on mass, total length, body depth, fin length, finspan, and mortality during the first four months of life in two cohorts of larval lake sturgeon (*Acipenser fulvescens)*. Fish were subjected to one of three rearing environments designed to increase environmental complexity: simple, no flow and no substrate; moderate with flow and no substrate; and complex with flow and substrate. The moderate and complex treatments significantly decreased growth throughout the study period, regardless of year, however, by the end of the sampling period all treatments were equal in metrics measured. The most likely cause for differences in growth was attributed to reduced foraging efficiency and above optimal flows. Further, finspan (maximum length of pectoral fins measured from tip to tip) had a strong positive relationship with total length that did not differ with treatment demonstrating no influence from substrate and flow. Finally, mortality was found to be greatest in the complex treatment supporting a reduced foraging efficiency in this treatment post-hatch. Despite the increased mortality in the complex treatment group, inclusion of flow and substrate in a conservation hatchery environment should be researched further, as potential behavioral traits (foraging and station holding) gained from this enriched environment will be relevant to the wild environment into which they will be released.

2.2 Introduction

Conservation aquaculture has developed rapidly over the past decade and is being used in numerous ways to improve the status and condition of species and ecosystems that have been damaged due to anthropogenic causes (Anders, 1998; Froehlich et al., 2017). The smallest scale of conservation aquaculture is at the species level with the most common example being the use of hatcheries for enhancement of fish stocks (Schreier et al., 2012; Froehlich et al., 2017). Conservation hatcheries have been used for numerous species, and the success of these programs is measured against the management objectives which can range from the addition of fish to supplement harvest to the recovery of extirpated species (Schreier et al., 2012). With respect to achieving these goals, it is important that hatcheries continue to develop a clearer understanding of the effects of the environment on phenotypic development as fish reared in a hatchery environment invariably develop a different phenotype from their wild counterparts (Chittenden et al., 2010, Johnsson et al., 2014).

Increasing complexity of the rearing condition through environmental enrichment is often used to replicate natural rearing environments and will influence phenotypic development (Newberry, 1995; Näslund and Johnsson, 2016). For example, previous studies have shown that the inclusion of tank substrate, at various life stages, had many beneficial impacts on development (Wagner et al., 1996; Arndt et al., 2001; Ottesen et al., 2007; Näslund and Johnsson, 2016). In rainbow trout (*Oncorynchus mykiss)*, and cutthroat trout (*Oncorhynchus clarkii),* the presence of cobble substrate in raceways during early rearing resulted in reduced fin damage (Wagner et al., 1996; Arndt et al., 2001) and in Atlantic halibut (*Hippoglossus hippoglossus),* juveniles showed increased skin lesions and limited healing capacity when raised in smooth tanks compared to fish raised over substrate (Ottesen et al., 2007). Furthermore, the

addition of substrate during the incubation and hatchling stages typically has positive effects on growth and survival in salmonids (Näslund and Johnsson, 2016). In yolk sac larval white sturgeon (*Acipenser transmontanus)*, inclusion of gravel substrate improved growth, condition, and survival (Boucher et al., 2014; Bates et al., 2014) and when given a choice between small (10-16mm), medium (30-40mm) and large (64-80mm) substrate, white sturgeon showed a preference for the smaller substrate as free embryos and hatchlings (Kynard et al., 2013). Additionally, Atlantic sturgeon (*Acipenser oxyrinchus)*, raised in gravel substrate showed a significant increase in larval survival but larval fish were shorter in length (Gessner et al., 2009). However, lake sturgeon (*Acipenser fulvescens)*, raised over substrate at the yolk sac and larval stage had no difference in mass and length compared to those raised over smooth bottom (Zubair et al., 2012) but in another study the presence of substrate during the transition to exogenous feeding resulted in a reduced growth rate compared to those raised over smooth bottom (Yoon et al., 2020a).

Changes in flow have also been used in aquaculture to influence growth and activity in farmed fish. In general, the addition of flow has been found to have a profound impact on metabolism, growth, behaviour, and the welfare of fish (Palstra and Planas, 2011; Nilsen et al., 2019). In salmonids, flow rate tends to be positively correlated with feed conversion ratios and growth rate (Jobling et al., 1993; Davison, 1997; Castro et al., 2011). Higher velocities (~0.5-1 body lengths$\cdot s^{-1}$) resulted in higher growth rates (Jobling et al., 1993; Castro et al., 2011; Ytrestøyl et al., 2017) and variable flow compared to constant increased flow had no effect on growth rate but significantly improved disease resistance in Atlantic salmon (Castro et al., 2011). As station holding is a behaviour exhibited in numerous species that live within high flow environments, flow may also influence pectoral fin length. Indeed, a strong relationship between

pectoral fin length and fork length has been shown in Atlantic salmon parr and parr reared in the complex flow environments of a stream had significantly longer fin lengths than hatchery reared parr (Pelis and McCormick, 2003). There are risks of having too much flow, which results in reduced growth which may be seen as a result of increased energy demand to maintain station in higher flow (Palstra and Planas, 2011)

Combining enrichment methods can result in a more naturalized environment than the single variable. For example, Atlantic salmon smolts subjected to variable salinity and velocity had improved growth rate in reduced salinity, but increased velocity had a positive effect on growth in all salinities (Ytrestøyl et al., 2017). Furthermore, Atlantic salmon raised over gravel substrate in elevated temperatures had better survival than those raised in elevated temperature with no substrate (Bamberger, 2009). Additionally, white sturgeon larvae raised over substrate in elevated temperatures grew larger than fish in no substrate (Boucher et al., 2014). In lake sturgeon, enhanced condition factor was reported for fish that were raised with substrate and 100% dissolved oxygen (Yoon et al., 2018). Furthermore, lake sturgeon raised with substrate and increased temperature during the yolk-sac stage demonstrated improved growth compared to traditional hatchery methods (Brandt et al., 2022). Thus, the influence of variable environmental factors on phenotypic development are complex and are likely dependent on species and life stage.

Due to human pressures of overfishing, habitat fragmentation and loss over the past century, lake sturgeon populations have drastically declined throughout their natural range. This combined with a slow rate of maturity and intermittent spawning has made recovery efforts challenging, leading to many populations being threatened or endangered (Peterson et al., 2007).

To help restore and maintain current populations, conservation hatcheries have been used as one mitigating strategy; however, our understanding of the impact of flow and its complexity, in the early rearing environment on phenotypic development is not fully understood. In the present study we assessed growth and fin length in lake sturgeon in response to increasingly complex environments during early life. Specifically, we tested the hypothesis that fish reared in the more complex environment that included substrate and flow, would grow larger in total length, mass, body depth, and longer fin length and finspan due to the energetic requirements of station holding and foraging against a flow.

2.3 Methods

In 2019 and 2020, spawning adult lake sturgeon were captured using gill nets downstream of Pointe du Bois on the Winnipeg River (50° 18’ 06.6’’ N, 95° 32’ 29.5’’W) in Manitoba, Canada. Milt was collected immediately from males and stored at 4°C for a maximum of 24h or when needed. Females captured were transported to a riverside holding tank supplied with flow through river water at ambient temperature from two submersible pumps placed in the river adjacent to the holding tanks. To stimulate maturation and egg release, females were given two doses of gonadotropin releasing hormone (GnRH; 10 and 5 mg kg^{-1}), the first 36 hours prior to stripping eggs manually and the second 24 hours prior. After collection, gametes were transported separately to the University of Manitoba’s animal holding facility to undergo fertilization where approximately 15 ml of eggs were fertilized with approximately 200µl of mixed sperm as previously described (Loeppky et al., 2019). In 2019 a total of two females and four males were used while in 2020 one female and two males were used for similar genetic makeup across treatments. The fertilized eggs were evently distributed across all treatments to reduce genotypic effects.

In 2019, all fertilized eggs were transferred to MacDonald tumbling jars for incubation with a constant supply of fresh water starting at 12°C and increased to 16°C at 0.5°C a day. Once hatched (~7-10 days), the larval fish were transferred to flow-through holding tanks under normal hatchery conditions (16°C, 12 hr light:dark photoperiod). At 25 days post fertilization (dpf) fish were transferred to one of three treatments with increasing environmental complexity each in triplicate:

1) SIMPLE - Rearing environment that contained no substrate and no addition of flow, similar to existing hatchery conditions.
2) MODERATE - Rearing environment with flow and no substrate. Flow was created using electric submersible pumps at one end of the holding tank. Flow averaged 630 +/- 68.9 $mL \cdot s^{-1}$. Interior tank dimensions 5.08 x 13.97 x 34.93 cm
3) COMPLEX - Rearing environment with flow and substrate in the form of pebbles (~2 cm in diameter) covering ~90% of the bottom of the tank to create a variable flow regime. Flow averaged 630 +/- 68.9 $mL \cdot s^{-1}$. Interior tank dimensions 5.08 x 13.97 x 34.93 cm

In 2020 fertilized eggs for the simple treatment were transferred to MacDonald tumbling jars starting at 12°C and slowly increased to 16°C at 0.5°C a day until hatched (~7-10 days) and then placed into their respective treatment tanks. However, fertilized eggs for the moderate and complex treatments were evenly divided across those treatment tanks and allowed to adhere to the substrate/tank bottom. This change between years occurred to allow for the larvae to be exposed to the most natural incubation possible. All treatment tanks in both years maintained equivalent stocking density, temperature (16°C) and photoperiod (12 hr light:dark) for the

remainder of the experiment. Flow rate was manually calculated using the equation Q=w*h*(l/t), by measuring the tank dimensions (l= length, w= width, h=height) and surface velocity (88.8 +/- 8.97 $mm \cdot s^{-1}$). Surface velocity was taken by timing how long a uniform floating object took to go the known length of the tank. This was repeated ten times and averaged.

In both years, after yolk absorption (~14-17 days post fertilization, dpf) fish were fed three times a day with freshly hatched brine shrimp (Artemia International LLC, Texas, USA). At feeding, flow was stopped in all holding tanks and fish were fed *ad libitum* for 15-20 minutes, after which any excess food was carefully removed with a siphon and flow was returned to all tanks. Around 30 dpf fish began transition from brine shrimp to chopped bloodworm (Hakari USA, California, USA) using a ratio of 10:1 increasing to 100% bloodworm at 50 dpf. The pebbles were cleaned twice weekly by carefully removing them from the tank and rinsing them off with warm water prior to soaking in Oxivir solution (Diversey, Mississauga, ON, Canada) for a 24-hour period. After the Oxivir solution they were rinsed again until the water ran clear, then the pebbles were dried and subsequently reused the following week. Tanks were without substrate for a maximum of 5 minutes during cleaning throughout rearing.

A total of 1053 larval fish were sampled over both years. In 2019 fish were sampled weekly between 39 and 95 dpf. In 2020, due to reduced total number of fish available, fish were sampled every two weeks from 18 to 88 dpf. At each sampling timepoint 24 fish (numbers permitting) were haphazardly selected using a dip net and immediately euthanized by immersion in an overdose of Tricaine methanesulfonate (MS-222) buffered with an equal volume of sodium bicarbonate (250mg ml^{-1}). Body mass (g) and total length (mm) were taken using a digital scale and calipers. Body depth (mm) was measured by placing the fish on their right side and aligning

the calipers before the first scute where the operculum opening begins. Left pectoral fin length (mm) was measured by placing the fish on the ventral surface and maneuvering the left pectoral fin to open with the leading fin ray perpendicular to the body and measuring at the point of articulation to the tip of the fin. Finspan was also measured with the fish in the same position as fin length, except both fins were opened with the leading fin rays perpendicular to the body and the measurement was taken from the tip of one pectoral fin to the other. Due to the accuracy of the digital calipers and size of fish, fin length and finspan were only measured by one user and when they could ensure repeatability, as such in 2019 these morphometrics were taken starting at 53 dpf until 95 dpf, and in 2020 starting at 60 dpf until 88 dpf. Mortality was recorded three times a day during the duration of the study, by removing and counting the number of dead fish removed.

A linear mixed model (LMM) was used to analyze the data to account for the random effect of rearing tank and unbalanced design as previously described (Yoon et al., 2018; 2020b). Substrate and flow were considered discrete factor variables and are represented by S (absence or presence) and F (absence or presence), respectively. a_{ID} represents the intercept of random effects of rearing tank and e represents the residual errors. The full model was written as follows:

$$\hat{R} = a_0 + b_s \cdot S + b_f \cdot F + b_{sf} * SF + a_{ID} + e$$

Where the response variable ($\hat{R}$) represents either mass, total length, body depth, fin length or finspan. The model was used to assess all variables independently at each sampling point across treatments for each year. Assumptions of normality and homoscedasticity were visually assessed using methods previously described by Zuur et al., 2010. When assumptions were not met, the data was either a log or Tukey transformed to meet the assumptions. A likelihood ratio test was

performed to test significance of each discrete variable using the function ‘anova’ in the R package lme4 with backward elimination (Winter, 2013; Yoon et al., 2020b). When there was significance, *post hoc* comparisons were performed for each sampling point within treatments using the ‘glht’ function in the R package multicomp with significance determined at alpha = 0.05. Cumulative mortality rate was analyzed by a Cox proportional hazards model, conducted in R using the survival package (Therneau, 2020), to evaluate differences in mortality between treatments for the duration of the study. When the analysis indicated significance, a *post hoc* log-rank test was performed to determine differences between treatments 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. All statistical analysis was conducted using RStudio 1.2.5033 (R core Team, 2020).

All experimental protocols performed on fish were approved by the Animal Care Committee at the University of Manitoba permit# F15-007 under the guidelines of the Canadian Council for Animal Care.

2.4 Results

Mass

In 2019 and 2020 mass increased with age for fish in all three treatments (Figure 2.1.). In 2019 at 39, 46, and 67 dpf mass of fish was significantly influenced by substrate (P=0.003, 0.0058, and 0.0039 recpectivly) and in post hoc analysis, fish reared in the complex treatment were significantly lower in mass compared to fish raised in the moderate and simple treatments (P<0.01). At 53 dpf substrate significantly influenced fish mass (P=0.039), however, treatments showed no significant difference. There was also a significant influence of substrate on fish mass

at 88 dpf (P=0.024), with fish in the simple and complex treatments being significantly different from each other but not from fish in the moderate treatment (P<0.01). At 74 and 81 dpf flow significantly influenced mass (P=0.0011 and 0.025 recpectivly). At 60 and 81 dpf fish reared in the simple treatment and complex treatment were significantly different from each other (P<0.01) but not from moderate. At 74 dpf mass of fish reared in the simple treatment was significantly higher compared to fish raised in the moderate and complex treatments (P<0.01). At 95 dpf there was no significant effect on body mass from flow or substrate, and no treatment was significantly different from the other.

In 2020, at 18 and 32 dpf the use of substrate significantly influenced fish mass (P=0.025, 0.0073 recpectilvly) as the fish raised in the simple treatment had significantly higher mass compared to fish from the complex and moderate treatments (P< 0.01). At 46 and 60 dpf, flow was found to significantly influence mass (P=0.026 and 0.0018 recpectilvly), and fish reared in the complex treatment had significantly lower mass compared to the simple and moderate treatment fish (P<0.02). At 81 and 88 dpf there was no significant effect on body mass from flow or substrate, and no treatment was significantly different from the other.

Total Length

In 2019 and 2020 total length increased with age in a similar pattern as described for body mass (Figure 2.2.). In 2019, substrate significantly influenced total length at 39 (P=0.0067), 46 (P=0.004), 53 (P=0.0071), 67 (P=0.0025), and 88 (P=0.0077) dpf. Fish reared in the complex treatment were found to be significantly shorter in length compared to fish in the simple and moderate treatments (P<0.02). At 74 dpf flow significantly influenced total length (P=0.002) as fish reared in the simple treatment were significantly longer compared to fish reared in the

moderate and complex treatments (P<0.01). At 81 dpf both substrate and flow influenced total length (P=0.041 and 0.028 recpectivly) as fish reared in the complex and simple treatments were significantly different from each other (P<0.01) but not the moderate treatment fish. At 60 and 95 dpf there was no significant effect on total length in fish in flow or substrate treatments. At 60 dpf fish reared in the complex and simple treatments were significantly different from each other (P<0.04) but not from fish reared in the moderate treatment. At 96 dpf no treatment was significantly different from the other.

In 2020, at 46, 60, and 81 dpf substrate significantly influenced the total length of fish (P=0.014, 0.00019, and 0.024 recpectivly). In post hoc comparisons for 46 dpf, fish reared in the complex and moderate treatments were significantly different from each other (P<0.01) but not from the simple treatment. At 60 dpf fish reared in the complex treatment were significantly shorter compared to fish in both the moderate and simple treatments (P<0.01). At 81 dpf fish reared in the simple and complex treatments were significantly different from each other (P<0.04) but not from fish raised in the moderate treatment. At 18, 32, and 88 dpf there was no significant impact on total length from substrate or flow and no treatment was significantly different from each other.

Body depth

In 2019 and 2020 body depth increased with age (Figure 2.3.). In 2019 at 46, 53, and 88 dpf substrate had a significant effect on the body depth of fish (P=0.0086, 0.054, and 0.03 recpectivly). At 46 dpf fish reared in the complex and moderate treatments were significantly different from each other (P<0.01) but not from fish reared in the simple treatment. At 53 dpf no treatment was significantly different from each other. At 88 dpf fish reared in the simple and

complex treatments were significantly different from each other ($P<0.01$) but not from fish reared in the moderate treatment. At 67 dpf both substrate and flow were found to significantly influence body depth ($P=0.015$ and 0.02 recpectivly) as fish reared in the complex treatment were significantly shorter in body depth compared to fish in the simple and moderate treatments ($P<0.04$). At 74 and 81 dpf flow significantly influenced body depth ($P=0.05$ and 0.0094 recpectivly). At 74 dpf fish reared in the simple and complex treatments were found to be significantly different from each other ($P<0.01$) but not from fish in the moderate treatment. At 81 dpf fish reared in the simple treatment were significantly taller in body depth than fish reared in the complex and moderate treatments ($P<0.01$). At 39, 60, and 95 dpf flow and substrate did not significantly affect body depth. In post hoc comparisons at 60 dpf fish reared in the simple and complex treatments were significantly different from each other ($P<0.01$) but not from fish reared in the moderate treatment. At 39 and 95 dpf no significant difference was found between treatments.

In 2020, at 32 and 46 dpf substrate was found to significantly influence body depth ($P=0.033$ and 0.0028 recpectivly). At 32 dpf fish reared in the moderate treatment were found to be significantly taller compared to the fish reared in the simple and complex treatments ($P<0.02$). At 46 dpf a significant treatment effect was found between fish reared in the complex and moderate treatments ($P<0.01$) but not with the fish reared in the simple treatment. At 60 dpf both substrate and flow were found to have a significant effect on body depth ($P=0.049$ and 0.023 recpectivly) with no significance between treatments. At 18, 81, and 88 dpf there was no significant effect of substrate and flow on body depth and treatments were not significantly different from each other.

Fin length

In 2019 and 2020 fin length increased with age (Figure 2.4.). In 2019, at 88 dpf substrate was found to significantly influence fin length ($P=0.024$) with a significant difference found between fish reared in the simple and complex treatments ($P<0.04$) but not with fish reared in the moderate treatment. At 74, 81 and 95 dpf flow was found to significantly influence fin length ($P=0.026$, 0.043, and 0.024 recpectivly). In post hoc comparisons, at 74 dpf fish reared in the simple treatment were found to have significantly longer fins compared to fish reared in the moderate and complex treatments ($P<0.02$). At 81 dpf treatments showed no significant difference from each other. At 95 dpf fish reared in the simple and moderate treatments were significantly different from each other ($P<0.04$) but not from fish reared in the complex treatment. At 52, 60 and 67 dpf substrate and flow were found to have no significant effect on fin length. At 52 and 67 dpf fish in treatments showed no significant differences from each other. At 60 dpf fish reared in the simple and complex treatments were significantly different from each other ($P<0.01$) but not from fish reared in the modreate treatment.

In 2020, at 60 and 81 dpf substrate was found to significantly influence fin length ($P=0.016$ and 0.0082 recpectivly) as fish reared in the complex treatment were found to have significantly shorter fins than fish reared in the simple and moderate treatments ($P<0.04$). At 88 dpf substrate and flow did not significantly influence fin length, and no treatment was significantly different from the other.

Finspan

In 2019 and 2020 finspan increased with age (Figure 2.5.). In 2019 substrate was found to significantly influence finspan at 88 dpf ($P=0.038$) and a significant difference was found

between fish reared in the simple and complex treatments ($P<0.04$) but not from fish reared in the moderate treatment. At 74 dpf flow was found to significantly influence finspan ($P=0.0038$) as fish reared in the simple treatment had a significantly longer finspan than fish reared in the complex and moderate treatments ($P<0.01$). At 53, 60, 67, 81, and 95 dpf substrate and flow did not influence finspan. At 53, 81 and 95 dpf no treatment differences were found. At 60 and 67 dpf fish reared in the simple and complex treatments were found to be significantly different from each other ($P<0.03$) but not from fish reared in the moderate treatment.

In 2020, at 60 dpf substrate significantly influenced finspan ($P=0.0068$) as fish reared in the complex treatment had a significantly shorter finspan compared to fish reared in the simple and moderate treatments ($P<0.02$). At 81 and 88 dpf substrate and flow did not influence finspan and no treatment effects were found. The relationship between finspan and body length was examined in both 2019 and 2020 at 60 dpf (Figure 2.6.). There was no difference in the relationship with treatment.

Cumulative Mortality

In both 2019 and 2020, mortality was seen throughout the study (Figure 2.7.). In 2019, both flow and substrate impacted mortality during the study ($P< 0.01$). Differences in mortality were seen between fish in the simple and moderate treatments, and complex and moderate treatments ($P<0.007$). In 2020, there were a few high mortality events that did not allow the statistical analysis of this data as conducted in the previous year. However, when viewing the data, a similar trend in mortality is seen between the years with high mortality early in both years.

2.5 Discussion

In the present study substrate showed a significant impact on growth throughout the sampling points at a younger age, with fish reared in the complex treatment being smaller than fish in the simple and moderate treatments. Interestingly, Atlantic sturgeon showed a similar trend in growth with substrate (Gessner et al., 2009), however, Gessner et al., (2009) did not extend beyond the yolk-sac stage. In contrast, green sturgeon (*Acipenser medirostris)*, raised over substrate during the first year of life, showed decreased survival and growth in cobble/rounded substrate and sand, compared to flat surface substrates (Nguyen and Crocker, 2006). Upon feeding, Nguyen and Crocker (2006) described how food would settle under and in between the cobbles, leading to a more inefficient foraging effort and increased mortality. Similar observations were made with Lake Sturgeon raised over sinking bioballs and sand at the yolk-sac and larval life stages (Yoon et al., 2020b). These studies support our data, however, removal of substrate during key transitions can negate the potential negative impact substrate may have on growth and survival. For example, in white sturgeon, gravel substrate was added upon hatch to provide refuge for yolk-sac larvae and then removed when exogenous feeding began, which resulted in a prolonged increase in growth in the gravel reared fish (Boucher et al., 2014).

At varying sampling times, fish reared in the simple treatment were larger than the other treatments and the lack of flow was found to have a positive effect on growth. Optimal growth from induced exercise is considered when the cost of transport is lowest and energy efficiency is at its highest (Palstra and Planas, 2011), typically around a water velocity of 1 bl (body lengths)$\cdot s^{1}$ for salmonids (Jobling et al., 1993; Castro et al., 2011; Ytrestøyl et al., 2017). At swimming speeds less than this, a substantial amount of energy is lost due to higher spontaneous

activity (Palstra and Planas, 2011), and at speeds higher, energy expenditure may be 10-15 times higher than a fish at rest (Jobling et al., 1993) diverting energy to locomotion leading to stress and fatigue, both of which reduce growth (Palstra and Planas, 2011). Indeed, in brook charr (*Salvelinus fontinalis)*, fish swimming at speeds 0.85 $bl \cdot s^{1}$ were larger than fish swimming at higher and lower speeds (East and Magnan, 1987). Further, in Atlantic salmon a similar trend was seen with reduced growth both above and below swimming speeds of 1.5 $bl \cdot s^{-1}$ (Jørgensen and Jobling, 1993). All of these examples, demonstrate that suboptimal flow can reduce growth. We have not attempted to optimise flow regimes for growth in the present study, but it may be that the magnitude of flow was above optimal speeds in the moderate and complex treatment, leading to reduced growth particularly in the very young fish. The absolute velocity of the water during the experiment was maintained at 88+/- 8.97 $mm \cdot s^{-1}$, which equates to approximately 5.95 $bl \cdot s^{-1}$ and 1.20 $bl \cdot s^{-1}$ for the smallest and largest individual in the study, respectively, both exceeding presumptive optimal velocity for growth. Behavioural observations demonstrated that fish would get swept up in the current and actively seek out areas with lower currents in the edge of the tank for the moderate treatments and back eddies in the complex treatment. That said higher water velocities may have resulted in lower growth due to fatigue and expending energy moving to more desirable flow conditions.

Regarding fin length and finspan, we saw some instances where substrate and flow had a significant influence. During development fish raised in the complex treatment had smaller fins compared to the other treatments and fish raised in the simple treatment often had significantly larger fins than other treatments. However, the general lack of difference between treatments and a similar relationship between finspan and total length regardless of treatment did not support our prediction that increased complexity in the environment would result in longer fins. In Atlantic

salmon, and Mozambique tilapia (*Oreochromis mossambicus)*, there was a strong relationship between fin length and fork length (Pelis and McCormick, 2003; Naeem et al., 2011). Therefore, the differences observed in fin length at certain sampling timepoints is most likely a function of the differences in total length of individuals at the same timepoints and not a function of the environment. That said, we did observe station holding behaviour in fish reared in the moderate and complex treatments, however, we did not quantify this behaviour relative to treatments.

Interestingly, both years in the present study produced similar trends in the measured parameters. It is tempting to compare the differences in growth between years as they did have different rearing environments for the first three weeks of life; however, we must also consider the differences in genotypes between years that would have a strong influence on growth phenotypes between years (Dammerman et al., 2015). However, environment may have also influenced the inter-annual variation in growth. In a previous study lake sturgeon incubated in tumbling jars and on egg matts with increased flow, showed differences in growth, with fish being larger by mass and length in the egg matt treatment group for up to 70 dpf (Earhart et al., 2020). The egg matt fish in Earhart et al., (2020) would be similar to how fish were treated in our 2020 cohort, however, fish in this study were no different in size compared to fish incubated in MacDonald jars by 88dpf. The two studies differ in that Earhart et al., (2020) transferred fish to a common garden setup post-hatch, whereas fish were maintained in the different treatments for the duration of the present study suggesting subtle differences influencing growth rate that may persist beyond the egg incubation stage.

At the end of the study period, regardless of year and treatment no significant differences were found, suggesting that once fish reached a certain size our environmental treatments had no

effect on growth. Fish in the complex treatment when larger, would no longer get trapped in the substrate, and gape size would presumably increase with size, ensuring effective foraging as fish transitioned to the larger bloodworm diet. In the gilthead seabream (*Sparus aurata)*, using different coloured glass substrates slightly smaller than the rocks used in the present study and smooth bottom tanks resulted in similar feeding behaviour between treatments for all yearlings (Batzina and Karakatsouli, 2012) suggesting that feeding behaviour and foraging efficiency may be similar across all treatments in the present study. Alternatively, if fish were not able to successfully forage, one might anticipate differential mortality depending on the treatment which was observed with higher mortality in the complex treatment, leading to only the larger individuals surviving to the end of the study.

Despite the similarities in growth across treatments in the present study the presence of flow and substrate would undoubtedly have introduced the need to develop appropriate foraging skills that the simple fish would not. For example, a study with lab and pond reared yearling rainbow trout (*Oncorhynchus mykiss)* lab fish initially choose a darker substrate to hide, but by 24 hours, the fish were distributed evenly across dark and white substrate, however, the pond reared fish continued to select a darker substrate indicating long term behavioral response based on rearing substrate (Ritter and MacCrimmon, 1973). Furthermore, lake sturgeon raised in elevated temperatures during early rearing demonstrated differences in growth that persisted post-winter in the following year (Brandt et al., 2022) indicating that early rearing environment produced long-term physiological and behavioural effects. In a previous study looking at escape behaviour with lake sturgeon, fish in clear water fled and then sought out cover in the rocky substrate but in turbid water fish immediately sought out cover demonstrating a low-cost/high-benefit response (Wishingrad et al., 2014). Thus, it is reasonable to assume fish raised in

substrate and flow would have gained valuable behavioural skills that fish in other treatments did not.

Despite the increased mortality in the complex treatment in the present study, we would recommend further research into the use of flow and substrate during early rearing. If wildtype phenotypes are the goal of conservation hatcheries, then a potential trade-off may need to be made between increased mortality during early rearing for a fish that may be better suited for the wild with potential behavioural adaptations such as foraging efficiency, predator avoidance, and swimming performance. Furthermore, investigating the timing of the removal or change in substrate would be a useful extension of this work as juvenile Lake Sturgeon are known to prefer a sand substrate (Peake, 1999). However, when sand substrate is provided too early in green and lake sturgeon, it led to a decreased growth rate (Nguyen and Crocker, 2006; Yoon et al., 2020a,b). Therefore, while enrichment methods are clearly desirable in conservation hatcheries significant research is required to understand optimal timing and duration of flow and substrate during early life history of lake sturgeon.

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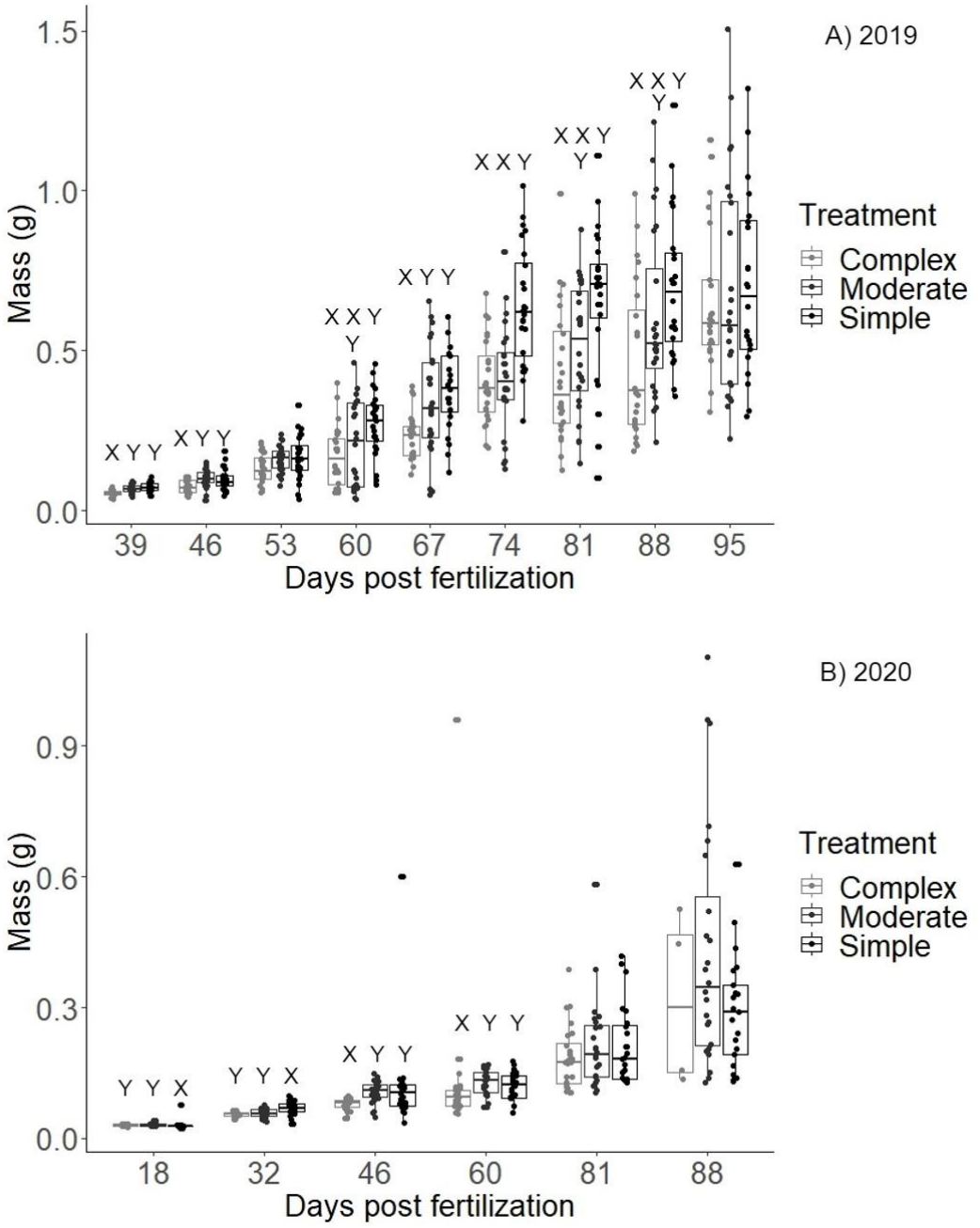

Figure 2. 1. Body mass (g) of larval lake sturgeon (*Acipenser fulvescens*) at age (days post fertilization) in A) 2019 and B) 2020 for complex (light grey), moderate (medium grey), and simple (black) treatments. Data are presented in box plots with the horizontal line representing the median value for each sampling timepoint the upper and lower lines represent the first and third quartile. The lower whiskers show the lower quartile minus 1.5 times the upper minus the lower quartile and the upper whisker show the upper quartile plus 1.5 times the upper minus the lower quartile. Individual points represent individual measurements. Different letters represent significant differences between treatments at each sampling point.

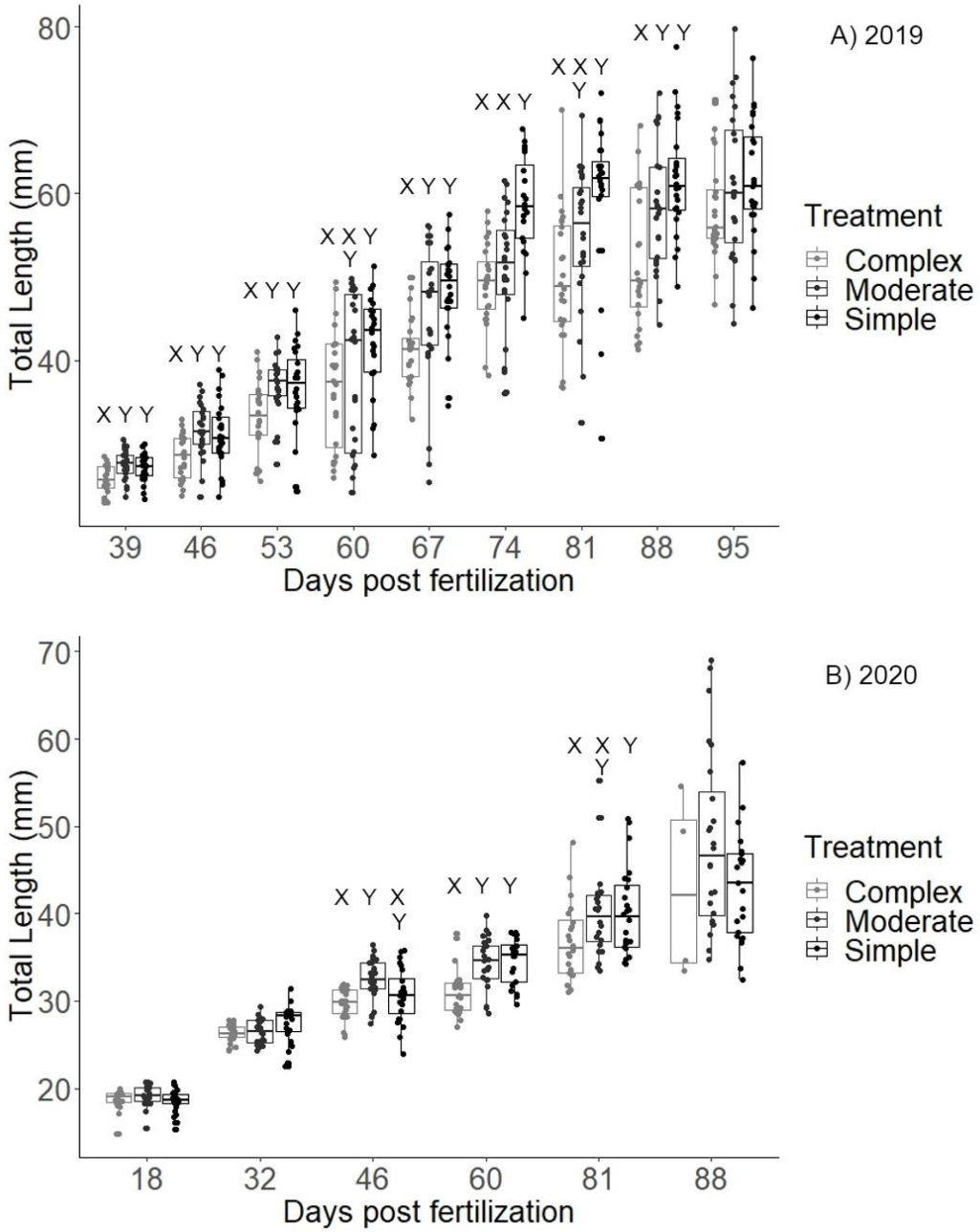

Figure 2. 2. Total length (mm) of larval lake sturgeon (*Acipenser fulvescens*) at age (days post fertilization) in A) 2019 and B) 2020 for complex (light grey), moderate (medium grey), and simple (black) treatments. Data are presented in box plots with the horizontal lin line representing the median value for each sampling timepoint the upper and lower lines represent the first and third quartile. The lower whiskers show the lower quartile minus 1.5 times the upper minus the lower quartile and the upper whisker show the upper quartile plus 1.5 times the upper minus the lower quartile. Individual points represent individual measurements. Different letters represent significant differences between treatments at each sampling point.

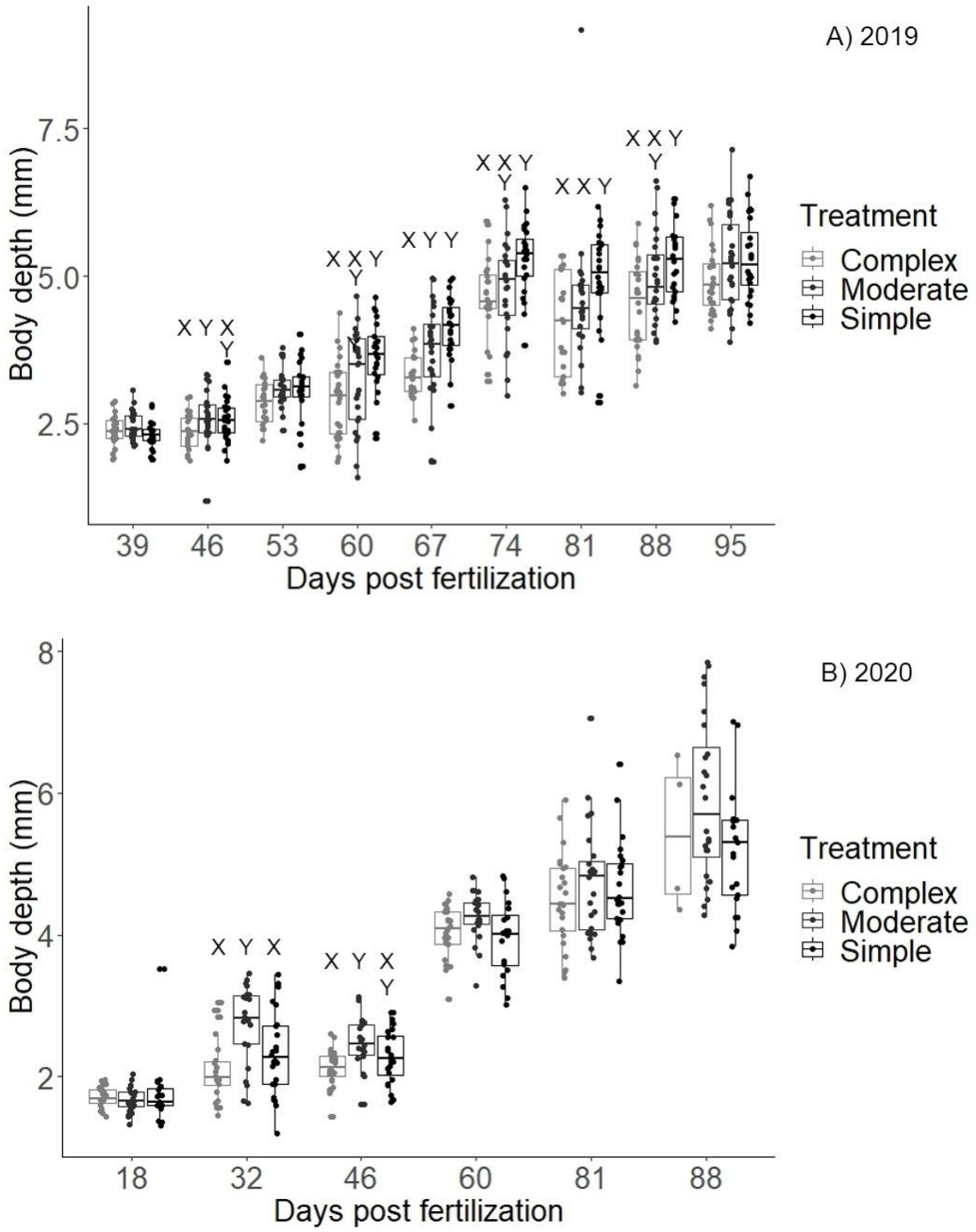

Figure 2. 3. Body depth (mm) of larval lake sturgeon (*Acipenser fulvescens*) at age (days post fertilization) in A) 2019 and B) 2020 for complex (light grey), moderate (medium grey), and simple (black) treatments. Data are presented in box plots with the horizontal line representing the median value for each sampling timepoint the upper and lower lines represent the first and third quartile. The lower whiskers show the lower quartile minus 1.5 times the upper minus the lower quartile and the upper whisker show the upper quartile plus 1.5 times the upper minus the lower quartile. Individual points represent individual measurements. Different letters represent significant differences between treatments at each sampling point.

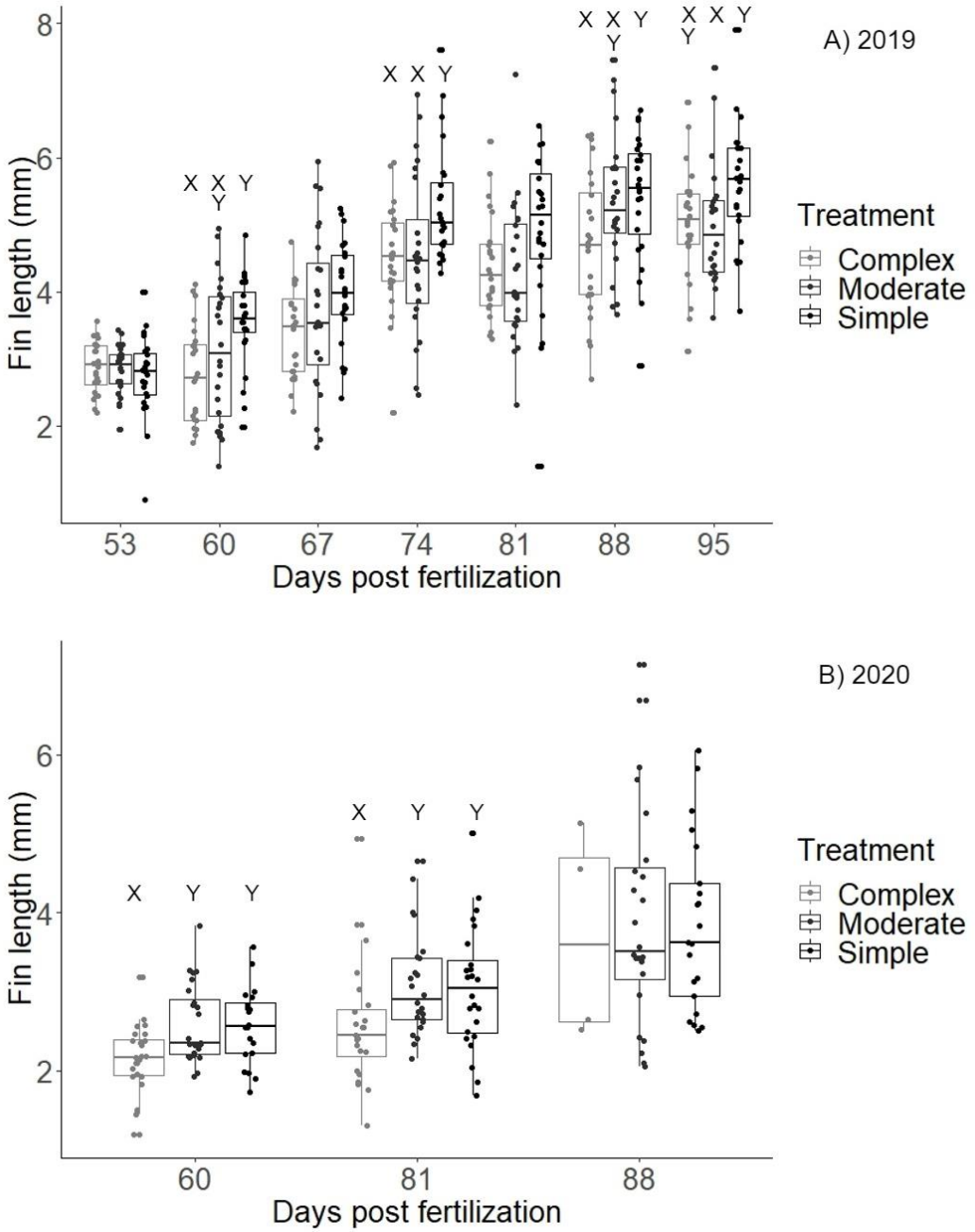

Figure 2. 4. Fin length (mm) of larval lake sturgeon (*Acipenser fulvescens*) at age (days post fertilization) in A) 2019 and B) 2020 for complex (light grey), moderate (medium grey), and simple (black) treatments. Data are presented in box plots with the horizontal line representing the median value for each sampling timepoint the upper and lower lines represent the first and third quartile. The lower whiskers show the lower quartile minus 1.5 times the upper minus the lower quartile and the upper whisker show the upper quartile plus 1.5 times the upper minus the lower quartile. Individual points represent individual measurements. Different letters represent significant differences between treatments at each sampling point.

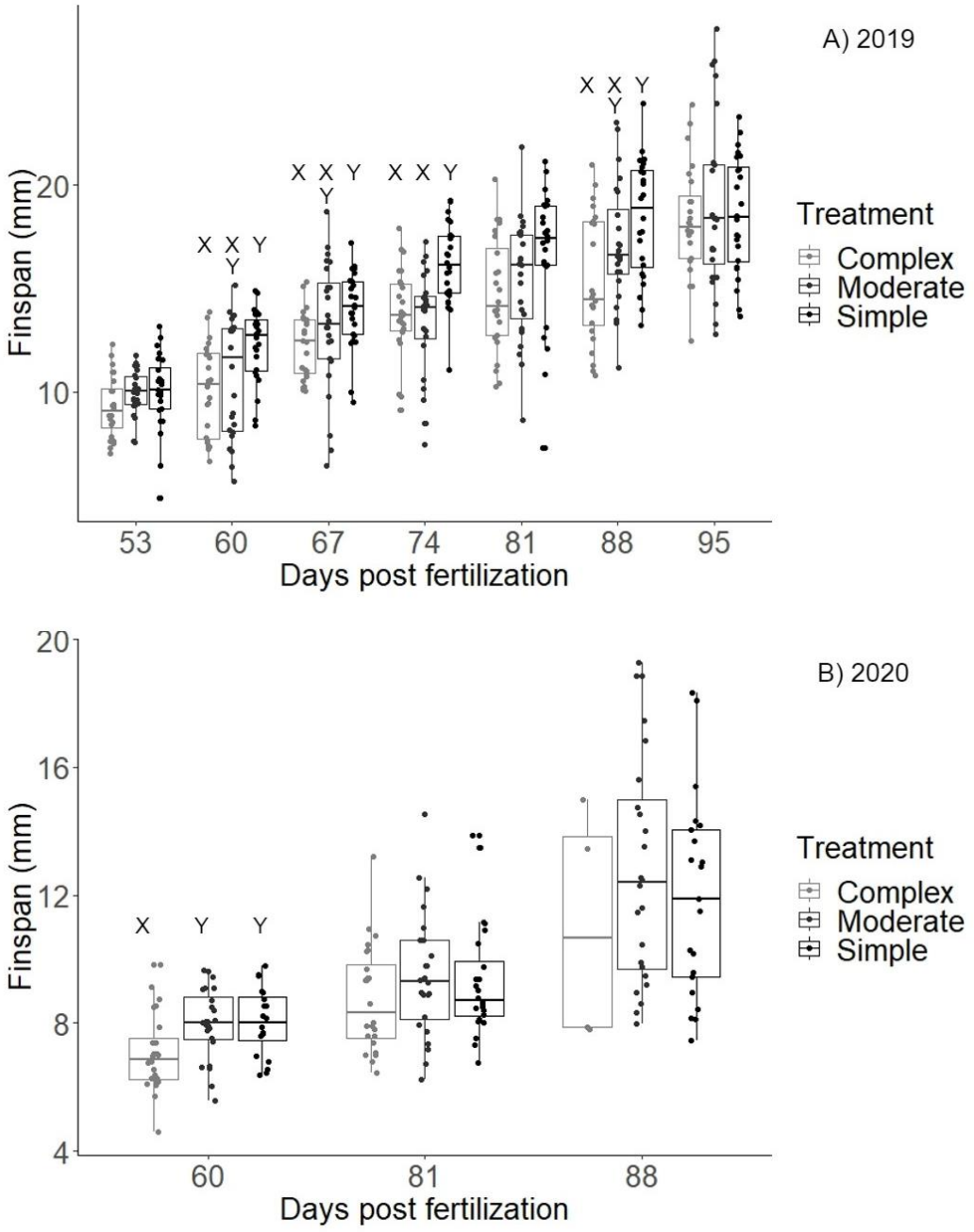

Figure 2. 5. Finspan (mm) of larval lake sturgeon (*Acipenser fulvescens*) at age (days post fertilization) in A) 2019 and B) 2020 for complex (light grey), moderate (medium grey), and simple (black) treatments. Data are presented in box plots with the horizontal line representing the median value for each sampling timepoint the upper and lower lines represent the first and third quartile. The lower whiskers show the lower quartile minus 1.5 times the upper minus the lower quartile and the upper whisker show the upper quartile plus 1.5 times the upper minus the lower quartile. Individual points represent individual measurements. Different letters represent significant differences between treatments at each sampling point.

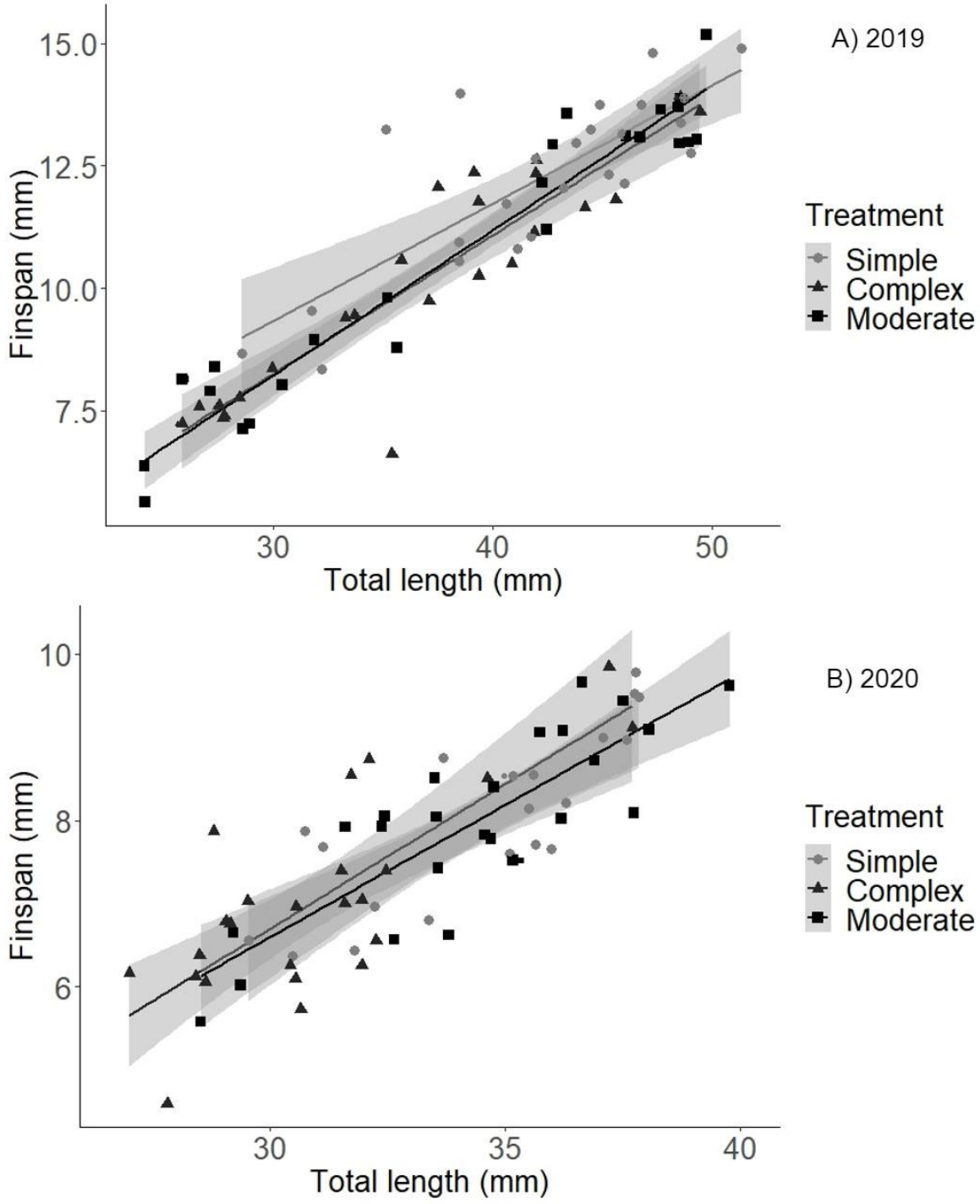

Figure 2. 6. Relationship between total length (mm) and Finspan (mm) of larval lake sturgeon (*Acipenser fulvescens*) at 60 days post fertilization in A) 2019 and B) 2020 for simple (light grey, circle), complex (medium grey, triangle), and moderate (black, square) treatments.

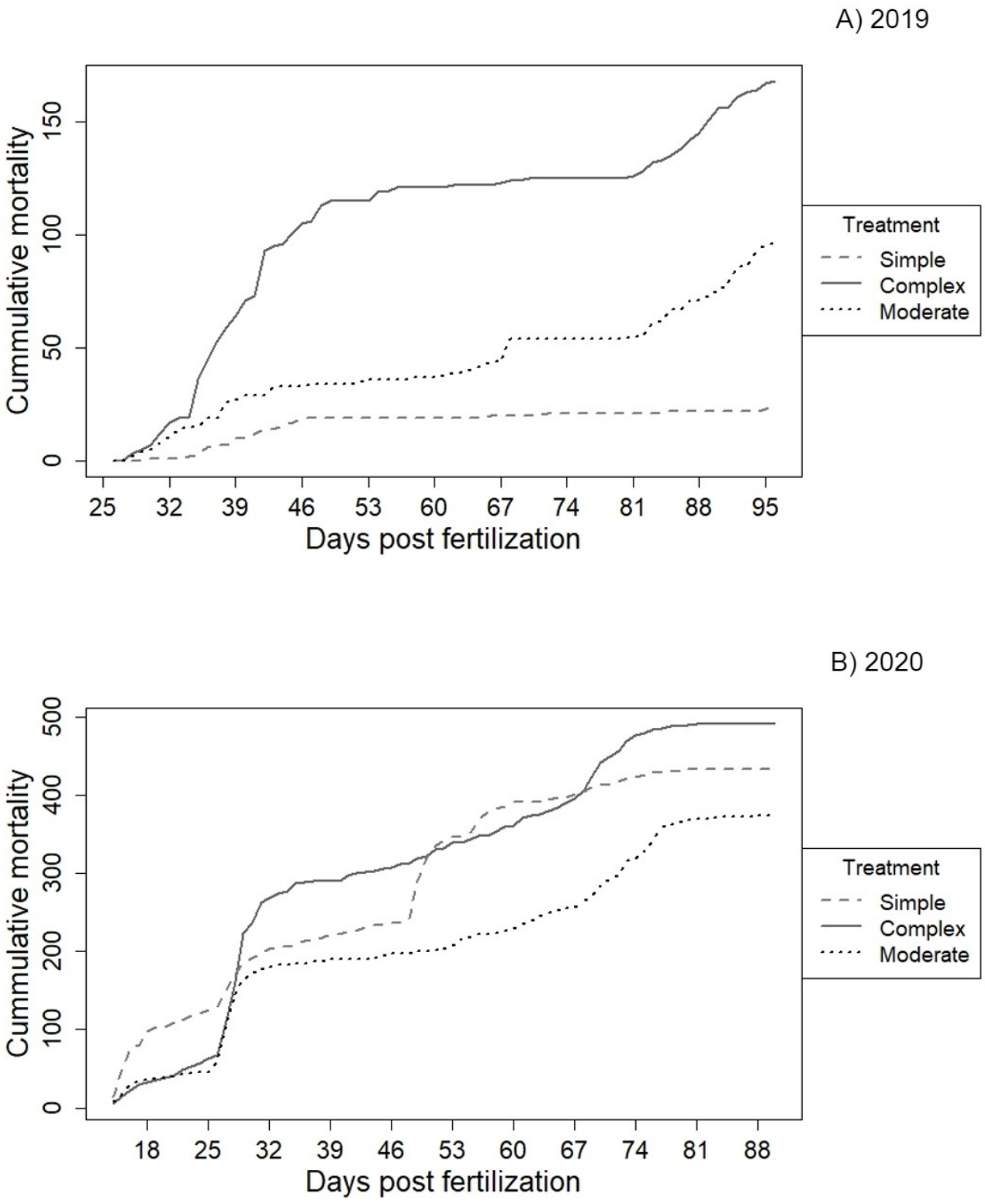

Figure 2. 7. Cumulative mortality of larval lake sturgeon (*Acipenser fulvescens*) in A) 2019 and B) 2020 for simple (dashed light grey), complex (solid medium grey), and moderate (dotted black) treatments.

Chapter 3. Effects of habitat complexity on otolith shape and polymorph composition in hatchery reared larval lake sturgeon, *Acipenser fulvescens*

*Kassandra M. Merks[1], Aaron J. Lussier[2], Ralph Rowe[2], Brenda M. Pracheil[3], Bryan C. Chakoumakos[4], and W. Gary Anderson[1]

[1] Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada, R3T 2N2

[2] Canadian Museum of Nature, P.O. Box 3443, Station D, Ottawa, ON, Canada, K1P 6P4,

[3] Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA

[4] Neutron Scattering Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA

KMM – study conception and design; data collection, curation and analysis; drafting of manuscript; AJL, RR, BMP, BCC sample analysis, data curation and manuscript review and editing; WGA study conception and design; manuscript and data review and editing; funding acquisition

*Corresponding author (Merksk1@myumanitoba.ca)

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3.1 Abstract

A changing environment during early life history influences development of numerous traits in all organisms, which ultimately can influence the fitness of the individual. The otolith is found within all fishes and is responsible for hearing and balance. Differences in otolith development have been reported for teleosts reared in a hatchery environment compared to the wild environment. Understanding which specific environmental factor that may influence otolith development is of interest in conservation programs. In the present study, we examined the effects of water flow and tank substrate on otolith size and polymorph composition during the first four months of life in two cohorts of larval lake sturgeon (*Acipenser fulvescens)*. We had three treatments designed to increase environmental complexity: a simple treatment with no flow or substrate, a moderate treatment with the addition of flow, and a complex treatment with the addition of both flow and substrate. We found that the chosen environmental parameters influenced otolith size. However, there was no effect on otolith polymorph composition, furthermore we found no difference between the left and right otoliths both in size and polymorph composition throughout development. These data suggest that polymorph composition may be a highly conserved phenotype while otolith size may be a more variable phenotype in response to environmental changes. Finally, no deviations from bilateral symmetry were seen with otolith development, underscoring the importance of appropriate development of otoliths during early life history.

3.2 Introduction

The environment plays an important role in phenotypic development which ultimately may have a positive or negative influence on the fitness of an individual (Monaghan, 2008). Negative effects of hatchery rearing are evident in many species (Arki and Schmid, 2010) and this is of even greater importance when fish are raised for conservation purposes to stock populations at risk or endangered (Crossman et al., 2014). Conservation aquaculture programs aim to stock fish that have the best chance of survival with the ultimate result of a self-sustaining population (Froehlich et al., 2017). To achieve the intended outcome, it is critical to understand the effects of the environment on phenotypic development as fish reared in a hatchery environment will develop phenotypes that differ from their wild counterparts (Chittenden et al., 2010, Johnsson et al., 2014). In hatcheries, fish are raised in cages, tanks, or ponds; and the physical environment is simple, temperature controlled, space restricted, has a lack of complex flow, and is devoid of structure and predators (Huntingford, 2004).

A number of environmental factors have been shown to influence phenotypic development in Lake sturgeon (*Acipenser fulvescens)*. For example fish reared under different flow conditions in the natural environment had greater variation in growth under higher flow conditions (Dammerman et al., 2015); in a hatchery environment, growth was reduced and the cortisol response to stress was retarded in fish initially incubated in hatching jars compared to those incubated on egg mats (Earhart et al., 2020); and fish raised at a higher temperature during early development demonstrated a persistent increase in growth rate one year later compared to those raised at lower temperatures (Brandt et al., 2022). Conversely some measured traits have little change in response to manipulation of early rearing environments; Yoon et al., (2020b) reported no significant effect of temperature, dissolved oxygen, and substrate on whole body

resting metabolic rate in young-of-the-year lake sturgeon pre and post a simulated winter event (Yoon et al., 2020b).

One trait that has been shown to differ in hatchery reared salmonids in comparison to wild counterparts is otolith structure. Otoliths are paired calcified structures formed in a non-collagenous organic matrix in the inner ear of fishes and function as balance and hearing systems in fishes (Campana, 1999). Three polymorphs of calcium carbonate ($CaCO_3$) — calcite, aragonite, and vaterite are known to exist in otoliths (Campana, 1999) each varying in density and crystal structure (Ni and Ratner, 2008); with aragonite being the predominant polymorph found in teleosts (Oxman et al. 2007; Pracheil et al., 2019). Interestingly, polymorph composition in fishes is influenced by development and rearing environment, and can vary by side (i.e., left vs right) (Bowen et al., 1999; Reimer et al., 2017; Loeppky et al., 2019). For example, vaterite inclusions were ten-fold higher in hatchery reared Atlantic salmon (*Salmo salar*) compared to wild Atlantic salmon (Reimer et al., 2016). Furthermore, in lake trout (*Salvelinus namaycush*) hatchery raised fish transitioned to vaterite from aragonite early in life, specifically within the first 3-4 months with 62% of stocked lake trout having 50% more vaterite while this was evident in only 37% of wild lake trout of a similar age (Bowen et al., 1999). Finally, polymorph substitution in the otolith of fishes has been shown to influence shape. For example otoliths from wild coho salmon (*Oncorhynchus kisutch*) were found to be small, dense, white and regularly shaped with growth rings, whereas the otoliths from hatchery reared coho salmon were larger, thin, crystalline and irregularly shaped with thick growth rings during the first winter of life (Chittenden et al. 2010). In addition, increases in vaterite inclusions in the otoliths of hatchery reared Atlantic salmon tended to be greater in the left sagittal otolith compared to the right (Reimer et al., 2016). Ear structures are expected to develop

symmetrically, and a deviation from that is known as fluctuating asymmetry with mixed results of significance in fishes (Allenbach, 2011). For example, alteration of otolith structure can result in a decrease in oscillation amplitude in response to sound in salmonoids which may impair functionality in hearing (Gagliano et al., 2007; Reimer et al., 2016) and possibly lead to inappropriate behavioural responses that could influence survival. Further differences between the left and right in shape and size may have major bearing on the ability to localise sound (Gagliano et al., 2007). At present, it is uncertain which specific environmental factor or combination of environmental factors and genotype may influence otolith polymorph composition and otolith size during early otolith development in hatchery reared fishes.

Lake sturgeon (*Acipenser fulvescens)* are designated as threatened or endangered throughout most of their native range due to human pressures such as overfishing and habitat fragmentation over the past century (Peterson et al., 2007). These pressures combined with their life history traits have made natural recovery slow and have led to the use of conservation aquaculture to encourage recovery. To enhance success of stocking efforts it is critical to promote development of wild type phenotypes by creating a more natural rearing environment during early life history within the hatchery. Sturgeon otoliths tend to differ from other species in polymorph composition, shape, and granularity (Carlstrom, 1963; Chalupnicki and Dittman, 2016) with much more irregularity in composition and shape compared to the teleost otolith. Furthermore, lake sturgeon otoliths were thought to be entirely composed of vaterite (Carlstrom, 1963). However, it was recently demonstrated that adults contained a large proportion of calcite within the sagittal otolith (Pracheil et al., 2017) and the larval and juvenile life stages havebeen shown to have a substantial proportion of aragonite within the sagittal otolith (Loeppky et al., 2019).

In this study we aimed to assess otolith size and polymorph composition in lake sturgeon in response to increasingly complex environments during early development. We examined the effect of flow and substrate on otolith composition testing the hypothesis that fish reared in the more complex environment that included substrate and flow would demonstrate smaller otoliths as well as a higher proportion of aragonite. We investigated these parameters throughout early development in two-year classes and also examined otolith symmetry by assessing differences in polymorph composition between the left and right sagittal otolith during early development in a single year class.

3.3 Methods

Fish were reared in habitat conditions over the course of three months to assess changes in otolith size and polymorph composition with treatment. Otolith size was assessed using otolith perimeter, surface area, and maximum diameter from imagery taken under a microscope. While otolith polymorph composition was assessed using x-ray diffraction (XRD) analysis. Otolith analysis was separated into two subsections one to assess development and the other to assess symmetry. Due to the large number of samples, each subsection underwent XRD at a different facility. No attempts to compare the XRD data between facilities were made due to slight differences in techniques and instrumentation.

Fish rearing

In 2019 and 2020, spawning adult lake sturgeon were captured using gill nets downstream of Pointe du Bois on the Winnipeg River (50° 18’ 06.6’’ N, 95° 32’ 29.5’’W) in Manitoba, Canada. Milt was collected immediately from males and stored at 4°C for a maximum of 24h or when needed. Females captured were transported to a riverside holding tank supplied with flow through river water at ambient temperature from two submersible pumps placed in the

river adjacent to the holding tanks. To stimulate maturation and egg release, females were given two doses of gonadotropin releasing hormone (GnRH; 10 and 20 $mg \cdot kg^{-1}$), the first 36 hours prior to stripping eggs manually and the second 24 hours prior. Once collected, gametes were transported separately to the University of Manitoba's animal holding facility to undergo fertilization and embryogenesis as previously described (Loeppky et al., 2019; Yoon et al., 2020b). In 2019 a total of two females and four males were used while in 2020 one female and two males were used for similar genetic makeup across treatments. The fertilized eggs were evently distributed across all treatments to reduce genotypic effects.

In 2019, all fertilized eggs were transferred to MacDonald tumbling jars for incubation with a constant supply of fresh water starting at 12°C increasing to 16°C at 0.5°C a day. Once hatched (~7-10 days), the larval fish were transferred to flow-through 9L holding tanks under normal hatchery conditions (16°C, 12 hr light:dark photoperiod). At 25 days post fertilization (dpf) fish were transferred to one of three treatments with increasing environmental complexity each in triplicate:

4) SIMPLE - Rearing environment that contained no substrate and no addition of flow, similar to existing hatchery conditions.

5) MODERATE - Rearing environment with flow and no substrate. Flow was created using electric submersible pumps at one end of the holding tank. Flow averaged 630 +/- 68.9 $mL \cdot s^{-1}$. Interior tank dimensions 5.08 x 13.97 x 34.93 cm

6) COMPLEX - Rearing environment with flow and substrate in the form of pebbles (~2 cm in diameter) covering ~90% of the bottom of the tank to create a variable flow

regime. Flow averaged 630 +/- 68.9 $mL \cdot s^{-1}$. Interior tank dimensions 5.08 x 13.97 x 34.93 cm

In 2020 fertilized eggs for the control treatment were transferred to MacDonald tumbling jars starting at 12°C and slowly increased to 16°C at 0.5°C a day until hatched (~7-10 days) and then placed into their respective treatment tanks. However, fertilized eggs for the moderate and complex treatments were evenly divided across those treatment tanks and allowed to adhere to the substrate/tank bottom. All treatment tanks in both years maintained equivalent stocking density, temperature (16°C) and photoperiod (12 hr light:dark) for the remainder of the experiment. Flow rate was manually calculated using the equation Q=w*h*(l/t), by measuring the tank dimensions (l= length, w= width, h=height) and surface velocity (88.8 +/- 8.97 $mm \cdot s^{1}$). Surface velocity was taken by timing how long a uniform floating object took to travel the length of the tank. This was repeated ten times and averaged.

In both years, after yolk absorption (~14-17 days post fertilization, dpf) fish were fed three times a day with freshly hatched brine shrimp (Artemia International LLC, Texas, USA). At feeding, flow was stopped in all holding tanks and fish were fed *ad libitum* for 15-20 minutes, after which any excess food was carefully removed with a siphon and flow was returned to all tanks. Around 30 dpf fish began transition from brine shrimp to chopped bloodworm (Hakari USA, California, USA) using a ratio of 10:1 increasing to 100% bloodworm at 50dpf. The pebbles were cleaned twice weekly by carefully removing them from the tank and rinsing them off with warm water prior to soaking in Oxivir solution (Diversey, Mississauga, ON, Canada) for a 24-hour period. After the Oxivir solution they were rinsed again until the water ran clear, then

the pebbles were dried and subsequently reused the following week. Tanks were without substrate for a maximum of 5 minutes during cleaning throughout rearing.

Otolith gross morphology

In 2019 fish were sampled weekly between 39 and 95 dpf and in 2020 fish were sampled biweekly from 18 to 88 dpf. At each sampling point a maximum of 24 fish, numbers permitting, were haphazardly sampled by dip net equally from each replicate from each treatment. Fish were immediately euthanized by immersion in an overdose of tricaine methanesulfonate (MS-222) buffered with an equal volume of sodium bicarbonate (250 $mg \cdot ml^{-1}$) then preserved in 95% ethanol. A single left or right sagittal otolith was removed from each individual in both 2019 and 2020 using an Olympus SZX7 dissecting microscope mounted with polarized lenses (Olympus SZX-PO) to aid in finding the otoliths. Once removed, otoliths were transferred to a microscope slide with a centimeter square grid divided into 25 equal squares, coated with a piece of double-sided tape as described in Leoppky et al. (2019). Each otolith was photographed under the polarized microscope at 10X magnification for analysis of gross morphology. Image analysis was conducted using freeware software – Fiji (Schindelin et al., 2012) first by scaling the image using the known distance of the grid placed on the microscope slide; enlarged to approximately 400% and the perimeter of each otolith was traced using the polygon tool. The software then calculated perimeter (mm), area (mm^2), and the maximum otolith diameter (mm) across each otolith (Figure 3.1.).

Otolith development and symmetry

To quantify whether differences in the environmental complexity result in differences in otolith $CaCO_3$ polymorph composition, we conducted two sets of X-ray diffraction (XRD)

analyses. The first analysis was used to determine if changes occurred throughout development. A subset of otoliths was chosen from 2019 and 2020 to undergo XRD to determine polymorph composition at the Canadian Museum of Nature (CMN) in Ottawa, Ontario. Three sampling timepoints, 32, 60, and 81 dpf were selected as the otoliths were sampled both years during these times and allowed for analysis across development and ~ 8 otoliths per treatment were chosen haphazardly from the different treatments. The diffraction data were collected with a Bruker D8 Discover microdiffractometer equipped with a DECTRIS EIGER2 R 500K detector and IμS microfocus Cu x-ray source (λCuKα = 1.5418454184 Å) running at 50kV 1mA. Polymorph identification and quantitative values were obtained with Bruker's DIFFRAC.EVA software using the ICDD PDF-4+ 2020 database and using the statistical calibration method of Rowe (2009).

The second XRD analysis was used to determine if there were any differences in symmetry between the left and right otolith. A second subset of otoliths was chosen from 2020 to undergo XRD. Four sampling timepoints, 32, 60, 81 and 88 dpf were chosen to examine symmetry across development with ~8 otolith pairs per treatment were chosen haphazardly to undergo quantitative phase analysis at Oak Ridge National Laboratory (ORNL) in Tennessee (CITES permit #20CA04368CWHQ). Data were collected using whole otoliths and a Rigaku XtaLab Pro diffractometer equipped with graphite monochromated Mo Kα radiation (0.7107 Å)) operating at 50 kV and 40 mA, with a HyPix-6000HE detector. Identification and quantification of polymorphs from both the otolith development and symmetry experiments were conducted by the Rietveld method using GSAS II (Toby and Von Dreele, 2013). The Debye-Scherrer diffraction patterns were used for phase analysis to determine the proportion of crystal polymorphs in each otolith (Loeppky et al., 2019).

Statistical analysis

Linear mixed models (LMM) were used to compare differences between treatments to account for the random effect of rearing tank. Flow and substrate are discrete factor variables and are represented by F and S (absence or presence), respectively. The intercept of random effects of rearing tank and residual error was represented by a_{ID} and e, respectively. The full model was written as follows:

$$\hat{R} = a_0 + b_s \cdot S + b_f \cdot F + b_{sf} * SF + a_{ID} + e$$

Where the response variable ($\hat{R}$) represents either otolith area, perimeter, maximum otolith diameter, or the difference (left minus right) in all of the above shape metrics between left and right otoliths. Each time point was assessed independently, and assumption of normality and homoscedasticity were assessed statistically and visually. When assumptions were not met the data underwent log or Tukey transformations. Likelihood ratio tests were performed to test significance using the function 'anova' in the R package lme4 with backwards elimination (Winter, 2013; Yoon et al., 2020b). When significance was found, *post hoc* comparisons on treatments were performed using 'glht' function in the R package multicomp with significance determined at alpha =0.05. All statistical analysis was conducted using RStudio 1.2.5033 (R core Team, 2020). Importantly, no comparisons were made between the two methods used to assess polymorph structure of developing otoliths and otolith symmetry.

All experimental protocols performed on fish were approved by the Animal Care Committee at the University of Manitoba permit# F15-007 under the guidelines of the Canadian Council for Animal Care.

3.4 Results

Perimeter

In 2019 and 2020 otolith perimeter increased with age for fish in all three treatments although it had varying results with habitat complexity (Figure 3.2.). In 2019 at 53 dpf perimeter was significantly influenced by substrate ($P=0.015$) with fish raised in complex and moderate treatments being significantly different from each other but not from the simple treatment ($P<0.05$). There was also a significant influence of substrate on otolith perimeter at 88 dpf ($P=0.0029$), with fish in the complex treatment having a smaller perimeter than the moderate and simple treatments ($P<0.01$). At 74 and 81 dpf perimeter was significantly influenced by flow ($P=0.012$ and 0.024 recpectivly). Also, at 74 dpf fish raised in the simple treatment had significantly larger otolith perimeters than fish raised in the moderate and complex treatments ($P<0.02$). At 81 dpf fish raised in the simple and complex treatments were found to be significantly different from each other but not from fish in the moderate treatment ($P<0.01$). At 67 dpf both flow and substrate were found to significantly influence otolith perimeter ($P=0.016$ and 0.02 recpectivly) with fish in complex, moderate, and simple treatments statistically different from each other ($P<0.05$). At 39 and 95 dpf there was no influence on otolith perimeter from flow or substrate, and fish reared in simple and complex treatments were significantly different from each other but not from fish in the moderate treatment ($P<0.02$). There was also no influence on otolith perimeter at 32, 46, and 60 dpf from flow or substrate and no treatment was significantly different from the other.

In 2020 at 60 dpf substrate was found to have an impact on otolith perimeter ($P=0.024$), however, there was no treatment significance. At 46 and 81 dpf both substrate ($P=0.0091$ and 0.00072 recpectivly) and flow ($P=0.0047$ and 0.00025 recpectivly) had a significant effect on

otolith perimeter with otoliths from fish raised in the moderate treatment having significantly larger perimeters than otoliths from fish raised in the simple and complex treatments ($P<0.03$). At 18 and 32 dpf otolith perimeter was not influenced by flow or substrate and there were no treatment differences.

Surface area

In 2019 and 2020 otolith surface area increased with age for all treatments (Figure 3.3.). In 2019 at 88 and 95 dpf otolith surface area was significantly influenced by substrate ($P=0.013$ and 0.018 recpectivly) where the fish reared in the complex treatment had significantly smaller otolith surface area than did those reared in the simple and moderate treatments ($P<0.04$). At 74 and 81 dpf otolith surface area was significantly influenced by flow ($P=0.011$ and 0.022 recpectivly) with fish reared in the simple treatment having a greater surface area than the complex and moderate treatments at 74 dpf ($P<0.02$) and otolith surface area from fish reared in the simple and complex treatments being significantly different from each other but not from the moderate treatment at 81 dpf ($P<0.01$). At 67 dpf both flow and substrate had a significant effect on surface area ($P=0.029$ and 0.0049 recpectivly) with the fish reared in the simple treatment having a greater surface area compared to the complex and moderate treatments ($P<0.01$). At 32, 39, 53, and 60 dpf otolith surface area was not influenced by flow or substrate and there was no treatment significance, except at 39 dpf where fish reared in the simple and complex treatments were different from each other but not from the moderate treatment ($P<0.03$).

In 2020 at 32 and 88 dpf otolith surface area was significantly influenced by flow ($P=0.0026$ and 0.016 recpectivly) with the otoliths from fish in the simple treatment having a smaller surface area compared to the complex and moderate treatments at 32 dpf ($P<0.01$) and simple and moderate treatments being significantly different from each other but not from the

complex treatment at 88 dpf (P<0.02). At 46 and 81 dpf both substrate (P=0.042 and 0.011 recpectivly) and flow (P=0.0051 and 0.0017 recpectivly) had a significant impact on surface area with fish reared in the simple and moderate treatments being different from each other but not from the complex treatment at 46 dpf (P<0.01) and at 81 dpf fish reared in the moderate treatment having a greater surface area compared to the simple and complex treatments at 81 dpf (P<0.01). At 18 and 60 dpf otolith surface area was not influenced by flow or substrate and there were no treatment differences.

Maximum otolith diameter

In 2019 and 2020 the maximum otolith diameter increased with age (Figure 3.4.). In 2019 at 53 dpf substrate influenced the maximum otolith diameter of the otolith (P=0.046) with no treatment differences. Substrate also influenced maximum diameter at 67 and 88 dpf (P=0.015 and 0.011 recpectivly), with the fish reared in the complex treatment having shorter maximum otolith diameter than the simple and moderate treatment fish (P<0.04). At 74 dpf flow was found to significantly influence maximum otolith diameter (P=0.014) with fish in the simple treatment having larger maximum otolith diameter (P<0.02) than the moderate and complex treatment fish. At 39 and 81 dpf maximum otolith diameter was not influenced by substrate or flow, however, there was a significant difference between the simple and complex treatments (P<0.01) but not with the moderate treatment. There was also no influence on maximum otolith diameter with substrate or flow at 32, 46 and 60 dpf, as well as there were no treatment differences.

In 2020 at 32 and 46 dpf flow was found to significantly impact maximum otolith diameter (P=0.024 and 0.018 recpectivly) with no treatment difference at 32 dpf, and fish reared in the simple and moderate treatments different from each other but not from the complex treatment at 46 dpf (P<0.04). At 81 dpf both flow and substrate were found to significantly

influence maximum otolith diameter (P=0.00084 and 0.0078 recpectivly) where fish reared in the moderate treatment having a larger maximum otolith diameter compared to simple and complex treatment fish (P<0.03). At 18, 60, and 88 dpf neither flow or substrate influenced maximum otolith diameter and there were no differences between treatments.

Differences in shape

In 2020 differences in left and right surface area, perimeter, and maximum otolith diameter were investigated during development (Figure 3.5.). The difference in otolith surface area was not influenced by substrate or flow at any point during development as well as there were no treatment differences found. The difference in otolith perimeter was found to be significantly influenced by substrate at 32 dpf (P=0.02), however there were no treatment differences found. At all other timepoints neither flow nor substrate influenced the difference in otolith perimeter, as well as there were no differences between treatments. The difference in maximum otolith diameter was also not influenced by flow or substrate, and there were no differences between treatments.

Polymorph composition

In 2019 and 2020 $CaCO_3$ polymorph composition was assessed at 32, 60, 81 dpf during development at CMN (Figure 3.6.). Both aragonite and vaterite were found in the samples but calcite was not detected. As the fish grew, the proportion of vaterite increased similarly in both years. Further, no differences were found with treatment. In 2020 $CaCO_3$ polymorph composition was assessed in both the left and right otoliths at 32,60, 81 and 88 dpf at ORNL (Figure 3.7.). There were no differences found in polymorph composition between left and right at each time point.

3.5 Discussion

We predicted that the changes in environmental complexity created with flow and substrate, would result in differences in otolith polymorph composition. However, contrary to our prediction no consistent differences were found in polymorph composition between treatments. Furthermore, we report aragonite as the dominant polymorph with smaller amounts of vaterite and no calcite being detected in the otoliths of developing lake sturgeon. In a previous study examining the effect of temperature and pCO_2 on lake sturgeon otolith development, it was shown that pCO_2 did not influence polymorph composition, but temperature did (Loeppky et al., 2021). Specifically, there were inclusions of calcite in the otoliths of the sturgeon reared in elevated temperatures (Loeppky et al., 2021) and the authors suggested that the increase in temperature may have increased the rate of reaction by raising kinetic energy as well as the solubility of Ca^{2+} and CO_3^{2-} allowing the precipitation of calcite (Loeppky et al., 2021). Interestingly Reimer et al. (2017) examined the effect of temperature and light on otolith development in hatchery raised Atlantic salmon, (*Salmo salar)* and reported that photoperiod but not temperature affected vaterite prevalence (Reimer et al., 2017). However, in that study identification of polymorph composition was conducted visually under a microscope and thus only identified presence/absence of vaterite on the surface of the structure (Reimer et al. 2017) unlike the techniques used in the present study that facilitated quantification of polymorph composition in the entire structure.

We found that a substantial proportion (90%) of all otoliths analysed at 32 dpf were comprised of 100% aragonite. This is the first time, to our knowledge that sturgeon otoliths have been found to be 100% aragonite. Historically it was believed that the sturgeon otolith was completely comprised of vaterite (Carlstrom, 1963), and recently it was shown that all three

polymorphs were present (Pracheil et al., 2017; Loeppky et al., 2019; Loeppky et al., 2021). To date adult lake sturgeon otoliths, have had no evidence of any aragonite present when examined using neutron diffraction (Pracheil et al., 2017). This leads to the conclusion that as the fish grows there is a shift in the primary polymorph being precipitated, such that the proportion of aragonite in adult fish may eventually fall below the detectable limits with the larger mass adult otoliths as suggested by Loeppky et al. (2021). An alternative explanation could be that wild sturgeon may never deposit aragonite in their otoliths as all larval lake sturgeon otoliths that have been analyzed thus far are lab reared while the adult otoliths were of wild origin. If there is an influence on otolith polymorph with rearing environment, and we know there are differences in the lab/hatchery environment compared to the wild, there could be additional undetermined environmental factor(s) affecting otolith polymorph composition. For example, in the hatchery, light and sound levels are both strongly manipulated, and it is known that both factors may influence sensory perception in fish (Popper and Carlson, 1998). Light has a high rate of transmission in water and sound attenuates slowly (Popper and Carlson, 1998) making them important factors to consider, however, not all species will perceive sound and light in the same manner. With respect to sound, not all species detect the same range, amplitudes or frequencies (Popper and Carlson, 1998). For example, in a study on 20 species of coral reef fish larvae, natural reef habitat noises as well as artificial sound conditions resulted in a range of responses in swimming behaviour from being attracted, repelled, or not influenced by the different sounds (Parmentier at al., 2015). Light also influences different species in different ways. For example, in a study on *Carassius carassius*, *Perccottus glenii,* and *Poecilia reticulata* they demonstrated that each species differed in their response to different colours of light, where some species grew better in green or blue light, while red light decreased all species growth rates (Ruchin, 2005).

Both light and sound levels within the hatchery could be affecting the otolith, as we strongly manipulate both within the hatchery environment. The sound levels are of particular interest given the otolith is the receptor for sound.

Our results for the ratio of aragonite to vaterite in the otoliths in both the 2019 and 2020 cohort can not be statistically compared due to differences in rearing techniques and broodstock, but the data clearly demonstrate that the ratio at each age is similar. A previous study using the same techniques to quantify the polymorph composition in lake sturgeon otoliths examined samples of a similar age and support the data reported in this study (Loeppky et al., 2019). In addition, Loeppky et al. (2021) also showed similarities in the patterns of percent vaterite and aragonite changes through ontogeny in both lake sturgeon and white sturgeon (*Acipenser transmontanus*). These species have quite disparate life cycles with lake sturgeon being largely a stenohaline freshwater fish but white sturgeon being an anadromous euryhaline fish. However, given the similarities in aragonite:vaterite ratios regardless of rearing environment in the present study alongside similar ratios in otoliths between species it suggests that otolith polymorph composition during early life history in sturgeons maybe a highly conserved trait. Fixed traits such as this, suggest strong selective pressure for a generalist phenotype that is suitable for a broad range of habitats (Hollander et al., 2006). However, while flow and substrate did not impact otolith polymorph composition, otolith size was affected perhaps suggesting more subtle environmentally dependent effects on otolith growth.

As described, the gross structure and polymorph content in otoliths of teleosts and sturgeon are quite different making comparisons between the two groups challenging. For example, the irregular shape of the sturgeon otolith makes metrics for shape such as circularity

and Fourier analysis (Tracey et al., 2006) impractical. However, previous studies have successfully used size metrics such as perimeter, area (DeVries et al., 2002; Aguera and Brophy, 2011; Begg and Brown, 2011), length, mass (Bakhshalizadeh et al., 2018), and axes measurements (Chalupnicki and Dittman, 2016) some of which have been applied to sturgeon otoliths. Specifically, axes measurement ratios were used to successfully distinguish between the eight species of sturgeon in North America (Chalupnicki and Dittman, 2016). In addition, differences in length and mass were used to identify between the great sturgeon (*Huso huso*), Persian sturgeon (*Acipenser persicus*), and the starry sturgeon (*Acipenser stellatus*) (Bakhshalizadeh et al., 2018). In our study axes measurements and length were not applied due to the size and extraction process and the irregularities in shape in larval otoliths. As an alternative we used maximum otolith diameter to ensure repeatability and retention of a metric of length. We were also unable to use otolith mass as a metric as available instrumentation did not facilitate accurate measurement of mass, further the fragility of the otoliths in these very young fish meant we could only remove and mount them for measurements in a single plane, thus perimeter, surface area, and maximum otolith diameter were reliably and repeatedly assessed.

Our results did show some differences in all three otolith size metrics during growth. Overall, the general trend in 2019 for perimeter, surface area and maximum otolith diameter was fish reared in the simple and complex treatments being different from each other with the simple treatment fish having a larger perimeter, surface area, and maximum otolith diameter than the moderate and complex treatment. In 2020, the general trend was the moderate treatment fish having a larger perimeter, surface area, and maximum otolith diameter than the simple and complex treatment. Our results in 2019 align with our prediction, where the most complex environment would promote smaller otoliths. This however only occurred at a few timepoints

across the timespan otoliths were measured and the polymorph data does not support this prediction. In a previous study manipulating rearing temperature with red drum (*Sciaenops ocellatus)* it was found that maximum length, projected area, volume and dry mass of otoliths all increased with temperature (Hoff and Fuiman, 1993). In another study examining otolith area, perimeter and maximum length in six species of the genus *Merluccius* and five species of the genus *Coelorhynchus*, they report a decrease in otolith size with an increase in body size which was correlated to water depth and temperature as larger individuals inhabit deeper and colder water temperatures (Lombarte and Lleonart, 1993). Additionally, a recent study examining lake sturgeon otolith development and the effect of water temperature and pH demonstrated that only temperature influenced otolith size (Loeppky et al., 2021). While another study manipulating pH demonstrated that differences in pH did alter otolith size in Atlantic cod (*Gadus morhua*) (Coll-Lladó et al., 2021). With these examples of changes as related to other environmental parameters, it suggests that otolith size may be more of a variable/plastic trait than polymorph composition. To further our understanding of how the environment might influence the otolith we investigated if there were differences in otolith symmetry.

Bilateral symmetry is where the left and right sides of an organism can be divided into mirror images of each other. Deviation from this is referred to as fluctuating asymmetry (Allenbach, 2011; Al-Mamry et al., 2011; Somarakis et al., 1997) where development does not follow the pre-determined symmetry in the organism (Allenbach, 2011) as a result of either environmental or genetic stress and can be of either natural or anthropogenic origin (Somarakis et al., 1997; Allenbach, 2011). Various studies have been conducted examining stressors such as pH, temperature and salinity but many studies have had confounding factors and could not specify a specific stressor (Allenbach, 2011). When comparing the symmetry of the left and right

otoliths in the present study, no differences were found in perimeter, surface area, and maximum otolith diameter. In pacific hake (*Merluccius productus*), shape measurements of otoliths appeared to be more sensitive to stress rather than linear or single dimension measurements (Alados et al., 1994) which provides one potential explanation as to why we might not have seen a difference between left and right with our metrics. Another reason could be that the treatments were not stressful enough to cause difference in symmetry. Fluctuating asymmetry is caused by stress and the treatments were not designed to stress the individual but rather to create a more naturalized environment, therefore no differences may have been seen due to a lack of environmental stress. In addition, no differences were found with the polymorph composition. Somewhat recently more attention has been given to the functional importance of the traits chosen. For example, in threespine stickleback (*Gasterosteus aculeatus*) a strong relationship between the asymmetry of the lateral plate number and parasite load was reported, in the habitat where the functional importance of the plates selected against their asymmetry (Bergstrom and Reimchen, 2005). Thus, it is reasonable to presume that morphological traits that are functionally important, undergo a stabilizing selection, and that this trait may be less likely to show fluctuating asymmetry (Allenbach, 2011). Due to the importance of otoliths in balance and hearing they may exhibit strong functional importance and therefore differences between the left and right otolith may not be tolerated in sturgeon. Indeed, in both *Sarotherodon melanotheron* and *Ethmalosa fimbriata*, no difference between the left and right otoliths in area, perimeter and diameter were found when fish were exposed to different salinities (Panfili et al., 2005). Furthermore, no difference in left and right otolith area, perimeter, maximum length, and minimum length was reported in golden shiners (*Notemigonus crysoleucas*) and channel catfish (*Ictalurus punctatus*) exposed to various sublethal isopropyl methylhosphonic acid

concentrations during embryogenesis (Green and Lochmann, 2006). Indeed, Allenbach (2011) concludes that many of the measurements used to assess the presence/absence of fluctuating asymmetry in otoliths (otolith length, diameter, area, perimeter, and radius) were less likely to be significantly different between the left and right otolith underscoring the functional importance of regular otolith growth on both sides.

Overall, our research has demonstrated that lake sturgeon otolith size may be a variable phenotype while the polymorph composition may be more of a fixed phenotype with respect to environmental complexity of flow. Our data also suggest that the functional importance of otoliths precludes deviations from bilateral symmetry. Given that otoliths are inert, and once deposited there is no resorption of elements (Campana, 1999) their early development may have significant impact on the fish's future hearing and orientation abilities. The consequences of variation in size and polymorph composition on the behaviour of sturgeons remains unknown, however, it is important to understand the survivability of fishes with different otoliths in relation to conservation aquaculture.

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Figure 3. 1. Photos of sagittal otoliths under dissecting microscope mounted with polarized lenses (Olympus SZX-PO) from larval lake sturgeon (*Acipenser fulvescens*) at age 46 days post fertilization from the 2020 cohort reared in A) simple, B) moderate and C) complex treatment. The black line indicates measurement of otolith perimeter and the, dark grey line illustrates measurement of maximum diameter. Scale = 1 mm.

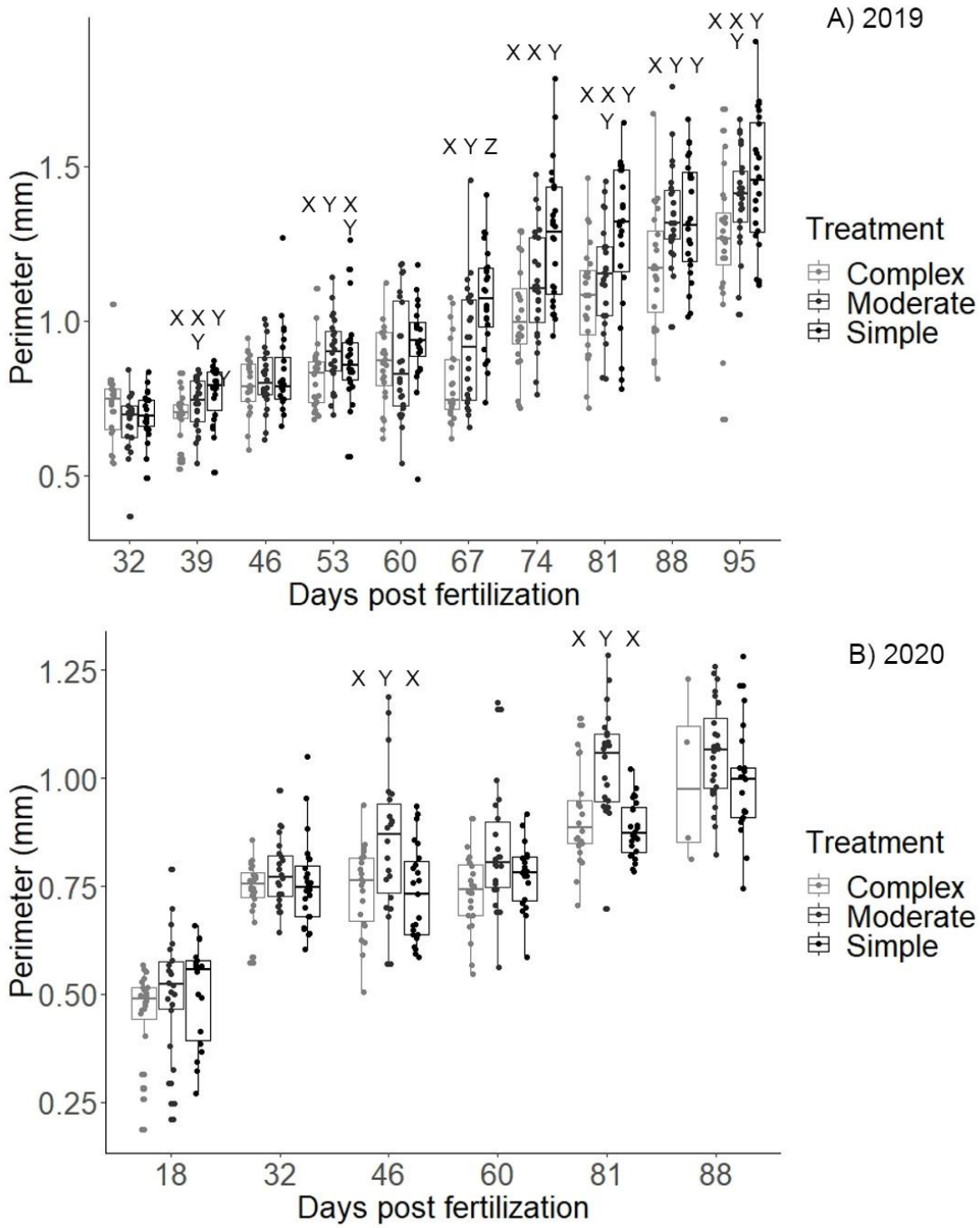

Figure 3. 2. Perimeter (mm) of larval lake sturgeon (*Acipenser fulvescens*) sagittal otoliths at age (days post fertilization) in A) 2019 and B) 2020 for complex (light grey), moderate (medium grey), and simple (black) treatments. Data are presented in box plots with the median represented by the horizontal line for each time point and the upper and lower lines represent the first and second quartile. The whiskers show the lower/upper quartile -/+ 1.5 times the upper minus the lower quartile. Each dot represents a single measurement within each treatment and different letters represent significant differences between treatments at each sampling point.

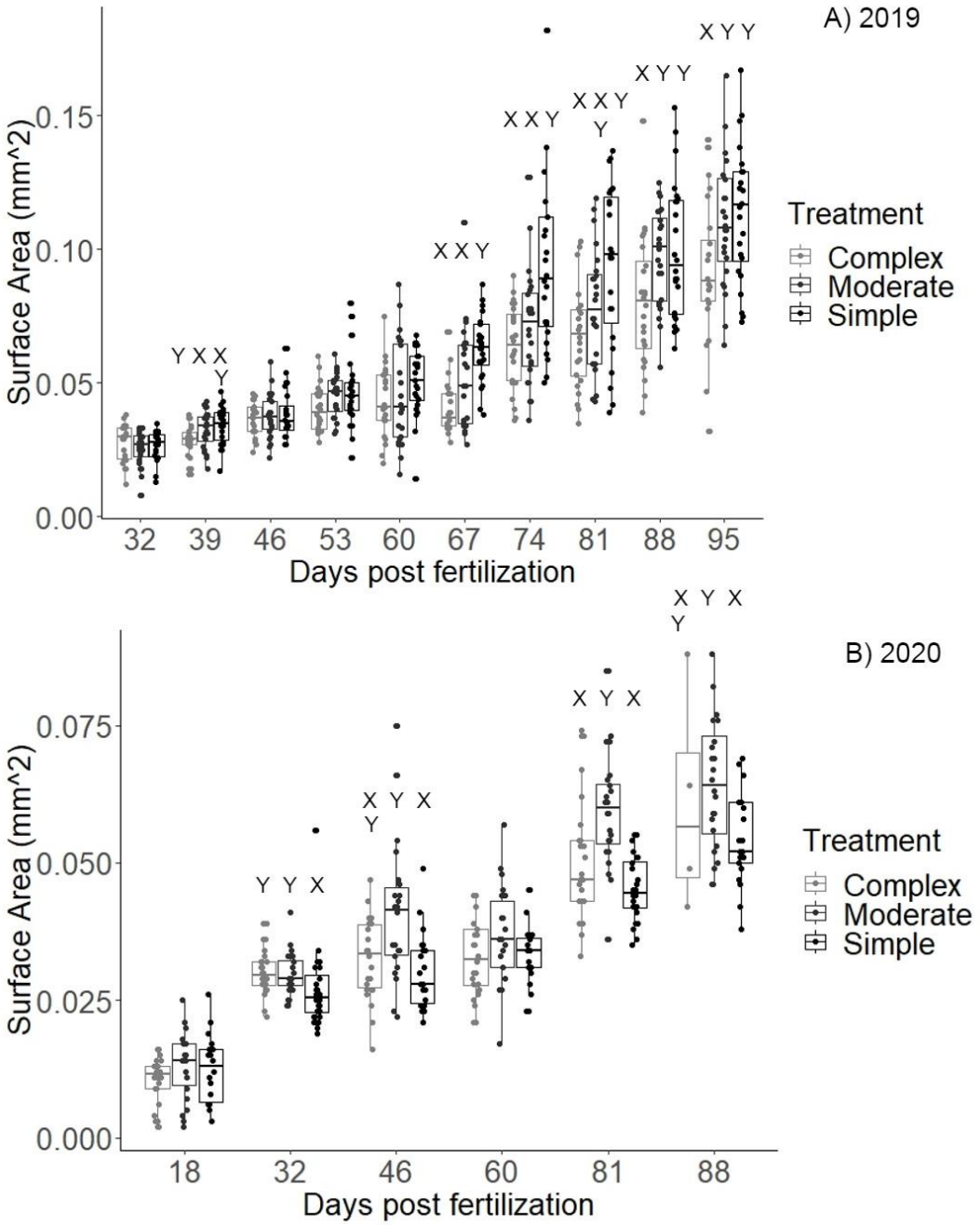

Figure 3. 3. Surface area (mm^2) of larval lake sturgeon (*Acipenser fulvescens*) sagittal otoliths at age (days post fertilization) in A)2019 and B)2020 for complex (light grey), moderate (medium grey), and simple (black) treatments. Data are presented in box plots with the median represented by the horizontal line for each time point and the upper and lower lines represent the first and second quartile. The whiskers show the lower/upper quartile -/+ 1.5 times the upper minus the lower quartile. Different letters represent significant differences between treatments at each sampling point.

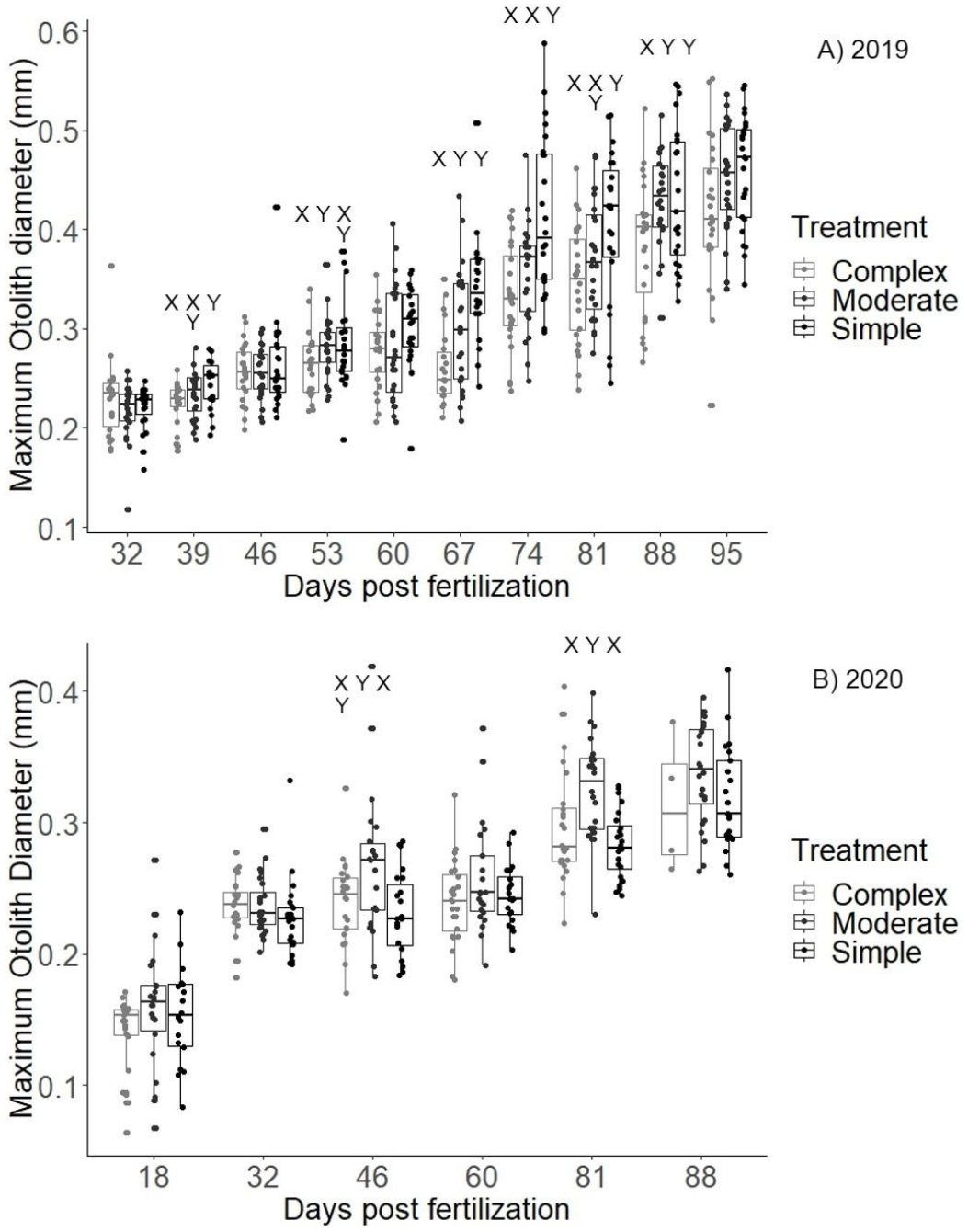

Figure 3. 4. Maximum otolith diameter (mm) of larval lake sturgeon (*Acipenser fulvescens*) sagittal otoliths at age (days post fertilization) in A)2019 and B)2020 for complex (light grey), moderate (medium grey), and simple (black) treatments. Data are presented in box plots with the median represented by the horizontal line for each time point and the upper and lower lines represent the first and second quartile. The whiskers show the lower/upper quartile -/+ 1.5 times the upper minus the lower quartile. Different letters represent significant differences between treatments at each sampling point.

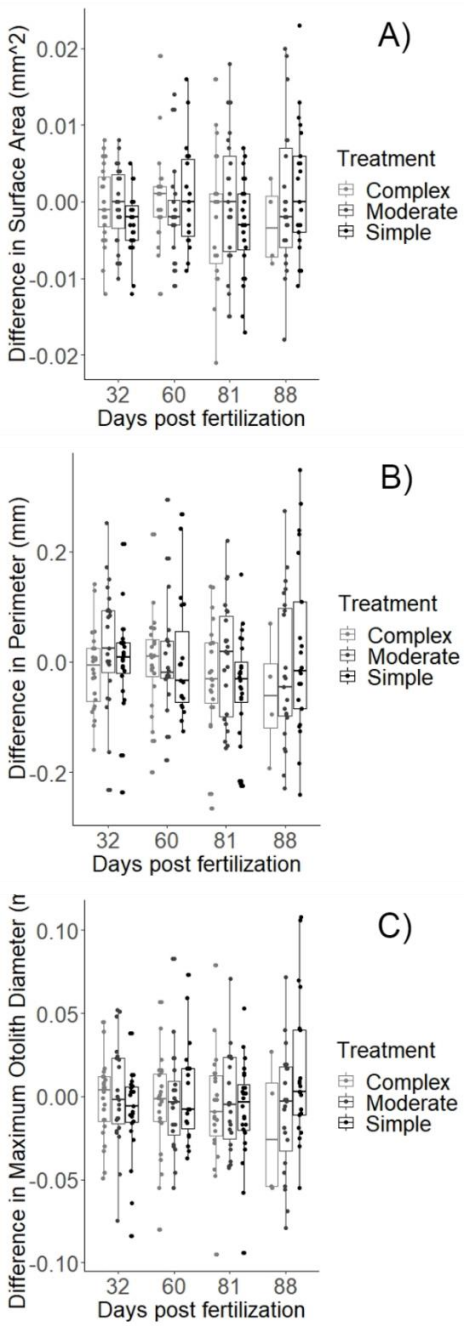

Figure 3. 5. The difference in a) surface area (mm^2) b) perimeter (mm) c) maximum otolith diameter (mm) between the left and right sagittal otolith of larval lake sturgeon (*Acipenser fulvescens*) at age (days post fertilization) for complex (light grey), moderate (medium grey), and simple (black) treatments. Data are presented in box plots with the median represented by the horizontal line for each time point and the upper and lower lines represent the first and second quartile. The whiskers show the lower/upper quartile -/+ 1.5 times the upper minus the lower quartile. Different letters represent significant differences between treatments at each sampling point.

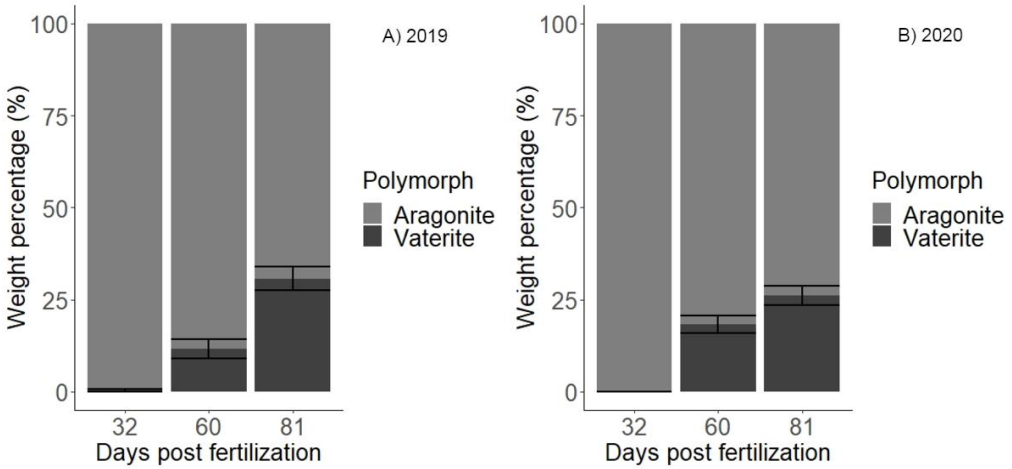

Figure 3. 6. The mean percent weight +/- se of $CaCO_3$ polymorph present in sagittal otoliths of larval lake sturgeon (*Acipenser fulvescens*) at age (days post fertilization) for A) 2019 and B) 2020.

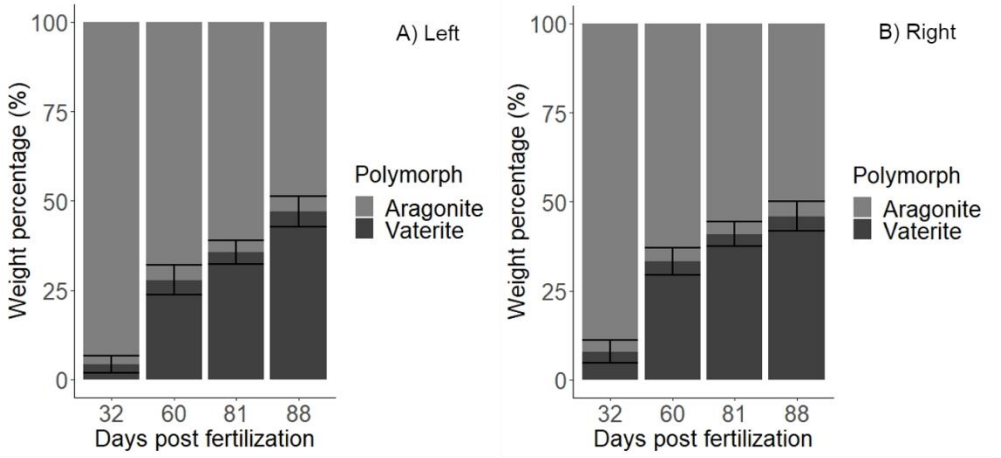

Figure 3. 7. The mean percent weight +/- se of $CaCO_3$ polymorph in A) left and B) right sagittal otoliths of larval lake sturgeon (*Acipenser fulvescens*) at age (days post fertilization).

Chapter 4: General Discussion

The environment, particularly during early rearing, plays an important role in the development of an individual, and will ultimately influence their fitness (Dammerman et al., 2015). Therefore, the habitat during embryonic and larval stages is critical in developing appropriate phenotypes. This is particularly important in the context of conservation aquaculture programs for conservation efforts of threatened and endangered species, including our study species, the lake sturgeon (Crossman et al., 2011). The practice of stocking fish has been a fisheries management tool for more than a hundred years (Pearson and Hopley, 1999). During this time stocking has evolved to address many concerns within the fishery; traditional supplementation primarily sought to increase abundance, while conservation aquaculture programs also seek to increase abundance, they also want to preserve genetic diversity, phenotypes, and behaviours (Schreier et al., 2012). The environment in a traditional hatchery is fully controlled and dissimilar to that of the wild, which allows for first year survival to be higher than in the wild (Alverson, 2002; Peterson et al., 2007), however, it also allows the development of alternative phenotypes and behaviours that may be maladaptive when fish are stocked into the wild. If we make changes to the hatchery rearing environment, we may facilitate a more natural phenotype. One potentially important abiotic environmental variable for sturgeons is water flow. Spawning sites are partially selected for based on flow (Dammerman et al., 2015), and therefore during the embryonic and larval stages the fish would naturally experience high water flows. The objective of the experiments conducted within chapters 2 and 3 were to examine how flow would influence growth, fin length, otolith size, and polymorph composition through early life-history in hatchery-reared larval lake sturgeon.

4.1 Growth

In chapter 2, we investigated the growth of larval lake sturgeon subjected to various flow conditions. We were able to determine that flow significantly influenced growth during the study period regardless of cohort. Throughout the sampling points we saw that a lack of flow had a positive effect on growth, where fish in the simple treatment grew largest. There is an optimal water velocity to have a positive effect on growth (Palstra and Planas, 2011). When above optimum, it leads to stress and fatigue reducing growth and when below, energy is lost to spontaneous swimming activity also reducing growth (Palstra and Planas, 2011). We did not attempt to optimize flow but rather mimic a naturalized flow rate and presumably flow was maintained at a velocity not at an optimum for this species. Indeed, observations of swimming individuals in treatments with flow demonstrated swimming behaviour to avoid direct current, expending energy moving to more desirable flow conditions i.e. seeking out edges in the no substrate treatments and back eddies in the substrate treatments.

We also determined that substrate influences growth, where fish were smallest in the complex treatment during a period of the study. Reduced growth had been previously seen in Atlantic sturgeon (*Acipenser oxtrinchus*) reared with substrate (Gessner et al., 2009). This is likely due to inefficient foraging, as other studies have described how food would settle under and in between the substrate making it more difficult to find the food (Nguyen and Crocker, 2006; Yoon et al., 2020). The potential negative growth effects of the substrate could be negated if it is removed upon exogenous feeding (Boucher et al., 2014; Yoon et al., 2020). Indeed, lake sturgeon naturally inhabit gravel type substrate from egg until they begin exogenous feeding, largely hiding within the interstitial spaces (Wishingrad et al., 2014a). In white sturgeon

(*Acipenser transmontanus*), the removal of substrate at exogenous feeding resulted in a prolonged increase in growth of the larval fish (Boucher et al., 2014).

There are potential behavioural benefits from rearing with substrate for lake sturgeon that might outweigh the initial growth differences. It has been identified that predation is one of the most important factors contributing to mortality early in life for fish (Hunter, 1976) and therefore antipredator behaviour is important for survival, and a skill hatchery fish will need once released into the wild. One way that sturgeons display antipredator behaviour is by using protective habitats, which involve hiding within the interstitial spaces in the bottom substrate. Usually, these habitats are often poor for foraging and residing in them may result in a cost to feeding (Ydenberg and Dill, 1986). Lake sturgeon have shown the ability to increase antipredator activity when exposed to alarm cues, specifically at the larval life stage (Wishingrad et al., 2014b). Additionally, cover seeking behaviour by hiding within substrate has been demonstrated in both clear and turbid waters, where perceived predator risks are different (Wishingrad et al., 2014a). Further, alarm cue responses in early exogenous feeding larvae and prewinter showed a difference in preference to substrate, where gravel was preferred by larval feeding sturgeon and pre-winter fish preferred the absence of substrate (Bjornson et al., 2020). The fish exposed to chronic predation risk spent significantly more time over the gravel substrate than acutely exposed fish (Bjornson et al., 2020). By continuing to rear fish in substrate post exogenous feeding, we may evoke a natural behavioural response, that sturgeons without substrate will not have, therefore better preparing them for their natural environment post release.

Additionally, flow and substrate may have initially reduced growth, but by the end of the study period no differences were seen between treatments. Fish in the complex treatment eventually grew larger and gape size would have increased with fish size, ensuring effective

foraging and fish that were unable to forage died, as seen by an increased mortality rate within the complex treatment. Although mortality may have increased, the potential benefits of the use of substrate and flow on phenotypes and behaviours are important for post-release fish. Investigations into these benefits and the timing of substrate removal are pertinent to adopting new hatchery protocols to increase fitness of released fish.

4.2 Fins

In chapter 2, we investigated how fin length and finspan of larval lake sturgeon may be affected by various flow conditions. We demonstrated that there were a few instances of differences between treatments for both fin length and finspan where the complex treatment had smaller fins and fish in the simple treatment had larger fins. However, there was a general lack of difference between treatments. We determined that there was a similar relationship between finspan and total length regardless of treatment and therefore we did not support our prediction that the increased environmental complexity would result in longer fins. The differences we found were likely due to the differences in total length, and therefore not a function of the environment. Interestingly, we did observe station holding behaviour in the fish reared in both the moderate and the complex treatments (i.e. treatments with flow).

Station holding behaviour is a technique typically used by sturgeon to maintain their position in flow by pressing the abdomen and pinning their pectoral fins to the substrate allowing the fish to conserve energy when in high flow habitat (Adams et al., 2003; Deslauriers and Kieffer, 2012). This behaviour is important once released from the hatchery as sturgeons reside in high flow environments and as such, variations in the functional morphology of the fin has the potential to impede the ability of the fish reducing the fitness of an individual. Although we did not see difference in fin length during the study period, in retrospect fin surface area and the

number of fin rays may have been additional measurements worth investigating. Organisms that live in high flow regimes exhibit adaptions to these conditions in numerous ways and not only would increasing fin length be beneficial but also the surface area to catch water and the number of fin rays may alter the flexibility of the fin. In a previous study, fin length was seen to differ between sand darters occupying different flow habitats (Carlson and Lauder, 2009). In a study on 9 species of sculpins (Scorpaeniformes: Cottoidea) the individuals exposed to high flow had a larger demand for station holding, and in turn they demonstrated they had a larger pectoral fin area (Kane and Higham, 2012). Further, the number of fin rays with webbing was seen to be reduced in *B. cirrhosus* in which the demand for station holding is minimal (Kane and Higham, 2012). The need to understand how flow effects the fins in any way is important for the hatchery fish upon release to increase the development of appropriate phenotypes and ultimately fitness of the individuals.

Additionally, a few individuals were left to grow onwards, and observations of fin curl were seen. Fin curl is a condition that can happen as soon the fin begins to develop in hatcheries where the fin bends in towards the body, diminishing the area of the fin (Deslauriers et al., 2016). In shovelnose sturgeon (*Scaphirhynchus platorynchus*), a positive relationship between diminished fin size and reduced swimming performance was observed (Deslauriers et al., 2016). The causes of fin curl are unknown, but perhaps a lack of flow may play a role in its development. Further investigations as to how this develops in lake sturgeon and how it may be mitigated in the hatchery is important for the fitness of the individual.

4.3 Otoliths

In chapter 3 we explored the otolith to determine if the different environments effected otolith size and polymorph composition. The otoliths exhibited no differences in polymorph

composition with the different treatments. Further, the dominant polymorph observed was aragonite, and in the youngest fish, aragonite made up 100% of the composition in 90% of the individuals. Some changes in polymorph have been reported with temperature in lake sturgeon otoliths (Loeppky et al., 2021). Our study also demonstrated that the ratio of aragonite to vaterite is fairly consistent both in our 2 cohorts and in Loeppkey et al. (2021). They also found it to be similar in white sturgeon, despite their life cycle being different than the lake sturgeon (Loeppkey et al., 2021). Thus, suggesting that otolith polymorph may be a highly conserved trait. While polymorph may be fixed, the otolith size data suggests that it may be a more variable trait.

We were able to demonstrate differences in otolith size during growth. However, in 2019, the perimeter, surface area, and maximum diameter were generally larger in fish in the simple treatment, while in 2020 otoliths from fish in the moderate treatment were larger. The 2019 data supports our prediction, but these differences only occurred at a few timepoints and the polymorph data does not support this hypothesis. There have been many instances of differences in size related metrics to temperature and other environmental parameters across multiple species (Hoff and Fuiman, 1993; Lombarte and Lleonart, 1993; Loeppky et al., 2021). While they do relate to different environmental parameters it does show that differences in otolith sizes appear to be more plastic than otolith polymorph composition.

Differences in otolith sizes may lead to behavioural consequences. Since the otolith is vital in hearing and balance/orientation, differences in sizes may affect the organism's ability to do so. Lake sturgeon hearing has been tested and showed they are responsive to the motion of water particles in a sound field in frequencies from 100 to 500 Hz (Lovell et al., 2005). Our understanding of sounds they may use and how they perceive it is still under speculation. Although we do not know how they may use their otolith for hearing, the otolith is still vital for

the ability of the fish to balance and orient themselves within their habitat. So, it is important to understand how differences in otolith size might affect this behaviour, especially as this will affect their fitness upon release from a hatchery. A study on red drum (*Sciaenops ocellatus*) demonstrated fish with greater otolith volume perceive their environment differently, where fish with the higher volume otoliths had a greater response to visual stimuli (Browning et al., 2012). The fish with higher otolith volume also had an increased stress response as seen by a significantly increased cortisol level compared to the normal fish (Browning et al., 2012). In zebrafish (*Danio rerio*) larvae exposed to cadmium, otolith surface area was reduced compared to fish not exposed and the fish showed impaired balance control and swimming activity (Han et al., 2019). Both these factors demonstrate that changes to the otolith do affect the swimming behaviour of the individual. Upon release from a hatchery, fish that have impaired balance and swimming ability will have a reduced fitness. They will likely not be able to effectively forage, navigate their habitat, or evade predators leading to mortality. Further research into changes in otolith size and how it effects the swimming ability of sturgeons is important to aid in the mitigation of this in the hatchery environment and avoid negative fitness consequences.

In chapter 3, we observed no differences in symmetry of the left and right otoliths both in size and polymorph composition. Due to the importance in balance and hearing, it is reasonable to assume that the otolith is functionally important and therefore deviations from symmetry are not tolerated. Interestingly, previous investigations have shown no differences in left and right otolith size by area, perimeter and diameter in several species of fish exposed to various environmental parameters (Panfili et al., 2005; Green and Lochmann, 2006; Allenbach, 2011). These studies further demonstrate that the otolith is functionally important across species and differences between the left and right are not tolerated. Further our data suggest that in future

studies sampling cost, time, and energy can be reduced as only one otolith per fish needs to be sampled.

4.4 Conclusion

Maladaptive phenotypes occur when there is an environmental mismatch where the phenotypes developed are adapted to one environment and a reduction of fitness occurs in an alternate environment (DeWitt and Yoshimura, 1998). Hatchery fish typically perform poorly in the wild due to this environmental mismatch (Stringwell et al., 2014). Most species develop phenotypes based on their early rearing environment to meet the challenges of that environment, therefore, the habitat for fish reared in conservation aquaculture is essential to avoid maladaptive phenotypes. For instance, coho salmon (*Oncorhynchus kisutch*) reared in a hatchery demonstrated differences in size, survival, and swimming endurance compared to naturally reared fish in a stream (Chittenden et al., 2010). Further, hatchery reared trout (*Salmo trutta*) were insensitive to predator proximity, while wild fish sought refuge (Álvarez and Nicieza, 2003). These phenotypic differences between hatchery and wild fish demonstrate maladaptive phenotypes are present within hatchery fish. As explored throughout this thesis, water flow is only one environmental variable that is different between hatchery and wild environments for lake sturgeon. Many consequences have been explored ranging from growth differences, behaviors like foraging and predator evasion, fin curl, ability to station hold, and swimming impairment and balance control. In a study on Atlantic salmon (*Salmo salar*), hatchery fish that were recaptured after release had longer heads, thicker caudal peduncles and more streamlined body as well as being more cryptic and had less occurrences of fin erosion than the fish kept in the hatchery (Stingwell et al., 2014). Thus, indicating that in some species some of the individuals released are able to adjust to the dissimilar environment while others may perish.

Our research indicates that the use of flow and substrate in the rearing environment does influence larval lake sturgeon development. We were specifically able to demonstrate that growth and otolith size was affected by the flow and substrate in the early rearing environment. Additionally, we gave several instances of potential behavioural benefits to its use that will likely affect the fitness of an individual once released from the hatchery. It is recommended to continue research into the use of flow and substrate in the hatchery before protocols can be widely adapted. Specifically, research conducted to investigate swimming behaviour and balance control with different otolith sizes as this will directly influence survival upon release from the hatchery. Further, research should also identify if behavioral benefits like foraging success, predator evasion, and station holding ability are seen within fish reared in this environment. Lastly, incidence of fin curl in lake sturgeon larvae should be quantified and monitored for and research conducted to determine the cause.

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