OVERWINTER MORTALITY OF YOUNG-OF-THE-YEAR STRIPED BASS (*Morone saxatilis*) IN FRESHWATER PONDS IN NOVA SCOTIA

by

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DEDICATION

For Vincent, without whom this thesis could not have been accomplished. Thank you for always being there with hugs, knowledge of Excel, and desserts. You believed in me when I didn't believe in myself and always reminded me that "on est une équipe".

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ABSTRACT

Commercial fishing for striped bass (*Morone saxatilis*) is very limited in Canada, therefore there is potential for pond-based aquaculture to fill the consumer market. One impediment to culture is high overwinter mortality among young-of-the-year (YOY) striped bass. In a culture setting, mortality is restricted to YOY bass transferred to cages in constructed freshwater ponds in November; larger bass (>500 g) typically survive well under the ice. Two trials were completed in consecutive winters (2016-2018) to investigate the effect of body size (range ca. 30-50 g), pre-winter diet, and pond characteristics on overwinter survival in 1m^3 cages. Mortality rates varied between ponds and years from 10 to 100 % and was inversely related to body size in some cases but not others. Diet had an insignificant effect on survival. Muscle total lipid and triacylglycerol (TAG) and liver TAG proportions on a wet-weight basis were significantly higher when samples from November and March were compared. Phosphoethanolamine (PE) mass in total muscle phospholipid was higher in striped bass sampled in the winter, but proportions of 18:0 and 22:6n-3 in PE and 18:1n-9 and 22:6n-3 in phosphatidylcholine were lower in March samples. Striped bass appear to rely on other lipid classes or body tissues than those analyzed in this study to survive the winter.

LIST OF ABBREVIATIONS USED

BW	Body weight
ca.	Approximately
Dal-AC	Dalhousie University Agricultural Campus
DO	Dissolved oxygen
dph	Days post hatch
DU	Designatable units
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
CHOL	Cholesterol
FA	Fatty acids
FAME	Fatty acid methyl esters
FL	Fork length
FW	Freshwater
GC	Gas chromatography
HPLC	High performance liquid chromatography
IM	Intra-muscularly
LC PUFA	Long chain polyunsaturated fatty acid
LDN	Natural day length
NB	New Brunswick
NS	Nova Scotia
NTU	Nephelometric Turbidity Units
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PL	Phospholipids
ppm	Parts per million
ppt	Parts per thousand
TAG	Triacylglycerols
TL	Total lipid
TLC	Thin layer chromatography
US	United States
YOY	Young-of-the-year

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CHAPTER 1 Introduction

1.1 Motivation

The Nova Scotia aquaculture finfish industry is currently dominated by the production of salmonid species; most notably Atlantic salmon (*Salmo salar*), which accounts for almost 86 % of the 116 million dollars in total sales (including shellfish) generated by aquaculture production across the province (DFA, 2017). Atlantic salmon production in ocean-based net pens has met considerable opposition due to its perceived impact on coastal environments and communities (Doelle and Lahey, 2014). The Doelle-Lahey Panel Report (2014) offered insight into the thoughts and opinions of Nova Scotians towards finfish marine-based aquaculture, especially that of Atlantic salmon, and introduced a proposed framework on how to move the aquaculture industry forward. The overarching theme of this framework is "low impact, high value aquaculture", indicating that if the aquaculture industry in this province is going to move forward successfully, it needs to adopt practices that offer the lowest negative impact on surrounding ecosystems and human communities while focusing on high value production (Doelle and Lahey, 2014).

The research presented in this thesis is directed towards the goal of "low impact, high value aquaculture", by investigating barriers to the possible commercial cultivation of striped bass (*Morone saxatilis*). In addition, the cultivating method does not involve controversial ocean net pens, but rather constructed freshwater (FW) ponds. Thus, this research addresses the challenges associated with growing striped bass in FW ponds, with a particular focus on the challenges of overwintering young-of-the-year (YOY) fish.

The work is a continuation of experimental trials that began in 2012 by investigators based at Dalhousie University's Faculty of Agriculture (Dal-AC; then known as the Nova Scotia Agricultural College). This early research consisted of placing YOY striped bass that had been reared in a laboratory setting into FW ponds located on land owned by a private Nova Scotia based aquaculture company, North River Fish Farms Ltd. (NRFF). This early experimentation into overwinter survival of YOY striped bass at NRFF is unpublished, but the data indicates that cohorts frequently experienced up to 100 % mortality during their first winter. In contrast, 1-year-old fish stocked into the same ponds in May (body weight ca. 100 g) that reached more than 400 g by the end of the growing season in November, suffered negligible mortality over the subsequent winter. Between 2012 and 2016, prior to my involvement in this work, various trials were conducted to determine the cause, or causes, of the high rates of YOY overwinter mortality. Experimental factors that were investigated included body size, the timing of stocking fish into ponds, and the depth at which monitoring cages were suspended in the ponds. Results of these trials proved inconclusive since mortality was typically 100 %. The thick ice covering the ponds from December to late-March were an important impediment, making sampling difficult. A noteworthy result was the YOY fish contained large amounts of visceral fat, even near the end of winter. Thus, when I joined the team in 2016, a hypothesis to explain the overwinter mortality was that fish were somehow unable to metabolise their energy reserves during the winter, perhaps due to an inappropriate pre-winter diet.

1.2 Thesis objectives

The aim of this thesis was to gain more knowledge on the sources of overwinter mortality of captive YOY striped bass in FW ponds. Specific objectives were:

1) To determine if YOY striped bass were exhausting their liver and muscle lipid reserves overwinter;
2) To describe changes in the fatty acid profiles of certain phospholipid classes found in the muscle tissues of YOY striped bass exposed to winter rearing conditions; and
3) To determine the effects of body size and pre-winter diet on overwinter survival rates of YOY striped bass.

These objectives were addressed in two trials, completed over the winters of 2016/17 and 2017/18. Through this research I hoped to advance the understanding of sources of overwinter mortality in YOY striped bass stocked in FW ponds.

1.3 Thesis structure

This thesis is comprised of four chapters. This first Chapter builds the rationale for the research objectives by reviewing the state of knowledge. The remainder of the Chapter introduces striped bass as a species, their historical importance, the current state of striped bass research locally, and reasons why this species could be included into Nova Scotian aquaculture. Chapter 2 presents the results of two consecutive overwinter survival trials that involved placing YOY striped bass in FW ponds for the duration of their first winter and concludes with a discussion on general causes of mortality and areas of potential future research. Chapter 3, intended as a potential journal article, reports explicitly on results of tissue lipid analyses on samples taken from fish over the winter of 2016/17 to assess changes in lipid characteristics. Specifically, it describes changes in lipids found in the white muscle tissue and liver of YOY striped bass from November to March. It is important to note that part of the methods section of Chapter 3 refers readers back to Chapter 2, as the work presented is a component of the larger trial occurring in the 2016/17 winter. Intended co-authors include Dr. Suzanne Budge, Mr. Juan Manriquez-Hernandez, Dr. Peter Tyedmers, and Dr. James Duston. At this time, a journal of interest for submission is the *Canadian Journal of Fisheries and Aquatic Sciences*. The final Discussion Chapter of this thesis, Chapter 4, synthesizes the major results and summarizes the advances in the state of knowledge from the research undertaken, along with recommendations for future research.

1.4 Overview of striped bass distribution and ecology

Striped bass is an anadromous, euryhaline species native to eastern North America. Historically, spawning has occurred as far south as the St. Johns River in Florida in the southern United States (US), to the St. Lawrence River in Canada (Scott and Crossman, 1973; Scott and Scott, 1988). Populations have also been introduced to the Pacific in Californian rivers, and their habitat ranges from British Columbia, Canada, to the border between Mexico and the US (Bonn et al., 1976; Hodson, 1989). Most wild populations of striped bass include a significant marine phase in their life histories, including estuaries and coastal marine environments, but a number of land-locked populations exist, primarily due to the stocking of many FW lakes and reservoirs across the US (Hodson, 1989; Bettoli, 2005).

Generally, striped bass spend most of their adult lives in seawater and embark upon extensive seasonal migrations in coastal waters (Rulifson and Dadswell, 1995). Spawning occurs during the spring and consists of an upriver migration to fresh or estuarine water where females will release upwards of 50,000 eggs per kilogram of body weight

(COSEWIC, 2012). Juveniles inhabit in estuaries and coastal areas for approximately one to two years before transitioning to an ocean habitat for much of the year (Rulifson and Dadswell, 1995). Winter habitat preferences vary; however, in the Maritimes evidence suggests that striped bass seek refuge in FW (Douglas et al. 2003; Bradford et al. 2012, 2014), whereas overwintering in coastal sea water has been reported in areas in parts of the US, such as off the coast of New Jersey (Able et al., 2012). Maturity typically occurs around age-3 in males and up to age-6 in females (Scott and Scott, 1988).

In Canada there are three distinct, or designatable units (DU), of striped bass that are reported by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC): the St. Lawrence River, the Southern Gulf of the St. Lawrence River, and the Bay of Fundy (COSEWIC, 2012). As of 2012, the Southern Gulf of the St. Lawrence River population was listed under special concern, whereas the Bay of Fundy and St. Lawrence River populations were listed as endangered and remain in such state currently (COSEWIC, 2012).

The Bay of Fundy DU includes the Annapolis River, Saint John River, and Shubenacadie River populations. Of these three, only the Shubenacadie River is considered to be a viable spawning population (COSEWIC, 2012). Importantly, LeBlanc et al. (2018) recently presented good evidence that genetically distinct striped bass in the Saint John River exist, when compared to Shubenacadie River, Hudson River, and Chesapeake Bay populations.

1.5 Historical importance of striped bass

Throughout their native range, humans have had a long-standing relationship with striped bass as a food fish (Karr, 1999; Richards and Rago, 1999; Nelson, 2018). Striped bass populations have supported substantial commercial and recreational fisheries, particularly in the eastern US, where they are both a highly prized game fish and table fish (Setzler et al., 1980; Nelson, 2018). Substantial directed commercial fisheries of striped bass have been maintained in US waters continuously for over a century, including a period of a substantial stock decline in the late 1970s and early 1980s (ASMFC, 2019a). In 2015, the reported commercial landings of striped bass in US waters was ca. 2100 tonnes (ASMFC, 2019a). In addition to commercial exploitation, striped bass are an enormously popular game fish in coastal and in-river settings in the US, with the highest reported landings occurring in Maryland, Massachusetts, New York, New Jersey, and Virginia (ASMFC, 2019b).

In Canada, early efforts to culture striped bass commercially were met with limited success (Cairns et al., 1999). The only known North American producer of pure striped bass is Pacifico Aquaculture based in Baja California, Mexico (FAO, 2014). At this facility, fish are spawned and reared in a hatchery setting, before being transferred to ocean-based net pens off the coast of Baja California, Mexico, for grow-out. In addition, hybrid striped bass, which are generally a cross between white bass (*Morone chrysops*) and striped bass (*Morone saxatilis*), have been routinely cultured for decades in the US (Harrell, 1997). Production of hybrids largely occurs in three steps including seed production, a nursery phase for the rearing of fingerlings, and a grow-out phase where fish reach market size (FAO, 2016). For hybrid striped bass, earthen ponds are the most common grow-out environment, followed by indoor recirculation systems (Hodson, 1989; Kohler, 2004; FAO, 2016). As of 2012, the most recent US aquaculture census available at this time, the annual production of hybrid striped bass in the US amounted to ca. 5600 tonnes of market-sized live weight annually (USDA, 2013).

In Atlantic Canada, striped bass have historically been harvested commercially in NS and New Brunswick (NB) in both targeted fisheries, or as bycatch in fisheries focused on other species such as American shad (*Alosa sapidissima*), Atlantic salmon, and gaspereau (*Alosa pseudoharengus*) (Dadswell, 1983; Rulifson and Dadswell, 1995; Andrews et al., 2017). These landings were generally much smaller than those reported in the US, with the largest being 50 tonnes in 1945 (Melvin, 1991; Rulifson and Dadswell, 1995). Historically, the largest annual landings of striped bass in Canadian waters are reported in NB with much of the harvest taken from populations that spawned primarily in the Miramichi River but also in various rivers throughout the southern Gulf of St. Lawrence (Jessop, 1991). The Saint John River population within the Bay of Fundy DU has also supported directed and incidental fisheries on and off since the late 1880s to 1978 (Jessop, 1991; Andrews et al., 2017). Between 1890 and the closure of the fishery in 1978, the largest annual landing, including targeted and non-targeted fisheries, was ca. 44 tonnes in 1959 (Andrews et al., 2017). However, annual landings fluctuated and could be as low as 45 kg, as reported in 1909 (Andrews et al., 2017). Fish found in the Saint John River could originate from the US due to the migratory tendencies of this species (Rulifson and Dadswell, 1995). In NS, the largest commercial landings were historically reported in Hants and Colchester counties, believed to be harvested predominantly from the Shubenacadie River population (Caddy and Chandler, 1976). In total, commercial landings in Nova Scotia were the greatest in 1943 and 1962 with 13 and 15 tonnes, respectively (Jessop, 1991; Rulifson and Dadswell, 1995). The commercial fishery in NS closed in 1970 (Rulifson and Dadswell, 1995). A noteworthy exception to closure of the directed striped bass fisheries in the Maritimes is the recent re-establishment of a small-scale commercial fishery that re-opened in 2018 for the Eel Ground First Nation. This fishery allows the community to harvest 50,000 individuals a year from the Southern Gulf DU found in the Miramichi River (CBC, Ibrahim, 2018).

In terms of recreation, both the Southern Gulf and Bay of Fundy DU support popular recreational fisheries, due to the fighting capacity of striped bass and their desirability as a table fish. In NS, it is possible to fish for striped bass in coastal waters throughout the year, whereas inland waters typically only have an open season during the months of April to October (DFO website, 2019). As of 2015, bass species, including striped and smallmouth bass (*Micropterus dolomieu*), were among the top five species targeted by recreational anglers in NS while striped bass were the third most fished species in NB (DFO, 2015).

Research on striped bass culture in eastern Canada has occurred since the 1980s. This was predominantly centered around rearing programs with the intention of determining how to successfully spawn striped bass for potential aquaculture purposes (Cairns et al., 1999). This endeavour was inspired by both the success in the 1980's with Atlantic salmon (*Salmo salar*; Boghen, 1995) and that of hybrid striped bass culture in the US (Cairns et al., 1999). A total of 15 rearing programs were initiated in the Maritimes region, in addition to four in the province of Québec during the 1980's (Cairns et al., 1999). Currently in NS, the primary experimental spawning and rearing facility of striped bass (working with Shubenacadie River fish) remains and is based at the Agricultural campus of Dalhousie University in Bible Hill, NS under the direction of Dr. J. Duston. This facility has over the last 20 years spawned and reared multiple generations of striped bass for experimental purposes. Successful production of larvae and juveniles enabled fundamental lab-based studies (Duston et al., 2004; Cook et al., 2006; MacIntosh and Duston 2007; Cook et al., 2010; Sampson et al. 2013; Manriquez-Hernandez et al., 2019), which extended to farm-based studies in collaboration with NRFF. Attention turned to overwinter survival of YOY striped bass, an important biological question of relevance to the production of this species in both the wild and farmed settings.

North River Fish Farms is located approximately 18 km north of Truro, NS and rears striped bass provided by Dal-AC in small net pens (ca. 12 m x 7 m) suspended in one of three constructed ponds on the property. Fish are typically fed a low fat commercially available salmonid diet, such as EWOS Vita (14 % crude fat) or Corey Aquasea (between 16 - 18 % crude fat) and are reared until they reach a market-size of approximately one kilogram.

Cultured production of striped bass in NS remains very small. In addition to NRFF, few locations have licenses to grow striped bass, and these include: Two Rivers Hatchery and Hamilton's Eel Fishery Limited, however these remain more on an experimental level and reliant on juveniles from the Dal-AC. Production statistics are not currently reported to the species level and are combined with data for a number of finfish species including brook trout (*Salvelinus fontinalis*) and Atlantic halibut (*Hippoglossus hippoglossus*). Together, these species accounted for a total of ca. 14 tonnes live weight in 2017 (DFA, 2017). In terms of value, this group only accounts for 0.26 % of the total provincial aquaculture (shellfish and finfish) production value that same year (DFA, 2017).

Looking to the future, there is great interest in advancing the aquaculture sector in NS in ways that could assist with its resiliency and sustainability, as it would not be relying as heavily on salmonids or on coastal net-pens (Doelle and Lahey, 2014). The inclusion of other finfish species could also provide opportunities for communities and attract new operators to the industry (Doelle and Lahey, 2014; Pers. comm., Dr. Peter Sykes, formerly Research and Development Coordinator for the Aquaculture Association of Nova Scotia, Nov. 29, 2016).

1.6 Shubenacadie River striped bass

Shubenacadie River striped bass typically spawn in the Stewiacke River, the major tributary of the Shubenacadie River, during the months of May and June (Bradford et al., 2012; Duston et al., 2018). In a nine-year study, most wild spawning events occurred in the river after 11 to 21 degree-days above 12 °C (Duston et al. 2018). Further, these spawning grounds are influenced by a tidal bore that is formed by the incoming tide against the river's current. This spawning location represents one of the only documented areas where striped bass successfully spawn under these dynamic tidal conditions where water quality can change very quickly (Lynch, 1982; Rulifson and Dadswell, 1995; Rulifson and Tull, 1999). After spawning events, the pelagic eggs drift with the tide and hatch in approximately two days (Duston et al., 2018). Larvae, also passively transported by the current, tend to be found in areas where salinity is between 0.1 and 10 ppt (parts per thousand; Duston et al., 2018). Their initial diet is copepods (Duston et al., 2018). As YOY striped bass grow in length, typically greater than 25 mm, their diet evolves to include mysids, shrimps, amphipods, and small fish, in addition to copepods (Duston et al., 2018). Throughout the summer YOY striped bass become widely dispersed from tidal areas of the lower Shubenacadie River to Cobequid Bay and Minas Basin (Bradford et al., 2012; Duston et al., 2018).

1.7 Young-of-the-year striped bass during the winter

Winter mortality in YOY fish is common among temperate regions. Examples include white perch (*Morone americana*; Post and Evans, 1989), Eurasian perch (*Perca fluviatilis*; Huss et al., 2008), Atlantic silversides (*Menidia menidia;* Conover 1984), Atlantic salmon (*Salmo salar*; Johnston et al., 2005; Boudreau and Bourdages 2000), and rainbow trout (*Oncorhynchus mykiss*; Biro et al., 2004). Striped bass also experience high mortality during their first winter (Hurst and Conover, 1998; Hurst et al., 2000; Bradford and Chaput, 1997).

Temperate regions are characterized as having long winter periods with relatively low prey availability, coupled with a short summer period associated with growth of organisms due to an increased abundance of resources (Shuter and Post, 1990; Hurst and Conover 2003; Biro et al. 2005). Due to this variety of seasons and conditions, temperate species of fish have evolved strategies to overcome a multitude of stresses (Hurst 2007). During the winter months, these stresses are due to an increased reliance on endogenous energy reserves (Robichaud-LeBlanc et al., 1997; Thompson et al., 1991; Hurst and Conover, 2001), adaptation to changing water temperatures (Beitinger et al., 2000), osmoregulatory stress (Johnson and Evans, 1996), and the susceptibility to pathogens and disease (Horning and Pearson, 1973; Bowden, 2008; Abram et al., 2017). Thus, overwinter mortality in YOY temperate fish can be attributed to a combination of these environmental stresses (Hurst, 2007).

Young-of-the-year striped bass in the Hudson River experience an energy deficit overwinter, which results in an increased vulnerability to stress when a combination of such stressors is present (Hurst et al., 2000). Although mortality and body size were negatively correlated, Hurst et al. (2000) indicate that starvation was not the ultimate cause of mortality because they were able to compare the energy levels in mortalities occurring in laboratory trials to energy levels of wild YOY striped bass during the winter. Alive, wild YOY striped bass sampled in late-March (1994) had lower energy levels than dead laboratory fish, which led Hurst et al. (2000) to propose starvation was not the cause of mortality. Instead, they pointed to the potential of lower osmoregulation abilities, prolonged periods of time exposed to severe cold temperature, and the increased potential of disease susceptibility during the winter (Hurst et al., 2000).

1.7.1 Utilization of lipid reserves

The appetite of fish decreases as temperatures become colder (Russell et al., 1996; Handeland et al., 2008). For YOY striped bass derived from the Shubenacadie River population, their interest in feeding activity tends to cease once water temperatures decline to ca. 10 °C (pers. comm. Dr. J. Duston). In the wild, this temperature and its related cessation of feeding in YOY striped bass generally coincides with times of food scarcity during the winter (Hurst and Conover, 2003). Since nutrients are not being consumed, there is no additional accumulation of energy, and fish must rely on their endogenous body energy reserves to maintain essential metabolic processes (van Dijk et al. 2005; Huss et al., 2008; Bar, 2014). Bar and Volkoff (2012) proposed three phases of energy use while fish are not feeding. Phase one is characterized by simultaneous mobilization of protein and fat (lipid) reserves, whereas phase two is described as being the longest period with a focus on lipid mobilization (Bar and Volkoff, 2012; Bar 2014). Finally, phase three consists of transitioning back to protein mobilization once lipid levels reach critical levels in the organism, reportedly at below 2 % body mass (Bar and Volkoff, 2012; Bar 2014).

Mass-specific metabolic rate increases as body size decreases; therefore, smaller fish within a cohort will utilize their reserves more rapidly during a time of thermal stress, such as during a temperate winter (Lindstedt and Boyce, 1985; Garvey and Marschall, 2003; Heermann et al., 2009). If smaller fish are utilizing their reserves faster than larger fish, they are potentially more vulnerable to starvation during the winter (Post and Evans 1989; Schultz and Conover 1999; Garvey et al. 2004; Heermann et al., 2009). Thus, a fish that is able to invest more resources into growth of energy reserves leading up to winter should have an advantage over others in its cohort in terms of survival (Post and Parkinson, 2001). Heermann et al. (2009) observed this in perch (*Perca fluviatilis*) in both pond and laboratory experiments comparing the survival rates of small (mean total length 43 – 70 mm) and large (mean total length 101 – 186 mm) fish. Prior to winter, smaller perch had accumulated lower levels of energy reserves than larger fish, such as fat (ca. 0.024 versus 0.510 g), glycogen (ca. 0.01 versus 0.148 g), and protein (ca. 0.233 versus 4.084 g), which resulted in size dependent mortality (Heermann et al., 2009).

Smaller relative energy reserves are a common and well documented characteristic in YOY fish. The size of an organism will generally dictate the size of their endogenous energy reserves; therefore, a smaller fish within a cohort will typically have smaller relative reserves available for energy purposes during the winter to avoid mortality due to starvation (Shuter and Post, 1990; Heintz and Vollenweider, 2010). An inverse relationship between body size, or length, of a fish and its ability to survive its first winter has been reported for numerous species (Oliver et al., 1979; Post and Evans, 1989; Tonn et al., 1992; Persson et al., 1996; Hurst and Conover, 1998; Post et al., 1999; Hales and Able, 2001; Hurst and Conover, 2003; Neuman and Able, 2009).

Information regarding the condition of YOY striped bass prior to winter is not documented for the Shubenacadie River population. However, Duston et al. (2018) report the growth and fork length (FL) of YOY striped bass of this population during the summer months and into September. Mean FL at the end of summer varied between ca. 3.5 and 7.6 cm during the years 2008 and 2016 (Duston et al., 2018). Similarly, YOY striped bass in the Miramichi River at the end of the first growing season exhibited considerable variation in body size from year to year (Douglas et al., 2001). For example, the mean FL from 1991-

1998 and 2000-2002 were between 10 and 13 cm, whereas in 1999 YOY had a mean 15.3 cm FL (Bradford et al., 2001; Douglas et al., 2003). Bernier (1996) suggested that a minimum FL of 10 cm is necessary for survival in YOY Miramichi River striped bass. Moreover, Hudson River striped bass subjected to a simulated overwinter trial had increased survival when part of the "large" size class experimental group that had a mean FL of ca. 9.8cm (Hurst and Conover, 1998).

Lipids are the one of the primary constituents of energy reserves that many fish species depend on during the winter (Adams, 1999; Schultz and Conover 1999; Huss et al., 2008). Multiple classes of lipids might contribute to survival of YOY fish overwinter. Triacylglycerols (TAG), often referred to as "energetic lipids", are an extremely important and dense source of energy available to organisms (Adams, 1999; Gibbons et al., 2000). They are stored in adipose tissue (Cowey and Sargent, 1979; Adams, 1999; Tocher, 2003), in addition to the liver, intestinal fat, muscle, subdermal tissue, and mesentery tissues of fish (Adams, 1999 van Dijk et al., 2005; Bar and Volkoff, 2012). In terms of structure, TAG are characterized as three fatty acids (FA) molecules esterified to a glycerol molecule (Figure 1; Tocher, 2003). During times of prolonged food deprivation, metabolic demands are filled by the catabolism of TAG. Fatty acids are enzymatically released from the glycerol backbone of the TAG molecule; catabolised into usable energy is accomplished through β-oxidation consisting of cleavage of 2-carbon units (Tocher, 2003). Ultimately, this breaks the fatty acid chain down into acetyl units before creating the final product of adenosine triphosphate (ATP; Tocher, 2003).

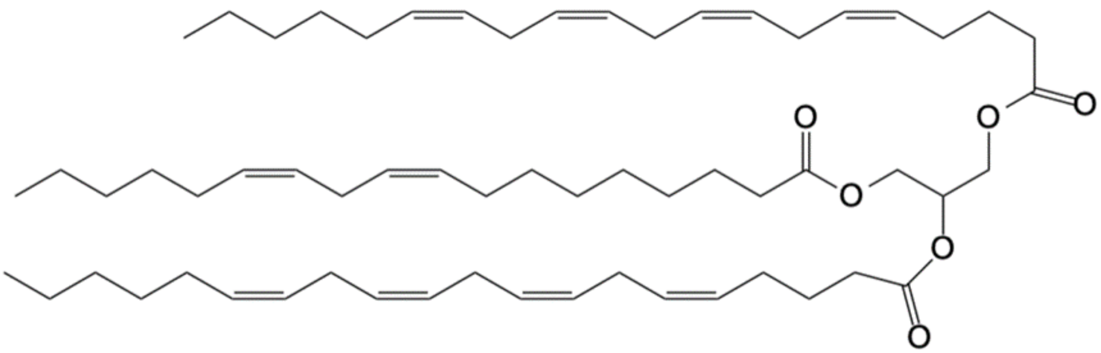

Figure 1. Structure of a non-polar triacylglycerol molecule consisting of three fatty acids esterified to a glycerol molecule.

In YOY fish, a minimum, or critical, level of TAG is required in order to avoid mortality from starvation. This level has been estimated at ca. 2 % of the dry body weight (Newsome and Leduc, 1975; Heermann et al., 2009; Bar and Volkoff, 2012). If YOY fish are able to opportunistically consume food during periods of low food availability, they can restore, or maintain adequate levels of lipids for energetic purposes (Hurst and Conover, 2001; Heermann et al., 2009). Striped bass appetite is generally thought to be depressed during the winter months, or when water temperatures decrease to ca. 10 °C (pers. comm. Dr. J. Duston); however, there is documentation of YOY Hudson River striped bass feeding during the winter in water temperatures of 2 to 10.4 °C (Hurst and Conover, 2001) as well as other age classes in other US populations (Walter et al., 2003).

1.7.2 Thermal acclimation

Given that populations of striped bass in Canada occur at the most northern extent of the species range, it is supported that they can survive colder temperatures than more southern populations (Cook et al., 2006). Further, the duration of winter in Canada's Maritime provinces is longer than those experienced in the southern US, as it can span from November to May (Andrews et al., 2019; pers. comm. Dr. J. Duston). It is unclear what the thermal tolerance, or the outer limits of the preferred temperature range, of a YOY striped bass is during the winter, since lethal temperatures have not been determined for wild YOY striped bass (Andrews et al., 2019). Generally, individuals within populations of temperate species that exist at the higher latitude edges of their ranges suffer from greater mortality during the winter (Shuter and Post, 1990; Oliver et al., 1979; Adams, 1999).

Phospholipids (PL) are another lipid class vital to survival in winter water temperatures. In general, their structure consists of two fatty acids esterified to a glycerol backbone; in the third position is a phosphate group to which a nitrogen-containing group is often bonded (Figure 2). Because they are amphiphilic, consisting of a non-polar (FA) end that is hydrophobic and a polar head (phosphate group) that is hydrophilic they are one of the primary components of membrane bilayers (Singer and Nicolson, 1972). The primary role of this lipid class (PL) is to maintain the proper fluidity and structure of an organism's cell, and consequently, they are referred to as structural lipids (Adams, 1999).

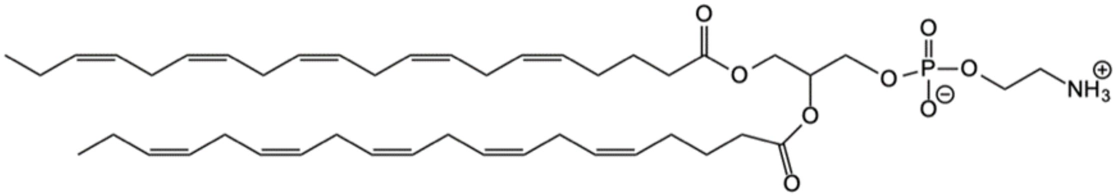

Figure 2. Structure of phosphatidylethanolamine, a phospholipid molecule consisting of two fatty acids, a phosphate group and an ethanolamine group.

Reconstruction in phospholipid bilayers occurs in colder temperatures as an adaptation to maintain proper cell fluidity (Dey et al., 1993). Phospholipids assist organisms in acclimation to winter temperatures. Thus, as temperatures become colder, an organism's cellular membranes must remain fluid instead of solidifying (Sinensky, 1974). Fluidity in cellular membranes of fish can be influenced by two structural aspects: 1) the FA contained in PL and 2) the types, or classes, of PL present (Farkas et al., 2001). In winter water temperatures, an increased unsaturation of FA in phospholipids within the cellular membrane can result in maintaining the proper fluidity of cells for function (Hazel and Prosser, 1974). The ability for PL to maintain solid membrane bilayers is also due to their shape. The FA tails vary in length and also in the number of *cis*-double bonds, which influences their shape, by introducing kinks in their FA tails (Alberts et al., 2002). Ultimately, this variation in shapes influences the packing ability of PL when forming bilayers (Alberts et al., 2002). Fluidity can also be accomplished by changing the composition of specific phospholipid classes within cellular membranes (Farkas et al. 2001; Guderly, 2004). Two of the dominant PL classes responsible for maintaining cellular membrane fluidity are phosphatidylethanolamine (PE) and phophatidylcholine (PC). The shape of these two classes of lipids is important, as it accounts for their structural packing abilities; PE are described as conic and PC as cylindrical (Dey et al., 1993; Farkas et al. 2001). In this regard, an increase of PE (conic in shape) in comparison to PC is one way of membranes are able to maintain proper fluidity during all thermal changes (Dey et al., 1993; Buda et al., 1994; Farkas et al., 2001).

1.7.3 Osmoregulation

The winter habitat of YOY Shubenacadie River striped bass is unknown (pers. comm. Dr. J. Duston; Andrews et al., 2019). For older (age-2 +) fish within this population, it is known that many of them spend the winter months in Grand Lake, a large FW lake that is part of the Shubenacadie River drainage. These older Shubenacadie River fish migrate from tidal waters into Grand Lake in the fall and then remain in the lake until April (Rulifson and Dadswell, 1995; Douglas et al. 2003; Bradford et al. 2012, 2014). Young-of-the-year fish on the other hand have not been documented utilizing this refuge during the winter and it has been speculated that they remain in estuarine or marine environments (Bradford and Cook, 2004; Bradford et al., 2014).

This presumed preference for estuarine and marine environments over FW could be related to salinity (Hurst and Conover, 2002). Evidence suggests that osmoregulation in FW places higher metabolic demands on individuals, which could be a limiting factor to YOY fish as they typically have smaller energy reserves leading up to their first winter (Hurst and Conover, 2002). Salinity levels significantly impacted survival of YOY striped bass of Hudson River origin during laboratory experiments (Hurst and Conover, 2002). Specifically, when temperature was decreased from 7 °C to -1 and -2 °C, fish in holding tanks with salinities ranging between 5 and 30 ppt demonstrated higher survival rates than those held in FW (Hurst and Conover, 2002). Further, YOY fish with higher total lipid content (ca. 3.48 % dry weight) had significantly better survival rates than those with a low lipid content (ca. 1.88 % dry weight) across all salinity treatments (0-35 ppt) when exposed to temperatures decreased from ca. 7 to 1 °C (Hurst and Conover, 2002). Further, Johnson and Evans (1996) proposed that when exposed to temperatures of 2.5 °C, white perch were not using as much of their energy reserves as fish that had been acclimated to 4 °C. Instead, fish at 2.5 °C suffered osmoregulation stress, which caused a greater proportion of mortality when compared to fish at 4 °C (Johnson and Evans, 1996).

Moreover, winter water temperatures and the restructuring of PL for the purpose of cellular fluidity (described in section *1.7.2 Thermal acclimation*) can affect a fish's osmoregulatory abilities. Cellular membranes become more unsaturated in cold temperatures (Hazel, 1995), therefore modifications to the properties of these membranes occur, most notably to permeability (Leray et al., 1986). This impacted permeability can cause a reduction in a fish's osmoregulatory ability (Davis and Simco, 1976; Toneys and Coble, 1980).

1.8 My Research

Over the next three chapters, I attempt to make connections between the gaps in the literature presented in this introductory Chapter and the results of the two trials carried out during the course of this thesis. Specifically, Chapter 2 aims to provide information on the overwinter requirements for the survival of YOY Shubenacadie River striped bass, whether they be related to pre-winter diet, size, or environment, as seen in sections *1.7.1* and *1.7.2*. In addition, section *1.7.3* indicates that the location preference of YOY striped bass during the winter is unknown. The monitoring of parameters such as temperature and dissolved oxygen at a pond level throughout the trials allowed for connections to be made in the discussion of Chapter 2 around the overwinter tolerances of Shubenacadie River YOY striped bass, which could help shed more light on winter habitat requirements.

The overwinter utilisation of lipids by YOY striped bass was explored in Chapter 3. The literature presented in sections *1.7.1* and *1.7.2* on both the energetic and structural lipids, led me to predict that TAG levels should decrease during the winter and certain PL classes should respond to cold temperatures to provide proper cellular fluidity. Chapter 3 also provides the first descriptions of the changes that occur in TAG and PL of YOY striped bass during the winter from the Shubenacadie population, to my knowledge.

Finally, Chapter 4, the thesis discussion, is a chapter whose main objectives are to synthesize the major findings of Chapters 2 and 3, and present further areas of research that were not addressed by this thesis. Future lines of research that were addressed in this first Chapter (Introduction) include the necessity of exploring how osmoregulation is impacted by certain environments, such as FW, during the winter (section *1.7.3*), as this was not completed in this thesis.

CHAPTER 2 Overwinter survival of young-of-the-year striped bass (*Morone saxatilis*) in constructed freshwater ponds in two winters (2016/17 and 2017/18)

2.1 Introduction

The province of Nova Scotia (NS) is currently home to many research projects working towards the expansion and diversification of the finfish aquaculture sector in the province (AANS, 2019). Further, the provincial government is looking to identify and promote lower environmental impact species and methods of production, while maintaining a high value industry (Doelle and Lahey, 2014). One species of interest is striped bass (*Morone saxatilis)* as they are a highly valued table fish native to the Maritime provinces and much of the United States (US) eastern seaboard (Scott and Scott, 1988).

Historically present in coastal Atlantic Canadian waters south of the estuary of the St. Lawrence River, striped bass populations have for centuries supported numerous directed commercial and recreational fisheries throughout the region (Jessop, 1991; Caddy and Chandler, 1976). However, due to population declines throughout much of their Canadian range over the latter half of the 20th century (Bradford and Chaput, 1996; Douglas and Chaput, 2011), including the extirpation of many spawning populations such as the St. Lawrence DU (COSEWIC, 2012), directed commercial harvest of striped bass in Canadian waters has been largely closed since 1996 (Bradford and Chaput, 1996; Douglas and Chaput, 2011). The one noteworthy exception to this is the recent re-opening of a small (maximum annual harvest of 50,000 fish) commercial striped bass fishery on the Miramichi River in eastern New Brunswick (NB) (CBC, Hadeel Ibrahim, 2018).

Reflecting their historical commercial importance, striped bass rearing programs were initiated in Atlantic Canada in the 1980s as a way to investigate the possibility of creating a profitable aquaculture industry, following the success of Atlantic salmon (*Salmo salar)* farming and the success of hybrid striped bass culture in the US (Boghen, 1995; Cairns et al., 1999; Harrell, 1999). However, of the early initiatives, only four remained active at of the turn of the Century (Cairns et al. 1999). Since then, the number of active striped bass research programs has decreased to two: efforts at the Dalhousie Agricultural Campus (formerly known as the Nova Scotia Agricultural College) in Truro, NS and the MacDonald[2] Aquaculture and Consultation (formerly known as Two Rivers Bass Hatchery) in Colchester County, NS. In addition, four farms in NS are currently licensed to grow striped bass though access to juvenile animals and hence overall production is extremely limited.

Across North America more generally, aquaculture production of pure striped bass is limited to one producer, Pacifico Aquaculture, located in Baja California, Mexico (FAO, 2014). Hybrid striped bass, on the other hand, occupy a portion of aquaculture production in the southern US. According to the 2013 Census of Agriculture results, hybrid striped bass were produced in 68 farms in the US and accounted for approximately 5600 tonnes of food fish that reached market size (USDA, 2013). The most common hybrid produced in the US is the sunshine bass, a cross between a female white bass (*Morone chrysops*) and a male striped bass (*Morone saxatilis*; Harrell, 1997).

2.1.1 Striped bass research and development in Nova Scotia

Successful artificial spawning and rearing of striped bass in captivity has taken place at the Agriculture Campus of Dalhousie University (Dal-AC) located in Truro, NS for the past 20 years. Resulting larvae and juvenile fish have been used in various past and ongoing experimental trials (for example: Duston et al., 2004; Cook et al., 2006; MacIntosh and Duston 2007; Cook et al., 2010; Duston and Astatkie, 2012; Sampson et al., 2013). The annual juvenile striped bass production at Dal-AC enabled researchers to explore viable grow-out locations as part of the process towards commercialization. This included investigating the phenomenon of overwinter mortality, a very common occurrence in YOY fish populations in temperate regions, including striped bass (Hurst and Conover, 1998; Hurst et al., 2000; Bradford and Chaput, 1997). Since 2009, the principal industrial partner in this research and development has been North River Fish Farms Ltd. (NRFF; 45º29’53” N, 63°12’31” W) located ca. 18 km north of Truro, NS. At NRFF, ca. 1500 striped bass per year have been successfully grown out in three constructed FW ponds.

Fish are generally transferred to ponds in early May at about one year of age and 80 g weight. By the end of their first growing season in the ponds, fish typically reach 500 g and ca. 1 kg by the time they are considered marketable size in the summer of their second season (pers. comm. Dr. J. Duston). There is substantial interest in transferring YOY striped bass in the fall of their first year (mid-November) when the fish are approximately six months of age and weigh on average between 30 and 50 g. Such a strategy would markedly increase the number of juvenile striped bass that could be transferred to commercial grow-out while simultaneously reducing costs of rearing juvenile fish in indoor facilities. Transferring YOY to ponds in the fall addressed two interesting questions: 1) could this be a viable production tactic to free up indoor space and potentially get them off to an advantageous start in the spring since they will be pond acclimated? and 2) how do YOY fish survive long winters?

Between 2012 and 2016, four small-scale trials were conducted at NRFF to investigate factors affecting survival of YOY striped bass during the winter in a commercial pond environment. The results of these trials are unpublished. The experimental factors in these trials were: body size, effect of pre-transfer thermal acclimation strategy, date of transfer to the ponds, and the depth at which experimental cages were suspended within the ponds. These trials resulted in significant mortality of YOY striped bass in all years across all experimental treatments. However, one important result was that the few survivors contained large amounts of visceral fat throughout winter, leading to the hypothesis that the type of fat included in pre-winter diets was unsuitable and could not be mobilized. At Dal-AC, striped bass in captivity were generally fed a commercial salmonid diet with a fat content of 14 - 18% (EWOS Vita: 14 % crude fat, or Corey Aquasea: between 16 - 18 % crude fat). Although dietary requirements of striped bass are not fully known, those of hybrid striped bass reflect those of marine fishes (Webster and Lovell, 1990; Clawson and Lovell, 1992; Nematipour and Gatlin, 1992, 1993; Barry, 2017), indicating that a diet formulated specifically for striped bass, or one more similar to a marine fish's diet, could be beneficial to overwinter survival of YOY fish.

Further, the survival of 150 g (juvenile) striped bass, when subjected to a simulated cold front decrease in temperature from 20 to 10 °C in 24 hours, was significantly improved when fish were fed increased dietary unsaturated fatty acids from a natural diet (fathead minnows, *Pimephales promelas*) for 89 days before being subjected to the thermal shock (Kelly and Kohler 1999). Fish fed diets with higher concentrations of unsaturated fatty acids were thought to be better prepared for colder thermal situations, suggesting that diet had an impact on a fish's ability to withstand abrupt, or acute, changes in temperature (Kelly and Kohler, 1999).

Reported here are the results of two overwinter survival trials spanning the winters of 2016/17 and 2017/18. The primary objective was to better quantify the influence of pre-winter diet and body size on overwinter survival of YOY striped bass. In addition, various ponds were used at both NRFF and other locations throughout the two trials to gain knowledge on whether pond environment had an impact on winter survival. Finally, we included the timing of sampling as an aspect of the experimental design used to estimate more accurately the time of death. Both trials tested the hypothesis that larger fish, and fish fed a diet more appropriate for striped bass, would be more successful in surviving the winter (see Chapter 1, Section *1.7*). The results of these consecutive trials increase the current state of knowledge on the timing of mortality through winter. Whereas most of the trials 2012-16 simply examined the cages when the ice melted in spring, the trials described here included serial sampling throughout the winter.

2.2 Materials and Methods

2.2.1 Experimental fish: rearing and thermal acclimation for both trials

The experimental fish used in the NRFF overwinter trials (2016-2018) were produced from domesticated broodstock (Shubenacadie River origin) spawned in May and June at the Dal-AC using methods developed in the US (Woods and Sullivan, 1993; Hodson et al. 1999). Spawning consisted of subjecting both female and male broodstock to an increase in water temperature from 12 to 17 °C in ca. 10 days. Thermal acclimation occurred in two 1.5 m^3 tanks with flow-through water supply (oxygen saturation > 80%) that each held between 4 and 12 fish of the same gender. On the first and final day of thermal acclimation, a biopsy of the ovaries of female fish selected for spawning (n=4) was conducted to assess the development stage of the oocytes. When water temperature reached 17 °C on day 10 of thermal acclimation, all broodstock were sedated (mixture of 50 ml clove oil, 50 ml acetic acid/vinegar, 900 ml water). Once sedated, male broodstock were injected intra-muscularly (IM) between the two dorsal fins with Chorulon (75 IU/kg body weight) to promote sperm production. Sedated females with oocytes at the >15-hour stage were implanted IM with one Ovaplant® pellet (150 μg GnRHa/pellet) using a Ralgun implanter. After injection, the fish were placed in spawning tanks (1.5 m^3) in a 3:1 male to female ratio. Temperature was maintained at 17 °C and oxygen saturation > 80% with fish exposed to natural day length (LDN; latitude 45ºN). Typically, spawning occurred about 48 hours after injection and placement in the allocated tanks.

After a spawning event, eggs were retrieved using a mesh sieve (1000 μm) and transferred in water to upwelling Krescel incubators (90 L) supplied with 16-18 °C brackish water (2 - 4 ppt) with an inflow rate of 200 mL min^{-1}. Hatching occurred about 48 hours post-spawning and larvae remained in the upwelling incubators until three days post-hatch (dph). Husbandry consisted primarily of removing dead eggs and associated oil from the water surface. At 3 dph, each cohort of yolk-sac larvae (ca. 4 mm total length) was transferred to a larger tank (1.5 m diameter, 0.4 m water depth, 800 L) also containing 2 - 4 ppt brackish water at 17 - 18 ºC. Swim-bladder inflation at 5 - 7 dph was facilitated by removing residual oil on the water surface using paper towel, applying a gentle water spray, and increasing the turbidity of the water to about 150 NTU (Nephelometric Turbidity Units) by addition of porcelain clay (Dragonfire Pottery and Supplies, Dartmouth, NS, Canada). Swim-bladder inflation was assessed under a dissecting scope (Leica EZ4). To stimulate the first feeding, water temperature was raised to 20 ℃ and stage I *Artemia* nauplii (Biomarine Aquafauna. Hawthorn, CA, USA) were offered every three hours between 07:00 and 22:00 up to 12 dph. Then stage II nauplii enriched with Algamac 3050 (Biomarine Aquafauna) was offered to larvae up to 33 dph. At approximately 15 dph fish were transferred into 500-L volume (1 m diameter) rearing tanks in a small recirculation system (Aquabiotech, Coaticook, Quebec). Salinity was maintained at 2 ppt until the fish reached about 5 cm TL, after which fish were reared in FW at about 20 °C. Weaning onto a dry diet began at 30 dph (Gemma 0.3 mm, Skretting, Saint Andrews, NB, Canada), progressing as body size increased to commercial salmonid diets (Nutra 0.5 and 1.0 mm, Skretting; Vita 1.5 and 2.0 mm, EWOS, Cargill, Surrey, BC, Canada). Once in the 500 L tanks, belt feeders were installed to provide feed continuously between about 7:30 and 23:00 h. In addition, fish were hand fed to apparent satiation approximately every three hours. Photoperiod was 24-hour light until late August, followed by simulated natural latitude 45 °N (LDN) daylength.

In preparation for each overwinter trial, early in September a sufficient number of YOY fish (N varied per trial) were randomly selected. These subsets were transferred to and distributed amongst tanks in a 12-tank recirculation system (150 L volume per tank) maintained at 20 - 23 °C and oxygen saturation > 80 %. Salinity was increased up to 10 ppt in the tanks if signs of a potential infection or pathogen outbreak, such as *Saprolegnia parasitica*, occurred, depending on trial. Feeding consisted of hand feeding to apparent satiation between two and five times per day.

Prior to transfer to outdoor ponds in November, each subset of YOY striped bass underwent a period of thermal acclimation. The average duration of thermal acclimation was two weeks, during which the temperature was gradually decreased to that of the pond water temperature at NRFF (ca. 6 - 7 °C). Temperature was reduced by 2 °C every two days from 20 to 16 °C then, followed by a more gradual decrease of 1 °C per day until reaching the desired temperature.

2.2.2 Experimental site: NRFF, cage set up, and sampling procedure

The primary experimental field site, NRFF, consisted of three, constructed ponds that line along a slope (Figure 3) and are fed seasonally by an intermittent stream and groundwater. Ponds range from ca. 86 to 138 m in diameter and are referred to as Upper, Middle, and Lower ponds respectively based on their relative elevation (altitude 122 to 137 m; Figure 3). At their deepest points, pond depths ranged from 3.9 m to 4.3 m and 4.8 m in the Upper, Middle and Lower ponds, respectively when sampled in 2018 (pond bathymetry maps in Appendix A, Figures A1-A3). Rates of flow into the Upper pond and between ponds vary seasonally but during winter the rate was at times 120 L per minute. Influent water to the Upper pond was primarily groundwater with additional water from a small brook (Figure 3). Since 2009, ponds have been used predominantly for striped bass grow-out, with the Lower and Middle ponds used most frequently given their greater depths.

Figure 3. Aerial photo of North River Fish Farms Ltd. indicating the three ponds, location of seasonal surface water influent source, direction of water flow in the pond system, and existing floating rafts surrounding net-pen arrays. Altitude 122 to 137 m. Source: Google Earth, Apr. 4, 2019.

The experimental unit for all trials was a plastic mesh ‘cubic’ cage 1 m^3 in volume, made from Vexar (rigid plastic 1.3 cm, ½ in, mesh; Rainbow Net and Rigging, Dartmouth) enclosing an ABS pipe frame (121.9 cm x 137.2 cm, 2 inch diameter; Figure 4). Cages were suspended individually with ropes at approximately 1 m under the surface from floating walkways surrounding four net-pens deployed in each pond (Figure 3).

Figure 4. Cubic meter Vexar cage for holding young-of-the-year striped bass overwinter

Transfer of fish took place on November 11 (2016/17 trial) and November 13 (2017/18 trial). The transfer process started with dip-netting fish from the rearing tanks into ca. 20 L plastic pails filled with rearing water. Fish were then carried to and gently poured into 250 L insulated tanks (Xactic^{TM}; Cornwall, Ontario) secured on the back of a flatbed truck. Prior to fish transfer, each Xactic tank had been filled to two thirds of its capacity with 7 ºC brackish water (10 ppt), matching the temperature of the pond water. Once all Xactics tanks were stocked and lids secured, the fish were driven approximately 18 km to NRFF. Oxygen was maintained >70 - 80 % saturation during trucking. At NRFF, fish were carefully transferred to their assigned cages five at a time in a 20-L bucket, then poured into the cage via a flexible funnel (Double Bubble Foil Insulation, RFOIL; Home Hardware, Truro). Cages were then sealed with zip ties and nylon braided twine to prevent escape and then submerged to 1 m below the surface with the aid of a concrete block attached to the base of the cage.

To facilitate sampling throughout the winter, insulated covers were placed above the cages once the ice was formed on the ponds (Figure 5). The covers reduced the thickness of the ice, allowing easier access to the cages during the winter sampling events. The rectangular frames (2.4 x 1.2 m), made from 5.1 x 15.2 cm spruce had a plywood cover that was insulated (Cladmate, Dow Chemical, R10; Figure 5). Once the holes froze over, the covers were solidified into place until sampling occurred.

Figure 5. Installation of an insulated plywood cover over Vexar cages in the Lower pond, North River Fish Farms Ltd., December 20, 2016.

Sampling consisted of bringing one or more cages to the surface, counting and removing the surviving and dead fish. To access the cages, first the insulated plywood cover was removed, and when present, the thin ice below broken, or cut through with a chainsaw (Husqvarna 357XP; vegetable oil as chain lubricant). Cages were brought to the surface by two, or three, people manually pulling suspension ropes aided by long-handled gaff hooks (Rainbow Net and Rigging, Dartmouth NS). The bottom half of the cage remained in the water and survivors and mortalities were removed with dip nets. Survivors from each cage were counted and depending on the trial either immediately euthanized (MS222, 0.1 - 0.15 g/L) or brought back to the lab alive in buckets. Mortalities in each cage were counted, and their fork length (cm) was measured or estimated when decomposition made accurate measurement difficult. All fish that died during the trial were brought back to the lab for disposal by incineration.

2.2.3 2016/17 Experimental design

Three diets were formulated based on the proximate body composition of two wild juvenile striped bass caught in the Shubenacadie River (Table 1). Diets were formulated by Dr. S. Collins (Dalhousie University) and identical except for fat composition (Table 2). In an attempt to adjust the ratio of saturated:unsaturated fatty acids, the ratio of poultry fat to fish oil in the three diets was altered. The ratios of fish oil:poultry fat in the three diets were : (1) 7% fish oil:1% poultry fat, (2) 4% fish oil: 4% poultry fat and (3) 1% fish oil: 7% poultry fat (diets will be referred to as “diet 1”, “diet 2”, and “diet 3” throughout the paper). Ingredients were sourced from Northeast Nutrition Inc. in Truro, NS, and diets (pellet diameter: 2mm) were produced at the Dalhousie Agricultural Campus Feedmill in September 2016.

Table 1. Whole body proximate composition analysis (% dry mass unless otherwise indicated) of two juvenile Shubenacadie River striped bass (*Morone saxatilis*; pooled dry sample weight 15 g) collected in the summer of 2016.

Parameter	**Wild SB Sample 1**	**Wild SB Sample 2**
Crude Protein	74.29	73.10
Crude Fat	11.69	11.73
Calcium	4.001	4.317
Potassium	1.439	1.437
Magnesium	0.159	0.160
Phosphorus	2.777	2.859
Sodium	0.502	0.482
Copper (ppm)	ND	ND
Manganese (ppm)	18.96	19.77
Zinc (ppm)	92.20	88.58

*ND indicates the analysis value is below reporting limits.

Table 2. Complete ingredient list and inclusion by mass for experimental diets fed to young-of-the year striped bass (*Morone saxatilis*) during a laboratory feeding trial period (September 20 – November 8, 2016) prior to transfer to freshwater ponds.

Ingredient Name	**g per kg**
Fish Meal	500
Empyreal	125
Blood Meal	111.5
Ground Wheat	100
Fat[a]	80
Wheat Gluten (corn/wheat starch)	50
Dicalcium Phosphate	20.5
Lysine HCI	5
Choline Chloride	3
*Vitamin/Mineral Premix[b]	2.5
*Special Premix[c]	2.5
Total	**1000**

[a] Fat ingredient content in three experimental diets varied as follows: Diet 1 (7 % fish oil, 1 % poultry fat), Diet 2 (4 % fish oil, 4 % poultry fat), and Diet 3 (1 % fish oil, 7 % poultry fat).

[b] Vitamin/mineral premix contained: manganese 125 mg, iron 84 mg, zinc 77.5 mg, copper 2.5 mg, iodine 7.5 mg, vitamin A 5000 IU, vitamin D 4000 IU, vitamin K 2 mg, vitamin B12 4 µg, thiamin 8 mg, riboflavin 18 mg, pantothenic acid 40 mg, niacin 100 mg, folic acid 4 mg, biotin 0.6 mg, pyridoxine 15 mg, inositol 100 mg, ethoxyquin 42 mg and wheat shorts 1372 mg (per kg).

[c] Special premix contained: vitamin E 250 mg, vitamin C 200 mg, astaxanthin 60 mg, selenium 0.22 mg and wheat shorts 1988 mg (per kg).

On September 20, 2016, 840 YOY striped bass (mean body wt. 21 g) were distributed at random (70 fish per tank) among 12 tanks (each 150 L volume) within the recirculation system described above (Section *2.2.1*), resulting in four replicate tanks per diet treatment. Feeding of the three experimental diets began September 20 and each tank of fish was hand fed between two and four meals per day, each to apparent satiation. Fork length and body weight of 30 randomly selected fish per tank was recorded biweekly following anesthesia (MS-222 (0.1 - 0.15 g/L), then recovered in brackish water (10 ppt) at the same temperature as the rearing water before being returned to their original tank.

When fish were measured in early November, prior to transfer to NRFF, it was determined that body size varied insufficiently to achieve two distinct size classes as originally intended (2 levels; small and large). Instead, each cage was stocked with fish randomly selected from across the body size range of those available. The mean FL of these fish was 14.3 cm and the mean BW was 46.4 g.

Further, analysis of the three experimental diets in early November 2016 (Marine Lipids Lab. Dalhousie University, Halifax) revealed that the lipid profile of the three experimental diets formulated with differing proportions of fish oil and poultry fat were not sufficiently different to be of biological significance (pers. comm. Dr. S. Budge, Dalhousie University; see Appendix B, Table B1). Due to this, diet was no longer included as an experimental factor.

Fish were transported to NRFF on November 11, 2016 and stocked in a split-plot factorial design consisting of 18 cages spread across two ponds, the Lower and Middle (blocks), at NRFF. The cages were stocked at a stocking density of 30 fish of the same average mean body weight of 46.4 g and fork length of 14.3 cm per cage, based on measurements taken on October 25. Each pond contained nine cages (three for each sampling event). Sampling dates were November 15, 2016 in the lab with remaining fish and then February 3, March 19, and April 19, 2017 at NRFF.

Water temperature and dissolved oxygen (DO) levels in each pond at the same depth (2m) as the floor of the experimental cages was monitored every 30 minutes with a HOBO data logger (Onset U26, Hoskin Scientific, Burlington ON). Prior to deployment on November 15, each logger was fitted with a new sensor cap (U26-RDOB-1), an anti-fouling guard (U26-Guard-2) and calibrated at 0 and 100% oxygen saturation. In addition, both DO and temperature were measured at each sampling event through the winter with a calibrated hand-held meter (YSI Professional Plus).

Water quality analysis of the Middle and Lower ponds was conducted on samples collected on March 19 and April 19, 2017. Samples were stored at -20 °C until analysis. LaMotte Laboratory Kits (LaMotte Company, Chestertown, Maryland, USA) were used to determine pH and concentrations (parts per million, ppm) of calcium carbonate (water hardness), nitrite nitrogen, and ammonia nitrogen. Water quality was similar between March and April in both ponds and was considered suitable (for these parameters) during the trials. pH was between 5.5 and 6.6 (Table 3). Nitrite in March and April was below detectable limit (< 0.165 ppm; Table 3). Total ammonia was < 0.25 ppm in both ponds. Unionized ammonia was < 0.001 mg L^{-1} (Trussell, 1972) and no threat to fish health since safe levels are < 0.019 mg L^{-1} in FW (CCME, 2010). Hardness was between 10.5 and 13 mg/L in both ponds (Table 3).

Table 3. Water quality parameters in the Lower and Middle ponds of North River Fish Farms Ltd. in March and April of the 2016/17 trial. Units are mg L^{-1}, except for pH.

	Lower Pond		**Middle Pond**	
Parameter	**Mar. 19**	**Apr. 19**	**Mar. 19**	**Apr.19**
pH	5.5	6.5	6.5	6
Nitrite nitrogen	<0.165	<0.165	<0.165	<0.165
Ammonia nitrogen	0.1-0.25	0.25	0.1-0.25	0.1-0.25
Unionized ammonia	<0.001	<0.001	<0.001	<0.001
Hardness	13	13	10.5	13

2.2.4 2017/18 Experimental design

In addition to diet and body size, pond was included in this trial as an experimental factor. These additional ponds were exploratory; therefore, the statistical analysis of their results was not incorporated with the NRFF ponds. This consisted of using all three ponds at NRFF (Figure 3) in addition to two exploratory trials at two ponds within 5 km of the Dal-AC Campus. Their water chemistry, thermal profile and winter ice conditions differed from NRFF, providing a valuable comparison between pond locations. The Faulkner pond, named after the surname of the property owner, is entirely spring fed. The Faulkner pond also remains ice-free throughout winter. It is about 150 meters long, 5 to 15 m wide, and 2 m deep at the west end where the cages were located (Figure 6). Effluent water exits via a ca. 41 cm plastic pipe at the west end of the pond at a rate of >100 L/minute. Escape of rainbow trout stocked in the pond was prevented by a metal grill (2.5 cm mesh) at the effluent outflow pipe. Wild fish were prevented from entering the pond by a flap valve on the outlet side. Effluent then drained out through a narrow ditch that connects to the Salmon River. The Dixon pond, also named after the surname of the property owner, is a constructed pond of approximately 42 x 32 m (1,344 m^2), located near North River (Figure 7). The estimated depth was between 3 to 4 m. Groundwater was the sole source of influent water. The pond is within 50 m of North River which has some tidal influence from the

Salmon River estuary about 2 km downstream. Large gravel deposits run along North River allowing water to percolate through the gravel into the pond. Conductivity remained low and stable throughout the trial (mean 0.16 microSiemens/cm; range 0 to 0.28 mS/cm) confirming the pond was FW; there was no influx of brackish water on spring tides, as speculated by the owner.

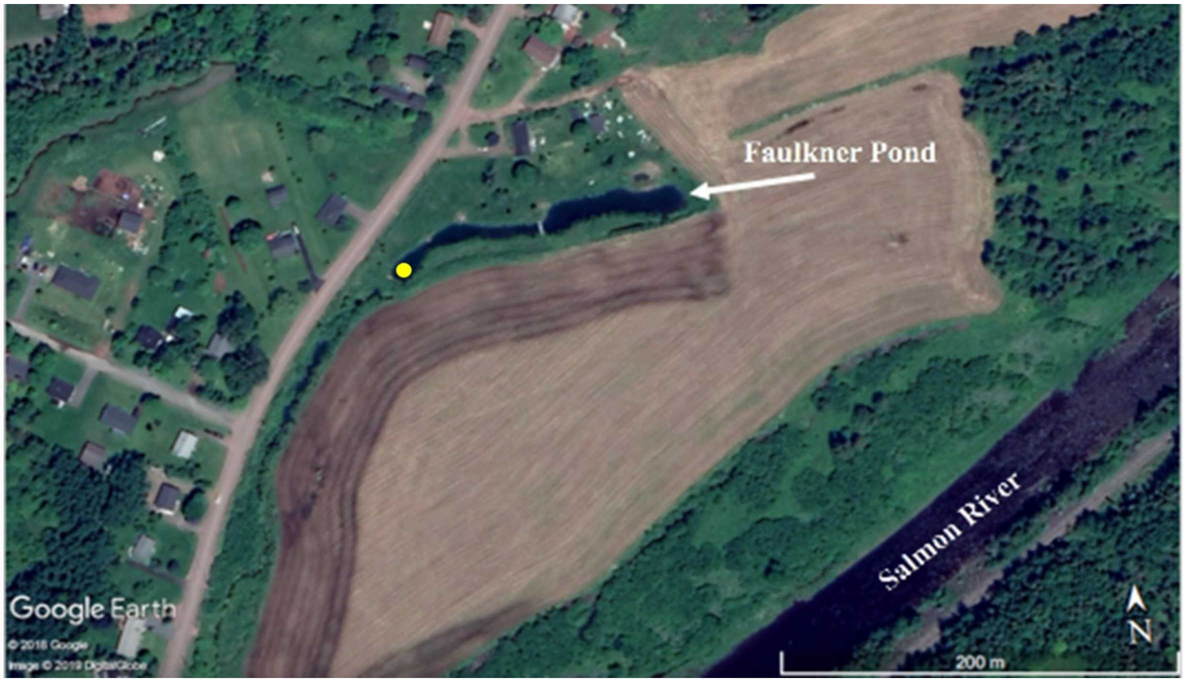

Figure 6. Aerial photo of Faulkner pond (45°22’21.57” N, 63°12’29.69” W) used during the 2017/18 overwinter trial. Altitude 25 m. Yellow dot indicates the location of all overwinter monitoring cages Source: Google Earth, June 29, 2018.

Figure 7. Aerial photo of Dixon pond (45°23’14.79” N, 63°17’1.12” W) used during the 2017/18 overwinter trial. Altitude 9 m. Yellow dot indicates the location of all overwinter monitoring cages. Source: Google Earth, June 29, 2018.

Two diets were formulated by Dr. S. Colombo (Dalhousie University) in the summer of 2017. One diet was based on commercially available salmonid feeds, whereas the second was designed as a tailored diet for striped bass dietary requirements (Table 4). Diets were prepared at the Dal-AC Feedmill in early September 2017 and made into two pellet sizes, 2 and 3 mm, to accommodate the growing of experimental fish. Both proximate and fatty acid analysis were conducted for both diets to confirm that there was significant variation between both (results for both analyses in Appendix C, Table C1 and Appendix D, Table D1).

Table 4. Composition of the experimental diets fed to young-of-the year striped bass (*Morone saxatilis*) during the laboratory feeding treatment period (September-November 2017) prior to placing fish in freshwater ponds overwinter.

	Tailored Diet	Commercial Diet
Ingredient	**% Ingredient Inclusion**	
Fish Meal	55	17
Herring Oil	10	3
Ground Wheat	19	14
Empyreal	0	25
Canola oil	0	9
Poultry By-product Meal	5	10
Soybean Meal	6	17
Vitamin Mix[a]	1.25	1.25
Mineral Mix[a]	1.25	1.25
Choline Chloride	1	1
Dicalcium Phosphate	1	1
Lysine HCl	0.5	0.5
Total	100	100

[a] Vitamin/mineral premix contained: manganese 125 mg, iron 84 mg, zinc 77.5 mg, copper 2.5 mg, iodine 7.5 mg, vitamin A 5000 IU, vitamin D 4000 IU, vitamin K 2 mg, vitamin B12 4 µg, thiamin 8 mg, riboflavin 18 mg, pantothenic acid 40 mg, niacin 100 mg, folic acid 4 mg, biotin 0.6 mg, pyridoxine 15 mg, inositol 100 mg, ethoxyquin 42 mg and wheat shorts 1372 mg (per kg).

In early September 2017, fish were placed in six tanks of the same recirculation system used the previous year with the same rearing parameters (section *2.1*). Two tanks were stocked with smaller fish (one to be subsequently reared on the tailored diet and the other on the commercial diet) and four tanks for larger sized fish (two tanks of each diet treatment). The amount of feed offered to each tank was recorded and tanks of small fish received less feed to maintain size differences between the two classes. Mean body size of the fish was recorded biweekly from September 14 to October 31, 2017. On October 31, the fish were graded by fork length and the cut-offs for the size of each diet treatment were determined: small commercial maximum 12.0 cm, small tailored maximum 12.3 cm, large commercial minimum 12.8, and large tailored minimum 13.0 cm. Between October 30 and

November 13, prior to transfer to the ponds, the fish underwent a period of thermal acclimation stepping the temperature down (section *2.1*) to that of the experimental ponds.

At NRFF 18 cages were arranged in a split-plot factorial design. Pond served as a ‘block’, with three levels (Upper, Middle, and Lower pond). The whole-plot was timing of sampling, three levels (February, March, and April) with a sub-plot with 2 x 2 factorial of diet (commercial and tailored) and body size (fork length; FL, two levels, ‘small’ and ‘large’).

Fish were transferred to all ponds on November 13, 2017. At NRFF, each cage was stocked with fish from two size classes of the same diet treatment (small commercial n = 15; large commercial n = 13; small tailored n = 14; and large tailored n = 13). Three cages stocked with fish fed the same diet were placed in each of the three ponds at NRFF to permit sampling from each pond at each of three sampling events: February 10, March 3, and April 10, 2018. At both the Faulkner and Dixon ponds, three Vexar, 1m^3 cages were deployed. At each pond, one cage was stocked with 28 bass reared on the commercial diet, n=13 large and n=15 small, and a second cage with 27 bass reared on the tailored diet, n=13 large and n=14 small. The third cage at each pond was stocked with 1+ year-old juvenile striped bass (> 250 g, n = 8) which were predicted to survive winter given their relatively large body size, and the good survival of similar sized fish at NRFF ponds in previous years.

As the Faulkner and Dixon ponds each only held three cages of striped bass, survival through the winter was assessed by visual inspection. Attempts to observe the fish using an underwater GoPro camera transmitting to a cell phone were unsuccessful. On January 27 the Faulkner pond was ice-free and water clarity was very good, but the GoPro camera would only focus on the Vexar mesh, not the fish within. Hence the cages were manually hauled to the surface by two operators in a boat and held semi-submerged to allow fish to be observed. On February 2 at the Dixon pond, several holes were drilled through the ice to allow the GoPro to be lowered, but light levels were too low, and nothing could be seen. On March 11 and April 10, 2018, the Dixon pond was ice-free allowing the cages to be accessed from a flat-bottomed wooden boat. Cages were manually hauled to the surface and held semi-submerged to allow fish to be counted and sampled without exposing the fish to the air. Live fish were brought back to the lab in 20 L buckets to facilitate the collection of blood for plasma osmolality analysis; however, the results are not reported because most of the plasma samples had coagulated and could not be analyzed in the osmometer.

A HOBO logger was deployed on November 13, 2017 in both the Middle and Upper ponds of NRFF, and in both the Dixon and Faulkner ponds. The Lower pond did not contain a HOBO logger during this winter as previous trials indicated that DO was not limiting throughout the winter. Water quality was assessed for each pond during, or near the time of all sampling events (NRFF: November 22, 2017, February 10, March 3, and April 10, 2018; Faulkner/Dixon: November 22, 2017, February 2, March 3, and April 10, 2018). Standard water analysis plus total metals were analyzed by AGAT Laboratories (Dartmouth, NS). Pond water was collected in plastic bottles supplied by the laboratory. Once filled, bottles were transported back to lab and kept refrigerated (4 ºC) until being shipped on ice to AGAT laboratories. A full list of the water quality parameters analyzed appears in Appendix E, Tables E1-E5. Table 5 below presents a subset of selected parameters that indicate that water quality posed no apparent threats to fish health during the overwinter trial. The pH in all ponds remained between 6.5 and 7.6. At NRFF, alkalinity ranged from 6 to 20 mg L^{-1} in all ponds (Table 5). Alkalinity was higher in both the Dixon and Faulkner ponds by comparison, ranging from 7 to 48 mg L^{-1} and 51 to 61 mg L^{-1}, respectively (Table 5). Nitrate nitrogen was between 0.05 and 0.81 mg L^{-1} and nitrite nitrogen remained $\leq$ 0.05 mg L^{-1} in all experimental ponds. Ammonia nitrogen (total ammonia) concentrations were consistent in all ponds throughout the winter of 2017/18, ranging between 0.10 to < 0.03 mg L^{-1} (Table 5). Unionized ammonia was consistently < 0.001 mg L^{-1} during all sampling events in all ponds other than the Lower pond, indicating it posed no threat to the fish (Trussell, 1972; CCME, 2010). Temperature data was not available for the Lower pond, therefore unionized ammonia was unable to be calculated. From the previous trial, it can be inferred that unionized ammonia levels were acceptable for fish survival.

Table 5. Water quality parameter concentrations in the Lower, Middle, and Upper ponds of North River Fish Farms Ltd. and the Dixon and Faulkner ponds during all sampling events in the 2017/18 trial. Units mg L^{-1}, except for pH.

Pond	**mg L^{-1}**	**Nov. 22**	**Feb. 10**	**Mar. 03**	**April. 10**
Lower	pH	7.1	6.6	7.0	7.1
	Alkalinity	16	6	16	8
	Nitrate nitrogen	0.17	0.14	0.44	0.11
	Nitrite nitrogen	<0.05	<0.05	<0.05	<0.05
	Ammonia nitrogen	0.05	0.06	<0.03	0.09
	Unionized ammonia	-	-	-	-
	Hardness	12.1	11.2	25.4	11.4
Middle	pH	7.1	6.5	6.7	7.0
	Alkalinity	14	7	7	8
	Nitrate nitrogen	0.18	0.16	0.13	0.17
	Nitrite nitrogen	<0.05	<0.05	<0.05	<0.05
	Ammonia nitrogen	0.11	0.08	0.06	0.05
	Unionized ammonia	<0.001	<0.001	<0.001	<0.001
	Hardness	18.8	11.6	12.1	12.8
Upper	pH	7.2	6.6	6.9	7.0
	Alkalinity	20	7	8	10
	Nitrate nitrogen	0.10	0.13	0.08	0.11
	Nitrite nitrogen	<0.05	<0.05	<0.05	<0.05
	Ammonia nitrogen	0.03	0.05	<0.03	0.05
	Unionized ammonia	<0.001	<0.001	<0.001	<0.001
	Hardness	15.8	12.8	14.2	13.3
Dixon	pH	7.5	7.4	6.8	7.6
	Alkalinity	48	42	7	30
	Nitrate nitrogen	<0.05	0.29	0.14	0.22
	Nitrite nitrogen	<0.05	<0.05	<0.05	<0.05
	Ammonia nitrogen	<0.03	<0.03	0.08	0.04
	Unionized ammonia	<0.001	<0.001	<0.001	<0.001
	Hardness	69.7	51.5	11.9	36.9
Faulkner	pH	7.5	7.5	7.4	7.7
	Alkalinity	61	52	51	51
	Nitrate nitrogen	0.59	0.83	0.71	0.81
	Nitrite nitrogen	0.05	<0.05	<0.05	<0.05
	Ammonia nitrogen	0.10	<0.03	<0.03	0.06
	Unionized ammonia	<0.001	<0.001	<0.001	<0.001
	Hardness	56.3	68.1	77.8	71.9

2.2.5 Statistical analysis

Survival data from the 2016/17 trial was analyzed as a 2 x 2 factorial model. The two factors were pond (two levels: Middle and Lower pond), and sampling date (two levels: March and April). February sampling was not included in the analysis since the survival was 100 %. Diet and body size were not considered as experimental factors (see Section *2.2.3*). This model allows determination of the differences in the means of the response variables. The sampling conducted in February 2017 was not included in the statistical analysis because the survival in all the treatments was the same, 100 %. If this sampling was included, the model did not meet the normality requirement to run the Mixed procedure (Littell et al. 1998; Elliott and Woodward 2010). The Anderson-Darling normality test (Anderson and Darling 1952) was conducted in Minitab 18 (Minitab Inc., State College, PA, USA), using the residuals of the Mixed procedure of SAS statistical software (SAS 9.4, Institute Inc., Cary, NC, USA). Least-squares means analysis ($p = 0.05$) was used as a *post hoc* test to determine pairwise relationships among all treatment combinations. To determine the relationship between body size and survival, size categories were divided into 0.5 cm increments (e.g. 13 - 13.4 cm FL) and the percent survival was determined for each.

Data of the 2017/18 trial was analyzed using a mixed effects model with block (pond) as the random factor. The fixed factors were the whole plot (sampling time, two levels; February and March), and the factorial sub-plot: two diets (commercial and tailored) x two body size categories ('small' and 'large'). 'Small' fish were defined as ≤ 12 and 12.3 cm FL among the commercial and tailored diet treatments; and 'large' fish were defined as ≥ 12.8 and 13.0 cm FL among the commercial and tailored diet treatments, respectively. The sampling in April was not included in the statistical analysis because almost all the treatments had the same response, zero percent survival. Normality and *post hoc* tests were conducted as explained above. Statistical analysis was not run on the Dixon and Faulkner ponds as they were considered exploratory.

Mean daily temperature and DO were calculated from the 48 data values from each 24h period. Statistical analysis of temperature and DO changes both within and between ponds was conducted on the arithmetic means for each month using Analysis of Variance (General Linear Model; Minitab 18).

2.3 Results

2.3.1 2016/17 Trial

2.3.1.1 Temperature and oxygen

Daily mean temperatures of both Lower and Middle ponds were the same through November, increasing from 6 to 8.3 ºC, then cooling to 3.1 ºC (Figure 8). Ice began to form at the end of November and thereafter, for the duration of ice cover, the Lower pond was significantly colder than the Middle pond by between 0.3 and 0.8 °C (ANOVA, $F(1,300) = 16.8$, $p < 0.001$). From mid-December to the end of February, both ponds cooled slowly by ca. 0.2 ºC a week, reaching their coldest temperatures on February 27, 2017, 1.6 ºC and 1.2 ºC in the Middle and Lower pond, respectively. During March they warmed to 3.2 ºC and 2.6 ºC, respectively, on March 22 and then cooled. Both ponds were at 1.9 ºC on April 8, 2017. The rapid warming that commenced April 8, 2017 was associated with 45 mm of rain on April 6 and 7, 2017 and the ponds becoming ice-free. Subsequently, they warmed quickly to more than 6 ºC on April 16th (Figure 8).

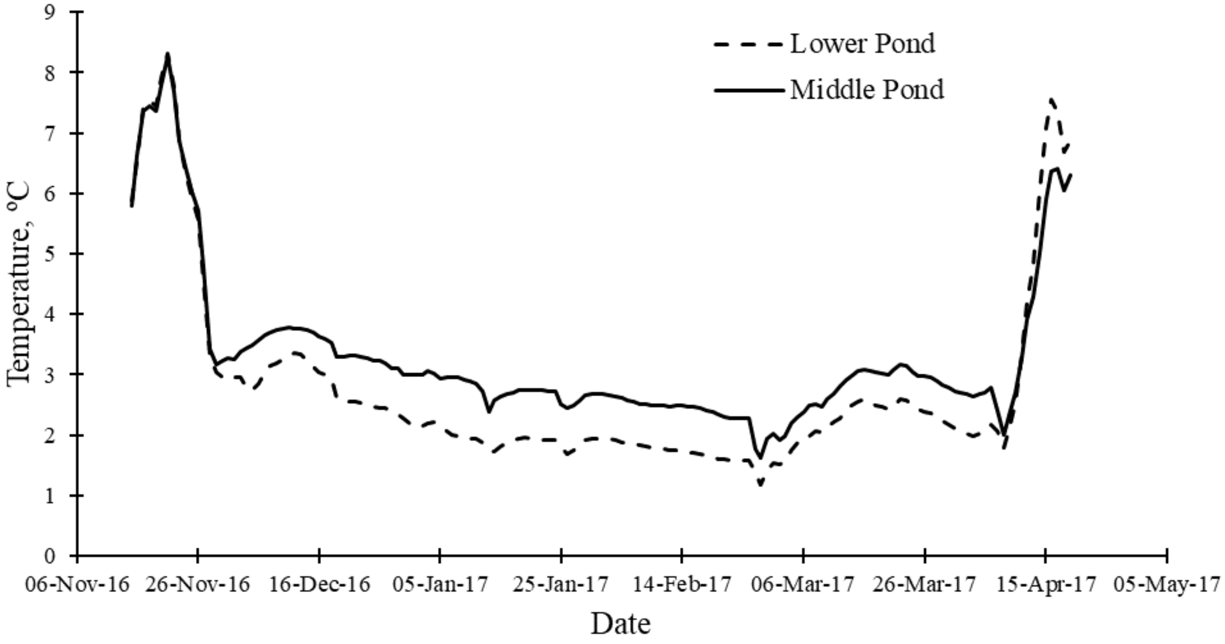

Figure 8. Daily mean temperatures (°C) from November 11, 2016 to April 19, 2017 in both the Middle and Lower ponds at North River Fish Farms Ltd.

Dissolved oxygen in both ponds ranged between about 5 and 11 mg L^{-1} overwinter (Figure 9). Dissolved oxygen in the Lower pond was significantly higher than the Middle pond by at least 1mg L^{-1} through most of the winter (ANOVA, $F(1,300) = 129.42$, $p < 0.001$). Temporal changes in dissolved oxygen in both ponds were highly significant (ANOVA, $F(5,300) = 106.38$, $p < 0.001$), falling progressively through January to less than 6 mg L^{-1} around February 26, 2017 and then abruptly increasing by more than 3 mg L^{-1} over two days. This was matched by a change in temperature (Figures 8 and 9).

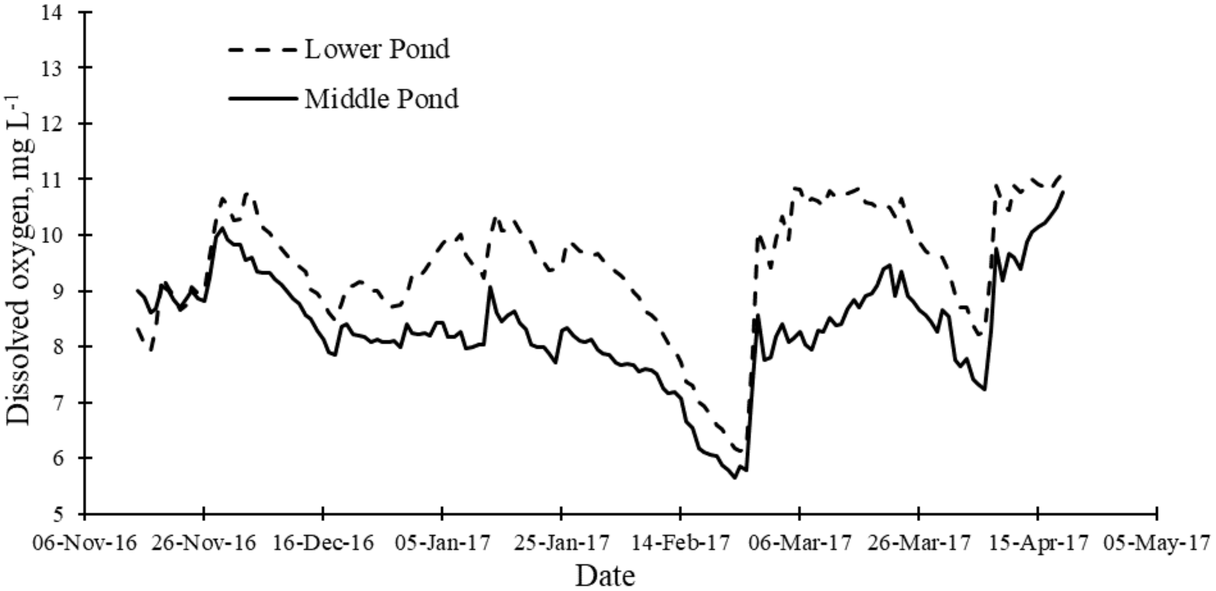

Figure 9. Daily mean dissolved oxygen concentrations (mg L^{-1}) from November 11, 2016 to April 19, 2017 in both the Middle and Lower pond located at North River Fish Farms Ltd.

2.3.1.2 Survival

On February 3, survival was 100 % in all treatments (Figure 10). On March 19, survival rates remained high, with 89 % (n=80) and 99 % (n=89) of the total of 90 fish stocked in the cages at the beginning of winter alive in the Lower and Middle ponds, respectively (Figure 10). On April 19, the final sampling event of the trial, survival rates differed markedly between ponds. Survival in the Lower pond was only 10 % (n=9), compared to 86 % (n=77) in the Middle pond (Figure 10). The overwinter survival rate of YOY striped bass was significantly affected by the interaction between pond and timing of sampling (ANOVA, $F(1,8) = 65.78, p < 0.001$).

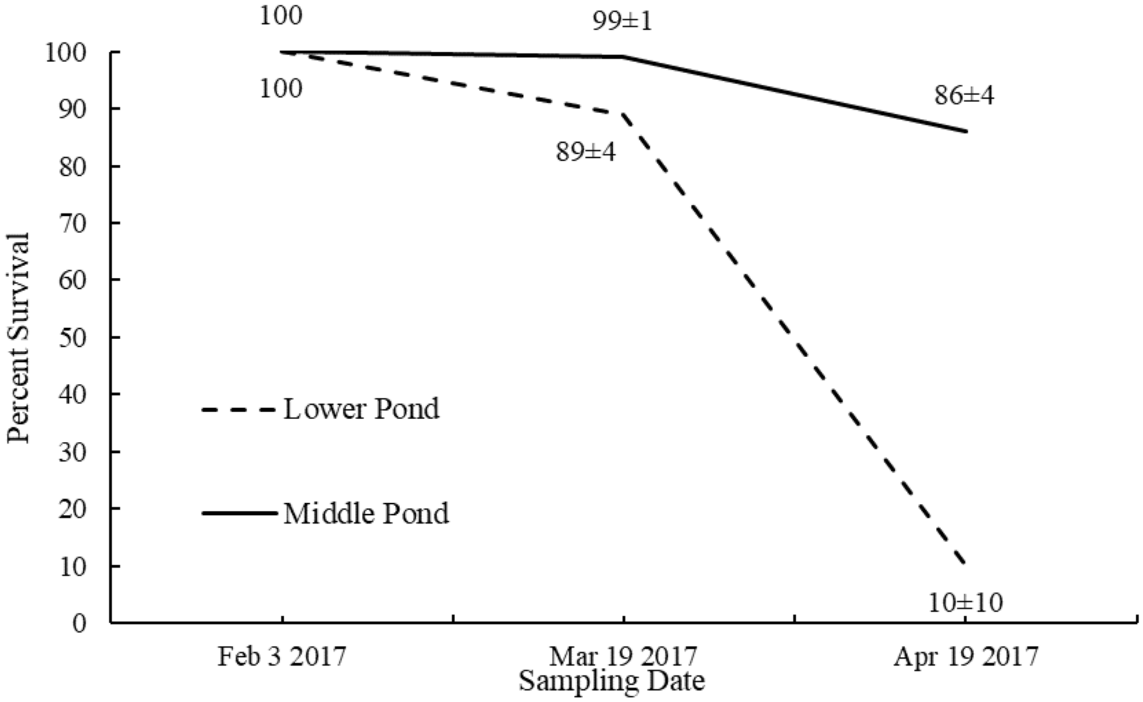

Figure 10. Pooled mean percent survival (± SE) in all cages during three sampling events for young-of-the-year striped bass (*Morone saxatilis*) stocked in cages in constructed freshwater ponds at North River Fish Farms Ltd. from Nov. 11, 2016 to Apr 19, 2017.

2.3.1.3 Fork length of mortalities vs. survivors

In the Middle pond on April 19, the mean FL of survivors and mortalities were similar, 15.1 vs. 14.7 cm (two sample *t*(0.72) = 0.72, *p* = 0.494; Table 6). In the Lower pond on March 19, the mean FL of survivors was 0.8 cm greater than that of the mortalities, but the difference was not statistically significant (survivors: 14.8 cm FL mortalities: 14.0 cm FL; two sample *t*(9) = 1.94, *p* = 0.080; Table 6). On April 19, by comparison, the difference was significant (two sample *t*(12) = 3.48, *p* = 0.005), as the survivors were 1 cm longer than the mortalities on average (Table 6).

Table 6. Mean fork length (cm) of young-of-the-year striped bass (*Morone saxatilis*) retrieved from cages in freshwater ponds in March 19 and April 19, 2017 at North River Fish Farms Ltd. Nova Scotia. *P* values indicate differences between survivors and mortalities within each pond derived from a 2-sample *t*-test.

	Middle Pond					**Lower Pond**				
	Survivors		**Mortalities**			**Survivors**		**Mortalities**		
Date	**n**	**FL**	**n**	**FL**	**P**	**n**	**FL**	**n**	**FL**	**P**
Mar 19	89	15.0	0	*		80	14.8	10	14.0	0.08
Apr 19	76	15.1	8	14.7	0.494	9	15.7	62	14.7	0.005

Pooling the data from both ponds on April 19, 2017 yielded further evidence of a positive relationship between body size and survival (Figure 11). The survival rate (%) was less than 30 % among striped bass smaller than 13.5 cm FL. Whereas, the survival rate increased to around 50 % for fish that were between 14 and 16 cm FL. For fish that were even larger (> 16.5 cm FL), the proportion of fish alive in April was > 80 % (Figure 11). The bodies of mortalities were in different states of decomposition during sampling events and the recording of FL. The caudal fins on some individuals were partially eroded, which required the FL to be estimated. Most of the mortalities, however, were fully intact, allowing FL to be measured accurately.

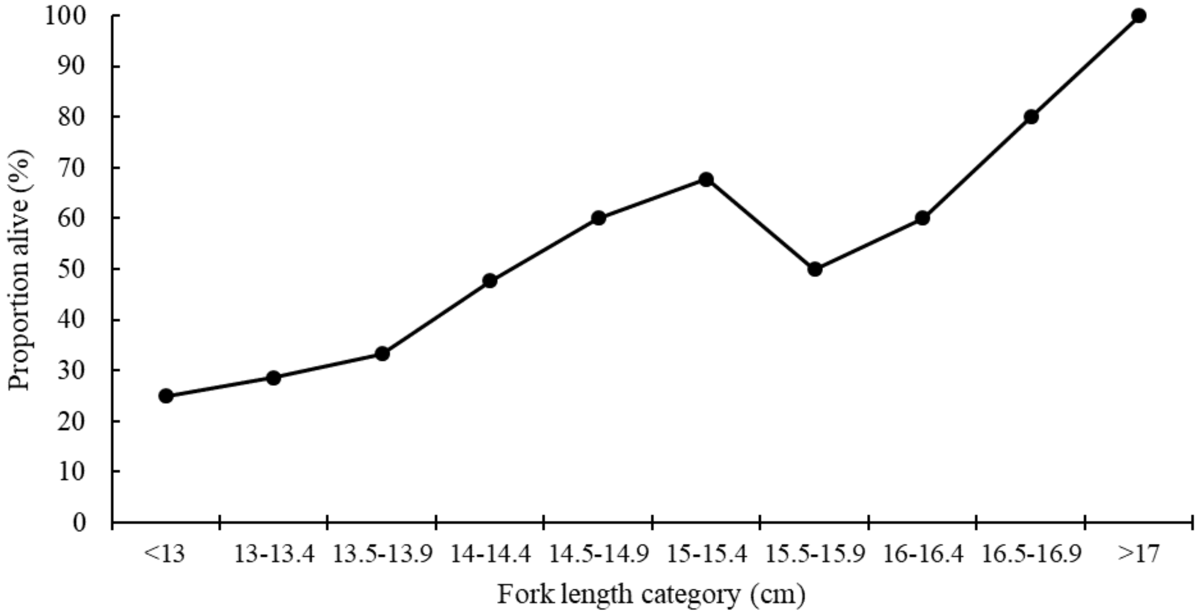

Figure 11. Relationship between fork length on April 19, 2017 and the incidence of survival among 0.5 cm size classes of striped bass (*Morone saxatilis*) reared in 1 m^3 cages in freshwater ponds (Middle and Lower) at North River Fish Farms Ltd. stocked on November 11, 2016.

2.3.2 2017/18 Trial

2.3.2.1 Water quality parameters 2017/18

At NRFF, daily mean temperature throughout the winter of 2017/18 was significantly warmer in the Middle pond compared to the Upper pond by about 0.3 to 0.6 °C (ANOVA, $F(1,286) = 50.05$, $p < 0.001$); Figure 12). The temperature of both ponds changed significantly overwinter, decreasing from about 6 °C in early November to < 2 °C in mid-December (Figure 12). At this point, ice on the ponds was thick enough to walk on. In the Upper pond, temperatures remained below 3 ºC until early April. The Middle pond warmed from February 24 until the end of the trial on April 10, 2018. During the final sampling event (April), the ice was still thick enough to stand on; however, it melted within the following week. The Middle and Upper ponds were coldest on December 15, 2017 at 1.7 and 1.1 °C, respectively.

The Faulkner pond remained ice-free and was about 3 °C warmer than the Dixon pond throughout the entire winter (Figure 12). The Faulkner pond remained above 2.6 °C throughout winter, whereas the Dixon pond was below 1 °C for the period of February 6 to 9, 2018 (Figure 12). This period was the coldest the water reached in the Dixon pond during the trial. Ice at the Dixon pond was about 10cm thick in February, but by March was very thin and patchy.

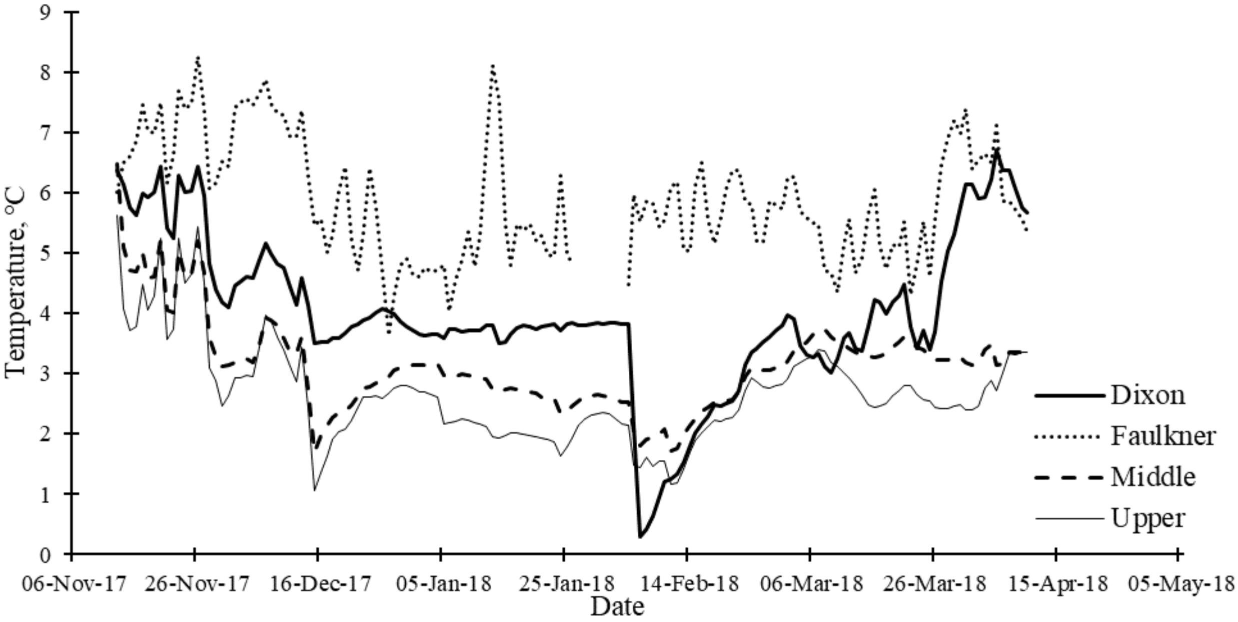

Figure 12. Daily mean water temperature (°C) from November 13, 2017 to April 10, 2018 in the Middle and Upper ponds located at North River Fish Farms Ltd. and the Faulkner and Dixon ponds. Data is missing from January 28, 2018 to February 2, 2018 in the Faulkner pond because the logger was removed to download data.

Dissolved oxygen at NRFF differed overwinter with a significant interaction between time and pond (ANOVA, *F*(1,286) = 230.22, $p < 0.001$). The Upper pond had higher DO concentrations (mg L^{-1}) than the Middle pond throughout the winter (Figure 13). From November 13, 2017 and April 10, 2018, DO concentrations at NRFF remained between about 7 and 12.5 mg L^{-1}. The Dixon pond DO from November through February was consistently higher than the Faulkner pond (Figure 13). For the duration of the trial, oxygen levels in the Dixon pond remained between 6.3 and 14 mg L^{-1} compared to the Faulkner pond that remained in the range of 4.4 – 12.9 mg L^{-1} (Figure 13). Dissolved oxygen in both ponds increased to between 11 and 12 mg L^{-1} in April, the final month of the trial.

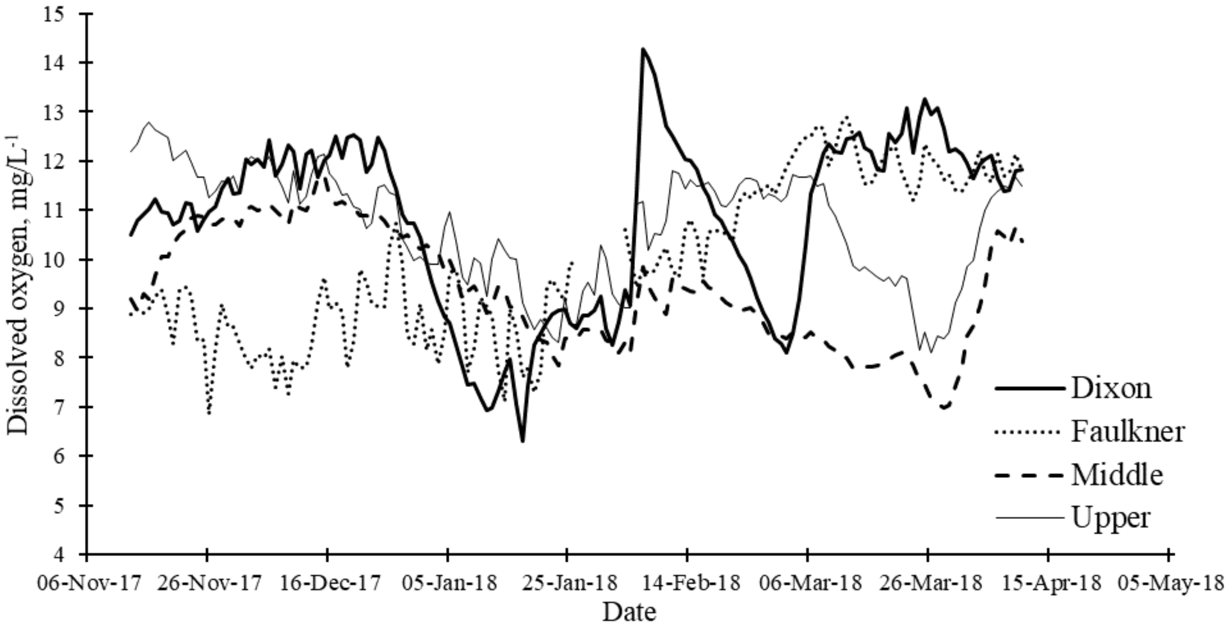

Figure 13. Daily mean dissolved oxygen concentrations (mg L^{-1}) from November 13, 2017 to April 10, 2018 in the Middle and Upper ponds located at North River Fish Farms Ltd. and the Faulkner and Dixon ponds. Data is missing from January 28, 2018 to February 2, 2018 in the Dixon pond because the logger was removed to download data.

2.3.2.2 Survival 2017/18

Survival during the 2017/18 trial at NRFF was much lower than the previous year in all ponds. An effect of 'pond' on survival was evident (Table 7). Highest survival occurred consistently in the Upper pond among small-grade fish: in February ranging from 57 to 93 %, and by April 7 to 13 % survival, compared to 0% survival in all other treatment combinations (Table 7).

The effect of body size on survival was not significant (ANOVA, $F(1, 12) = 3.48$, $p > 0.05$); however, small grade fish typically survived better than large in most cages (Table 7). Most notably, in the Upper pond, most survivors were small grade fish on both February 10 and March 3 (Table 7). Also, on February 10 in the Middle pond 7 of 11 survivors, and in the Lower pond only 3 of 8 survivors, were small grade (Table 7). On March 3 in the Middle pond, there were only two survivors, one of each size category and in the Lower pond on the same date, there were no survivors.

Table 7. Overwinter survival of young-of-the-year striped bass (*Morone saxatilis*) of two size-classes stocked into 1m^3 Vexar cages in three ponds at North River Fish Farms Ltd. Pre-winter diet was either commercial or tailored. Small grade fork length was ≤12 and 12.3 cm among the commercial and tailored groups. Large grade fork length was ≥ 12.8 and 13 cm among the commercial and tailored groups, respectively.

	Upper Pond				**Middle Pond**				**Lower Pond**			
Diet	Commercial		Tailored		Commercial		Tailored		Commercial		Tailored	
Size class	small	large	small	large	small	large	small	large	small	large	small	large
N start	15	13	14	13	15	13	14	13	15	13	14	13
Alive Feb. 10	12	2	7	2	2	3	5	1	1	2	2	3
% survival	80	15	50	15	13	23	36	8	7	15	14	23
N start	15	13	14	13	15	13	14	13	15	13	15	13
Alive Mar. 3	4	3	5	0	1	0	0	1	0	0	0	0
% survival	27	23	36	0	7	0	0	8	0	0	0	0
N start	15	13	14	13	15	13	14	13	15	13	14	13
Alive Apr. 10	1	1	1	0	0	0	0	0	0	0	0	0
% survival	7	8	7	0	0	0	0	0	0	0	0	0

Analysis of survival included body size and diet as factors. Pond was not considered an experimental factor for the analysis due to low, or no survival in all ponds for multiple sampling events; therefore, data was pooled across ponds. Following transfer of the fish to NRFF on November 13, 2017, survival on February 10 was very poor ranging from 15 to 33 % when comparing all ponds (Table 8). Survival declined further through March, and April to less than 5 %. Overall, survival was independent of diet and the effect of body size was not significant (ANOVA, $F(1,12) = 0.07$, $p = 0.795$).

Table 8. Pooled mean (± SE) survival (%) in all three NRFF ponds of small (commercial diet fork length max.12 cm; tailored diet fork length max.12.3 cm) and large (commercial diet fork length min. 12.8; tailored diet fork length min. 13 cm) young-of-the-year striped bass (*Morone saxatilis*) stocked in three constructed freshwater ponds at North River Fish Farms Ltd. from Nov. 13, 2017 to Apr. 10, 2018.

	Small		**Large**	
Month	**Commercial**	**Tailored**	**Commercial**	**Tailored**
February 10	33 ± 23a	33 ± 10a	18 ± 3ab	15 ± 4ab
March 3	11 ± 8ab	12 ± 12ab	8 ± 8ab	3 ± 3b
April 10	2 ± 2	2 ± 2	3 ± 3	0

Means sharing the same letter are not significantly different (Least-square means test, $p < 0.05$). April's data was not included in the ANOVA.

In the Dixon pond on March 11, survival among YOY fish fed the commercial diet pre-winter was very good at 80 % (12 of 15) among small fish, and 92.3 % (12 of 13) among the large fish (Table 9). Among fish reared on the tailored diet, survival of small fish was 21.4 % (3 of 14) and large fish was 92.3 % (12 of 13; Table 9). Of the 14 fish reared on the commercial diet that were left in the pond until April 10, 2018, two were found to be alive (ca. 14 %), with a mean FL of 12.6 cm. Survival of the 1+ year fish (> 250 g, n = 8) in the Dixon pond was 100 % on April 10, 2018.

In the Faulkner pond, when the cages were first examined on January 27, 2018, survival among fish from the tailored diet treatment was 0 %, compared to 7.7 % (1 of 28) among the fish fed a commercial diet. The one survivor was a relatively large fish (15.0 cm

FL and 40.7g BW). In the third cage, survival was 100 % among the eight 1+ year-old fish (> 250 g) on January 27th. However, on April 10, survival was zero for this size class (Table 9).

Table 9. Overwinter survival of small (commercial diet fork length max. 12 cm; tailored diet fork length max. 12.3 cm) and large (commercial diet fork length min. 12.8; tailored diet fork length min. 13 cm) young-of-the-year (*Morone saxatilis*) from either the formulated commercial diet or tailored diet treatments stocked in 1m^3 Vexar cages in two freshwater ponds (Dixon and Faulkner) near Truro, Nova Scotia.

	Dixon pond				**Faulkner pond**			
Diet	Commercial		Tailored		Commercial		Tailored	
Size class	small	large	small	large	small	large	small	large
N start	15	13	14	13	15	13	14	13
Alive Mar. 11	12	12	3	12	0	1	0	0
% survival	80	92	21	92	0	8	0	0
N sampled	5	5	4	11	*	*	*	*
N alive Apr. 10	0	2	*	*	*	*	*	*
% survival	0	15	*	*	*	*	*	*

2.4 Discussion

The trials conducted in two consecutive winters (2016/17 and 2017/8) at three sites confirmed that YOY striped bass held in FW ponds are at a high risk of dying. The use of periodic sampling during the winters of these two trials assisted in better understanding when YOY fish were dying. Most of the previous trials (except during the 2015/16 winter) conducted at NRFF simply waited until the ice melted at the end of winter, providing little insight into whether fish died acutely at the beginning of winter, or chronically throughout the experiment. In the trials reported here, mortality was progressive in most cases, occurring later in the winter. The cause of mortality remains unknown, but certain factors can be eliminated, such as hypoxia due to low DO concentrations and ammonia toxicity. Other factors require more study, including body size, diet, and pond environment. Body size remains a relevant factor; bigger fish generally had a better chance of survival, but some of the results were contradictory. Though the effect of diet on survival was inconclusive, the progressive decline in survival through the winter supports a hypothesis that the fish were exhausting some essential, but unknown, energy source. In the 2017/18 trial, diet was successfully tested, however it did not have a significant impact on survival of YOY fish. These conclusions are discussed below in the context of furthering the knowledge of YOY striped bass overwinter survival.

2.4.1 Influence of body size

Body size appeared to have an influence on mortality during both overwinter trials, but not always in the way hypothesized. Body size is one of the main influencers of overwinter survival and recruitment of YOY temperate fish and multiple studies have demonstrated evidence of size-dependent mortality. Specifically, in striped bass from the Hudson River population, Hurst and Conover (1998) determined that size-dependent mortality was a factor contributing to winter mortality in a 99-day simulated overwinter trial. Young-of-the-year striped bass were caught in beach seining efforts (October 6 and 20, 1993) in Haverstraw Bay of the Hudson River and divided into small (7.7 ± 0.4 cm) and large (9.8 ± 1.1 cm) size classes, in addition to fed (adult brine shrimp once daily) and unfed treatments for a total of eight cages. In this trial fish were exposed to winter ambient temperatures (not specified in the article) and photoperiod in addition to salinity of ca. 15-25 ppt, similar to those found in the overwintering areas. Survival of the large body size treatment (9.8 ± 1.1 cm) was significantly better than the smaller size class (7.7 ± 0.4 cm). The present study exhibited a similar trend although fish used were larger (mean FL was 14.3 cm in 2016/17 trial, and between 12 and 13 cm in 2017/18). In the 2016/17 trial, size was not successfully tested through specific size class treatments, but we were able to compare the FL of survivors and mortalities. By doing this, we observed significant difference between the FL of survivors and mortalities, supporting the hypothesis that larger YOY fish have higher rates of survival throughout the winter.

Although the survival results between our pond trials and the study by Hurst and Conover (1998) were similar, both the genotype and environmental conditions differed between the two studies. Further, water temperature during their laboratory trial was not stated in the Hurst and Conover paper, making it impossible to determine if temperatures were similar between both experiments (Hurst and Conover, 1998). The duration of the trials differed, 99 days for the laboratory experiment compared to 160 days in the ponds. This means that fish in our pond experiments were subjected to a longer winter period, which could have also impacted survival of YOY striped bass. In the 2016/17 trial, survival was >90 % in mid-March, after 128 days.

We are also able to compare the survival results of Hurst and Conover's (1998) experiment of fish in unfed treatments since fish in our trials were not offered food for the entirety of the winter. For the Hudson River YOY, 55 % of large fish survived compared to only 34 % of small fish when exposed to ambient temperature conditions during the winter from December 22, 1992 to March 25, 1993 (Hurst and Conover, 1998), indicating again that body size had an impact on winter survival. Fish in our trial were fed two pre-winter diets, commercial or tailored, whereas fish in the Hurst and Conover (1998) trial were all fed frozen adult brine shrimp leading up to the trial in their feeding period that consisted of three temperatures (5, 10, and 15 °C). This indicates that fish might not have been prepared for winter, in terms of diet, the same way and should be kept in mind when comparing survival results.

In the field portion of Hurst and Conover's (1998) experiment, including five years (1986-1990) of weekly trawl sampling in the Hudson River from around mid November to mid April, size-dependent mortality was not evident in all years (Hurst and Conover, 1998). Specifically, only in the winter of 1990 was mortality attributed to size selectively, based on the observation that the mean size of fish captured increased and the variance in size decreased during the winter (Hurst and Conover, 1998). They indicated that in the four other winters (1986-1989), these factors were not significant; therefore, mortality was not necessarily a result of small size (Hurst and Conover, 1998). When comparing our two trials, the positive influence of body size on survival was only evident in the 2016/17 trial. In the second trial (2017/18), results were contradictory to size-dependent mortality as we observed higher survival with smaller fish. Although size did not significantly impact survival in the 2017/18 trial, smaller fish surviving in higher numbers during the second winter could indicate that the minimum required FL for winter survival was smaller than the previous year. Specifically, YOY smaller than 12 cm (commercial diet treatment) and 12.3 cm (tailored diet treatment) survived better than the larger fish in this trial; a size more comparable, but still larger than those from the Hudson River in lab trial (minimum FL 9.8 ± 1.1 cm) and Gulf of St. Lawrence (minimum FL 10 cm) populations (Bernier 1996; Bradford and Chaput, 1997; Hurst and Conover, 1998).

Mean FL of YOY striped bass entering their first winter can fluctuate from year to year, as seen in the Gulf of St. Lawrence (Bradford et al., 2001; Douglas et al., 2003), the Hudson River (Hurst and Conover 1998), and the Shubenacadie River populations (Duston et al. 2018). For example, the FL from 1991-1998 and 2000-2002 was between 10 and 13 cm, whereas in 1999 YOY had a mean 15.3 cm FL in the Gulf of St. Lawrence designatable units (DU) (Bradford et al., 2001; Douglas et al., 2003). Further, Bernier (1996) suggested a minimum FL of 10 cm at the onset of winter is required to survive the winter. In the Hudson River population, mean fork or total length as of December 1 varied substantially over five consecutive years of sampling (1986-1990) from 10.3 to 12 cm. In a second study on Hudson River striped bass, the mean total lengths at the beginning of the 1996 and 1997 winters were 11.8 cm and 10.3 cm, respectively (Hurst and Conover, 2003). The Shubenacadie River population also exhibits large interannual variation in growth during the first summer, which has been attributed to differences in temperature from year to year since colder years often resulted in smaller fish (Duston et al., 2018). Between the years 2008 to 2016, the mean total length of age-0 striped bass in mid-August ranged from ca. 3.5 in 2011 to 7.6 cm in 2016 (Duston et al., 2018). The difference in temperatures between those two years is evident, as mean water temperature for the month of August were 18.6 °C in 2011 and 21.7 °C in 2016. Although lengths from these various populations were recorded at different times of the year, all appear smaller than the FL estimated was necessary for 50 % chance of survival during the Nova Scotian winter in FW ponds, even when comparing FL and total length. In the 2016/17 trial, the minimum FL required for 50 % chance of survival was between 14 and 16 cm. Further, fish that had a 13.5 cm FL had less than a 30 % chance of survival during the 2016/17 winter in our trial. The difference in FL of fish alive in the consecutive trials in addition to those in various populations indicates to us that attaining a certain length is not the only criteria required for successfully surviving a first winter as a temperate YOY fish. Other components in addition to size need to be considered when discussing the concept of overwinter mortality, including the duration and harshness of winter, and the presence of other stressors.

It is possible that the severity of winter in the different locations compared to Nova Scotia, such as the Gulf of St. Lawrence and Hudson River populations, can have an impact on the minimum body size required to survive the winter. In respects to our trial, the only real comparison of winter water temperatures we can make is within the Middle pond, as this is the only pond that had temperatures recorded for the entirety of both trials. Minimum temperature was very similar in the Middle pond for both the 2016/17 and 2017/18 trials, at 1.6 and 1.7 ℃, respectively; however, temperatures remained colder in the first trial until April, possibly having an impact on mortality of smaller fish. Hurst and Conover (1998) presented temperature data in the Hudson River from 1986 to 1990. The minimum temperature recorded and experienced by wild striped bass were comparable, and mostly less severe, to those in the Middle pond of NRFF, ranging from 1.4 to 4.5 ℃. Since water temperature seems to be colder in Nova Scotia, perhaps YOY striped bass require a larger body size to successfully survive the winter. Additionally, Hurst and Conover (1998) explained that the body size of mortalities increased as their simulated winter experiment progressed, indicating the duration of winter, or time exposed to winter temperatures, can have an impact on mortality of YOY fish.

Another possible source of mortality among YOY fish in the present study could be the ability to prepare for winter, specifically in terms of energy reserves. When studying perch (*Perca fluviatilis*), Huss et al. (2008) concluded that size-dependent mortality is a good explanation of survival variance within a cohort, but not always when comparing multiple cohorts. What can be more beneficial is to compare the growth rate near the end of the growing season, just before winter, as different cohorts may adopt different growth strategies (Huss et al., 2008). Often YOY fish experience a conflict between two growth strategies leading up to their first winter; to grow in length in order to avoid predation (Grant and Tonn 2002; Biro et al., 2005) or to accumulate more reserves, such as lipids, prior to winter (Post and Parkinson, 2001; Hurst and Conover 2003). Hurst and Conover

(2003) reported that lipid levels remain low during the summer and early fall as the focus is on somatic growth, most likely to avoid predation. As winter nears, there is an allometric shift as YOY striped bass tend to switch to storing lipid into energy reserves (Hurst and Conover, 2003). Between the winters of 1993 to 1998, the lipid energy reserves among YOY striped bass sampled in the Hudson River estuary varied significantly from 13 % to 23 % of the total energy content at the onset of winter (Hurst and Conover, 2003).

When looking at the total number of fish alive in April in both trials, survival results were higher in the 2016/17 winter; however, fish that survived the second winter were more likely to come from the small (mean BW 25.6 g) category of graded fish. This could indicate that the fish in our studies may have been able to allocate more to their body reserves, such as lipids, prior to the onset of the 2017/18 winter, compared to those in the 2016/17 study. Future research into the pre-winter storing of energy reserves adaptations would assist in determining the best practices for striped bass culture at this life stage if stocking in ponds prior to winter is desired.

Moreover, a more robust examination into the impacts of size-dependent mortality in a captive setting could be beneficial to determine the minimum size (FL) requirement for YOY striped bass being placed in a pond, or other commercial grow-out setting prior to winter. At this stage, it is unclear what is the minimum length required for overwinter survival of YOY striped bass. This size requirement can also vary from population to population, as the overwinter experience may not be the same depending on location. For striped bass from the Shubenacadie River population, we suggest focusing efforts on pre and post-winter sampling for consecutive years to determine lengths of fish at the onset of winter and once fish have survived until the spring. Quantifying energy reserves, specifically lipid, proportions during both these sampling times could also provide insight into how reserves are being used throughout the winter. Moreover, it could highlight the winter energy deficit experienced by YOY striped bass in the Shubenacadie River. Winters in NS and the Maritime provinces generally can extend from November to May (Andrews et al., 2019), something fish in captive pond setting such as NRFF will experience although not in their native river, or other, more southernly winter habitats. We need to have a better understanding of the condition of YOY striped bass that survive the winter before we can successfully begin rearing these fish on a commercial level.

2.4.2 Diet

The effect of diet on overwinter survival remains unresolved. Although only successfully tested in the 2017/18 trial, diet did not appear to have a clear impact on survival. The commercial diet had the highest survival rates in most ponds where fish were alive during sampling; however, diet had no statistically significant impact on survival. This is evident in all sampling events that occurred in the Upper pond of NRFF, as well as the Dixon pond. This result was somewhat counterintuitive, as the tailored diet was formulated specifically for the dietary requirements of striped bass, whereas the commercial diet was formulated to mimic the readily available commercial salmonid diets available on the market. Dietary requirements for pure striped bass for aquaculture purposes are not well known, probably due to their limited presence in commercial aquaculture. In North America, most farmed striped bass are hybrids; generally, a cross between white bass (*Morone chrysops*) and striped bass (*Morone saxatilis*) (Harrell, 1997). Dietary requirements of these hybrids have been known to reflect those of marine fishes (Nematipour and Gatlin, 1992, 1993; Barry, 2017), which typically are not the same as those necessary for salmonids. Historically in Canada, striped bass have been reared on commercially available salmonid diets (Cairns, 1999; J. Duston unpubl. data), and the survival results of our trial demonstrate that striped bass can survive on this type of feed.

The concept of YOY fish preparing for winter has been presented previously (sections *1.7.1* and *2.4.1*), however it may also offer further insight into the impact of diet formulation on winter survival. Fish store their energy reserves, such as lipids, in various body tissues such as adipose, liver, and muscle tissue (Cowey and Sargent, 1979; Adams, 1999; Tocher, 2003; van Dijk et al., 2005; Bar and Volkoff, 2012). When survivors from both trials were dissected, large amounts of visceral fat remained in the body cavity of YOY striped bass.

Further, salmonid species were present in the ponds at NRFF during both trials. In 2016/17, 15 brook trout (*Salvelinus fontinalis*) and 15 Arctic char (*Salvelinus alpinus*) were placed in monitoring cages in the Middle and Lower ponds from December to the end of the trial. In 2017/18, fingerling rainbow trout (*Oncorhynchus mykiss*) were present in one of the pre-existing net pens in the Upper pond. Not only did these fish survive the winters with extremely low, or no, overwinter mortality, but they also exhausted all their visceral fat by the end of the winter. This indicates that striped bass do not mobilise their lipid reserves in the same way as salmonid species. Moreover, it highlights that a better understanding of dietary lipid requirements for YOY striped bass is required to further extend our understanding of how young fish survive periods with a combination of no food consumption and winter water temperatures. Increased amounts of unsaturated fat in diets has been noted to improve survival of juvenile striped bass during simulated cold front trials (see section *2.1.1*; Kelly and Kohler, 1999), but we were unable to test this in our overwinter experiments. Therefore, testing this hypothesis could be a future line of inquiry utilized to better understand if the manipulation of the ratio of unsaturated:saturated fats can improve overwinter survival in YOY striped bass.

2.4.3 Pond effect

The use of ponds in addition to those present at NRFF allowed for the observation of survival success in different pond environments. However, at this point winter survival of YOY striped bass in FW is unpredictable and poor. Survival of YOY striped bass reared in our trials seems to be linked to something in their physical environment, but up to this point we have been unable to identify it. The effect of pond is more evident in some years than in others, as seen in the 2016/17 trial at NRFF. We cannot fully control the physical environment of the fish overwinter in these trials because YOY striped bass were not kept in a closed system. The pond system introduces multiple sources of stress, or a combination of environmental factors, that young fish must overcome to survive the winter, such as harsh winter conditions as well as possible osmoregulatory stress and exposure to toxins or pathogens (Hurst, 2007).

The observed pond effect on overwinter survival at NRFF could be linked to differing water quality in the ponds, especially in the 2016/17 trial, but a specific lethal factor was not identified. It also implies that water quality might have more of a role to play when it comes to overwinter survival, than both size and diet in our trials. Although in a winter setting characterized by cold-water temperatures and ice-covered ponds, neither temperature nor DO were considered to be limiting factors in any of the ponds included in either trial, even with dramatically different survival results between ponds. Striped bass from the Shubenacadie River are known to tolerate a wide range of temperatures, an adaptation potentially due to the significant influence of a tidal bore present in the river

(Rulifson and Dadswell, 1995; Cook et al., 2006). They are near the most northern limit of their ecological range, therefore have the ability to withstand colder temperatures compared to more southern populations (Cook et al., 2006). Cook et al. (2006) determined that the incipient lower lethal temperature of YOY striped bass was 0.9 °C lower than that of fish derived from a Maryland population when exposed to an acute temperature decrease (Kelly and Kohler, 1999), although a more extensive comparison of thermal tolerances of different striped bass stocks has not been accomplished to our knowledge.

Our study provides data to support that YOY striped bass can tolerate periods of cold-water temperatures, such as below 1 °C, as seen in the Dixon pond in February (Figure 12). Prior to this decreased water temperature, the Dixon pond was stable at approximately 3.5 - 3.8 °C, suggesting that when fish are acclimatized to lower temperatures, they can indeed tolerate a lower thermal change in the ponds. Cook et al. (2006) determined that acute thermal tolerance of a YOY striped bass descendant of the Shubenacadie River is related to the temperature at which they were acclimatized prior to the thermal change. In their study, the lower thermal tolerance of wild YOY (reared in lab from eggs collected in Shubenacadie River) acclimated to 15 °C was 2.4 °C (Cook et al., 2006); this tolerance grew as the acclimation temperature increased. Water temperatures in all experimental ponds in both trials was well below 15 °C during the winter, therefore supporting that YOY fish are able to tolerate temperatures below the 2.4 °C limit previously suggested in the literature (Cook et al., 2006).

Although not tested in our trials, another interesting source of mortality to point out is the different impact that acute and gradual changes in temperature can have on YOY fish survival. In the Kelly and Kohler (1999) paper discussed throughout this Chapter (sections

2.1.1 and *2.4.3*), they described the acute effects of a rapid cooling event on YOY striped bass. Survival results suggested death due to insufficient fluidity of cell membranes when fish (150 g) were subjected to a simulated cold front decrease in temperature from 20 to 10 °C in 24 hours (Kelly and Kohler, 1999). By contrast, in the ponds included in our trials, the decline in temperature was gradual; therefore, perhaps sufficiently slow enough for the cellular membranes to acclimate and alter their fluidity. The mortality observed in the pond experiments was most likely due to different reasons than in the Kelly and Kohler (1999) paper, as mortality in the ponds was chronic and overwinter temperatures did not seem to be a limiting factor.

Turning to DO, concentrations remained above 5 mg L^{-1} for the majority of both trials; therefore, none of the ponds were considered hypoxic (< 2.0 mg L^{-1} DO). The DO requirements specific to YOY striped bass in the Shubenacadie River during the winter are not described to our knowledge. Juvenile striped bass in the Delaware Estuary reportedly have a minimum DO requirement of 3.0 mg L^{-1} (Krouse, 1968; Bain and Bain, 1982); however, this seems to be at a temperature of 17 °C. Survival was consequently higher when DO concentration are at 5.0 mg L^{-1} or above (Krouse, 1968; Bain and Bain, 1982). It has been documented that adult striped bass in the wild avoid locations with 2 mg L^{-1} or less DO (Chittenden 1971; Coutant 1985; Thompson et al., 2010; Kraus et al., 2015). This tolerance, or threshold, of 2 mg L^{-1} has also been reported in largemouth bass (*Micropterus salmoides*) during the winter in Warner Lake, Ontario, Canada (Halser et al., 2009). As for younger striped bass, Coutant (1985) explained that juvenile fish in the Cherokee Reservoir, Tennessee, become stressed at DO concentrations of 3 mg L^{-1} and are unable to inhabit areas with 2 mg L^{-1} or less DO. Further, Brandt et al. (2009) observed a decrease in regular feeding activity and an increase in stress when DO concentrations were 4 mg L^{-1} or less in age-0 striped bass within their laboratory trial. All these examples demonstrate that the DO concentration tolerance of YOY striped bass tends to be impacted when levels reach ca. 4 mg L^{-1}, a situation that was not experienced by fish in our trial.

The setting of our trials also provides an alternate environment compared to the hypothesized preferred winter sites of YOY striped bass (see Chapter 1, section *1.7.3*), as ponds were covered by ice and fish were maintained in monitoring cages. This can influence the amount of DO present and also prohibit fish from relocating to areas of the pond with more preferable DO concentrations, or temperatures, as seen in wild striped bass and other fish species (Hasler et al., 2009; Kraus et al., 2015). However, the present trials offer evidence of the DO tolerance of YOY striped bass in winter temperature conditions, specifically in the trial with high survival (2016/17). Young-of-the-year striped bass are able to tolerate DO concentrations of at least 5.6 mg L^{-1}, as this was the lowest concentration survived by fish in the Middle pond with 80 - 90 % survival at the end of the winter. Indeed, significantly higher mortality was seen in the Lower pond during the same trial, which also had a higher DO concentration for the majority of the winter.

Pond water quality according to the parameters we measured were acceptable in all experimental ponds. Also, brook trout and Arctic char placed in monitoring cages in the ponds during the 2016/17 trial, and the rainbow trout present in the ponds in the 2017/18 survived the winter with limited or no mortality. Since salmonids are considered sensitive to pollutants (Environment Canada, 1988; Lazorchak and Smith, 2007), their overwinter survival suggests that water quality was not a factor causing mortality. Certain pollutants, such as ammonia, have been known to be associated with winter mortality in ponds (Hargreaves and Tucker, 2004); however, the risk of mortality due to ammonia poisoning is not a threat in the ponds utilized in our trials as unionized ammonia levels were <0.001 in all ponds throughout the winter. The Canadian Council of Ministers of the Environment (2010) stated that safe levels of unionized ammonia for fish health are < 0.019 mg L^{-1} in FW, a level much higher than those seen in all experimental ponds. It is possible that the cage specific environment provided different conditions to the open ponds, but future monitoring of conditions inside the cages is required to confirm their characteristics. In addition, the presence of brook trout and Arctic char in the same size cage ($1m^3$ Vexar) as the YOY striped bass indicates that the cage specific environment was probably not lethal.

Finally, the survival rates observed in the Dixon pond can offer some evidence that pond culture is an acceptable method to raise striped bass in captivity as survival rates were high during the March sampling. Although survival was lower in April, this is possibly due to the stress experienced by the fish when being previously sampled in March. The different survival response in the Dixon pond indicates the complexity of overwinter mortality in YOY striped bass. More trials in various ponds are needed to determine the viability of rearing YOY striped bass in FW ponds throughout the winter.

It can be argued that the best environment for YOY striped bass reared in a captive setting is one that resembles their natural winter environment in the wild. As this location is not known for YOY striped bass from the Shubenacadie River, further investigation is needed to fully understand their winter habitat requirements. It has been hypothesized that fish of this age remain in estuarine or marine environments (Bradford and Cook, 2004; Bradford et al., 2014), whereas older fish are able to overwinter in FW areas. Knowing the preferred environmental conditions of YOY striped bass will further our understanding of the winter habitat needs of these fish, in addition to pinpointing preferable locations for the captive rearing of young fish during the winter. This future research would require effort in locating winter habitats of YOY striped bass through sampling at various sites throughout the native range of YOY Shubenacadie River striped bass.

2.5 Conclusions

Winter mortality in YOY fish cannot be explained by one individual cause; instead, it is clear that a multitude of different components play a role in causing death overwinter. The results further the knowledge of YOY striped bass' ability to survive winter conditions, specifically temperatures and DO concentrations. Fish survived temperatures that were at times below 1 °C and DO concentrations of 5.6 mg L^{-1}. Size and pond environment had the most impact on survival of YOY striped bass in the present study. Evidence that YOY must have a FL of between 14 and 16 cm for 50 % survival was obtained, providing some guidance for rearing YOY striped bass in this sort of setting for aquaculture purposes. Additionally, March survival results in the Dixon pond indicate that YOY fish can survive in various locations. We suggest that future investigation should include additional ponds in order to gain more information on the type of environment required for YOY striped bass in the winter. Although diet did not seem to have an impact on survival in our trials, further research into the pre-winter diet requirements and storing of energy reserves could be beneficial in furthering the state of knowledge of the winter requirements and adaptations of YOY striped bass.

CHAPTER 3 Overwinter changes in the lipid profile of young-of-the-year striped bass (*Morone saxatilis*) in freshwater ponds

3.1 Introduction

High levels of overwinter mortality amongst young-of-the-year (YOY) fish is a common phenomenon among temperate species. Mortality in this young age class has been noted in both wild and laboratory trials on white perch (*Morone americana*; Post and Evans, 1989), northern populations of Atlantic silversides (*Menidia menidia)* (Schultz and Conover, 1997), and in age-0 rainbow trout (*Oncorhynchus mykiss*) in both laboratory and lake experiments (Biro et al., 2004). Similarly, the abundance of age-1 striped bass (*Morone saxatilis*) in the Miramichi and Hudson River has been connected to the severity of the winter that they experience as YOY fish (Bradford and Chaput, 1997; Hurst and Conover, 1998). Overwinter mortality in YOY fish is attributed to a combination of environmental stressors (Hurst, 2007), including starvation due to lack of prey, interest in food consumption, and dependency on energy reserves (Thompson et al., 1991; Handeland et al., 2008), predation (Woodhead, 1964), thermal stress (Beitinger et al., 2000), and the presence of parasites or pathogens (Horning and Pearson, 1973). Though single independent stresses are infrequently lethal, when a combination of stressors is present, YOY fish need to respond adequately in order to survive (Hurst et al., 2000).

Feeding frequency decreases when water temperatures cool (Handeland et al., 2008; Russell et al., 1996) and many fish species either reduce or cease feeding activity throughout winter (Bar, 2014). During periods of starvation, fish must rely on their endogenous energy reserves, primarily lipids, but are also known to utilize protein and glycogen (van Dijk et al. 2005; Kooka and Yamamura, 2011; Bar, 2014). Energetic demands are filled by the catabolism of fatty acids (FA) in triacylglycerols (TAG) which are stored mostly in tissues as energy reserves, including the liver and white muscle (Adams, 1999; van Dijk et al., 2005; Bar and Volkoff, 2012). YOY fish in temperate to cold waters experience a conflict between two different survival challenges relating to the size of these energy reserves prior to entering winter (Post and Parkinson, 2001). Early in their first year, metabolized energy is typically directed to somatic growth to limit the risk of predation (Post and Parkinson, 2001). However, at a certain point energy metabolism must shift to increase stored energy as lipids, since larger endogenous reserves are necessary to survive periods of starvation (Post and Parkinson, 2001). This evolved response to competing threats is well documented in YOY striped bass from the Hudson River estuary (New York), as YOY fish do not begin storing lipids for energy purposes until early September and extending into November (Hurst and Conover, 2003). This limits the resources available and the scale of energy reserves for winter, which is linked to the size-dependent mortality pattern seen in this and other species (Post and Evans, 1989; Thompson et al., 1991; Griffiths and Kirkwood, 1995; Hurst and Conover, 1998).

In addition to TAG, phospholipids (PL) contain FA that are vital during times of thermal acclimation experienced by YOY fish overwinter since they are key in maintaining cellular structure and are only metabolized during times of extreme and prolonged food scarcity, or no food consumption (Love, 1980; Adams, 1999). During the winter, PL assist in ensuring proper fluidity and structure of an organism’s cell membranes during periods of cold temperatures (Dey et al. 1993; Farkas et al. 2001; Guderley, 2004). PL are made up of a variety of different classes, some of the most abundant being phosphatidylethanolamine (PE) and phophatidylcholine (PC). Specifically, in the muscle tissue, these PL classes have demonstrated particular benefits to fish during the winter with respect to ensuring the structural integrity and function of tissues during times of low environmental temperatures (Farkas et al., 2001). Homeoviscous adaptation, or the maintenance of proper cellular fluidity across a range of environmental temperatures, can be accomplished by changing the FA composition of phospholipid classes, such as PE and PC, within cellular membranes as temperatures change (Hazel, 1995; Farkas et al., 2001; Guderly, 2004). Several studies have found an increase in total PE in addition to a rise in proportions of particular FA species such as 18:1/22:6 in a variety of warm and cold adapted marine fish (Wodtke, 1978; Dey et al., 1993; Farkas et al., 2001).

Striped bass are a temperate anadromous and euryhaline fish native to rivers and coastal waters adjacent to eastern North America that spawn in rivers as far south as northern Florida to rivers emptying into the southern Gulf of St. Lawrence in the north (Scott and Scott, 1988; Bradford et al., 2015). In NS, it is among the species of interest for commercial cultivation as they have a historical commercial presence and can be grown in FW. Unfortunately, similar overwinter mortality described above can be seen in this captive setting. For example, YOY striped bass have commonly suffered up to 100% overwinter mortality when stocked experimentally in FW ponds (trials occurring from 2012-2018) during the fall of their first winter (Pers. Comm. Dr. J. Duston, September 2016; Duston et al., unpubl. data).

The present study set out to investigate the role of lipids stored in body tissues in the overwinter survival of YOY striped bass stocked in FW ponds. Specifically, the objective was to determine the extent to which YOY striped bass in this setting were exhausting their lipid reserves prior to the end of the winter season. Since fish are not feeding throughout the winter, we hypothesized that the influence of lack of food intake would be primarily reflected in the liver lipids (primarily TAG). More specifically, we hypothesized that total lipid (TL) and TAG would decrease in the liver because the YOY fish were relying on it for energy to survive a period without available food. In addition, we anticipated that changes in tissue composition due to adaptation to winter water temperatures would occur in the white muscle lipids, specifically in the PL. PE/PC FA compositions were quantified overwinter to explore the hypothesis that the concentration of PE and specific FA, including 18:1n-9 and 22:6n-3 and other monoenic (1 double bond) and polyenic (more than 1 double bond) lipid species, would increase overwinter in the muscle tissue as an adaptation to thermal change. Consequently, we evaluated the concentration of TAG, PC, and PE in muscle tissue and TAG in the liver of YOY striped bass along with the FA profiles of each lipid class to determine the differences in each in fish collected in late fall (November) and mid-winter (March).

3.2 Materials and Methods

The work presented in this Chapter is part of a larger trial at NRFF occurring in the winter of 2016/17; therefore, all information concerning the experimental fish, feeding trial, fish transfer, overwinter rearing, and sampling procedures are documented in Chapter 2. Please see sections *2.2.2 Experimental site: North River Fish Farms, cage set up, and sampling procedure* and *2.2.3 2016/17 Experimental design* for specific details.

3.2.1 Sample selection for lipid analysis

For lipid analysis, only fish from Diet 1 (see Chapter 2, section *2.2.3 2016/17 Experimental design*), containing 7 % fish oil and 1 % poultry fat were selected. Initial pre-winter samples of fish (n=5) were taken on November 15 from fish remaining at the lab in tanks with water temperatures of ca. 7 °C. Late-winter samples were collected on March 19, 2017 from both ponds (n=5 in each pond). The five fish from a cage in each pond were euthanized (MS-222; 0.1-0.15 g/L) and transported back to the lab on ice in an insulated container. In the lab, fork length (cm) and body weight (g) were recorded, liver and white muscle tissue (approximately 1-2 cm wide ventral to the dorsal fins, above the lateral line) was dissected, wrapped in aluminum foil and stored at -80ºC.

3.2.2 Lipid analytical methods

Total lipid (TL) was determined for both liver and white muscle tissue (< 1 g liver and 1.5 g muscle) following a modified Folch et al. (1957) method using a 2:1 $CHCl_3$:MeOH extraction (Budge et al., 2006). This method requires a sample moisture content of 80%, so, when necessary, water was added to both homogenized liver and muscle samples. Then, 30 mL of 2:1 $CHCl_3$:MeOH was added to each sample and stored overnight in sealed 125 mL glass jars at 4 °C. Samples were then filtered to recover the liquid phase and 7 mL of 0.88 % NaCl was added to separate the mixture into aqueous and solvent phases. The lower, solvent phase containing TL was retained and the remaining solvent was evaporated under a nitrogen stream in a water bath (25-30 °C). Total lipid was determined gravimetrically on a wet weight basis. Recovered lipid was stored in 1.5 mL of methylene chloride containing butylated hydroxytoluene (BHT) at 0.01% mass/volume.

Individual lipid classes of liver and muscle TL (n=5 from each pond) were separated and recovered using thin layer chromatography (TLC) with silica gel on glass plates. Plates were first activated by heating overnight at 100 °C, then cooled in a desiccator. TL samples dissolved in 0.1 mL dichloromethane (15 mg/0.1 mL) were streaked 1 cm from the bottom edge of the plate. TAG, PE, and PC standards were also applied to the right-hand side of the plate for the purpose of lipid class identification. TAG, PE, and PC were isolated by a 2-solvent system TLC method (Kupke and Zeugner, 1978). Chamber 1 contained

$CHCl_3$:MeOH:acetic acid:water (50:30:8:3), and was used to separate polar lipids (PL, classes of interest: PE and PC), whereas chamber 2 contained hexane:diethyl ether: acetic acid (70:30:2), and was used to separate non-polar lipids (TAG).The plate was placed in the first chamber until the solvent rose approximately half the distance of the plate height. It was then air dried in a fume hood and placed in the second chamber until the solvent reached 1 cm from the top of the plate. The plate was removed from the chamber and air dried again. Visualization of individual lipid classes was accomplished by spraying the developed silica plate with dichlorofluorescein in ethanol (0.2 % in 96 % ethanol) and placing it under a UV light. Bands containing individual lipid classes were scraped from the plate and then placed in appropriate solvents in individual 10 mL test tubes to solubilize the lipids (TAG: chloroform; PE/PC: methanol:chloroform, 2:1). Samples were evaporated (under a nitrogen stream in a 25-30 °C water bath) and 1.5 mL methylene chloride with the addition of 0.01 % butylated hydroxytoluene (BHT) was added; lipid extracts were then stored in a -30 °C freezer.

To determine the entire fatty acid composition of liver and muscle TAG as well as muscle PE and PC, fatty acid methyl esters (FAME) were prepared by transmethylation of lipids using sulphuric acid as a catalyst with heating at 100 ℃ for an hour (Hilditch et al., 1935; Budge et al., 2006). The FAME were recovered with three hexane extractions and were dried over anhydrous Na_2SO_4. Hexane was then evaporated under a nitrogen stream in a 25-30 ℃ water bath. FAMEs were stored in hexane (20 mg mL^{-1}) prior to analysis with gas chromatography (GC) using a Bruker 436 GC equipped with a DB-23 column (50%-cyanopropylmethylpolysiloxane; 30 m, 0.25 mm ID, 0.25 µm film thickness), using helium (flow rate 1 ml min^{-1}) as carrier gas. Split injection (1/100) at 250 ℃ was used with flame ionization detection (280 °C with argon as make-up gas). The oven temperature was initially held at 150 °C for two minutes and then ramped at a rate of 5 °C min^{-1} until it reached 220 °C, at which it was held for three minutes for a total run time of 19 minutes. Fatty acids are expressed in shorthand notation as A:Bn-C, where A represents the number of carbon atoms, B the number of double bonds, and C the position of the first double bond relative to the terminal methyl group.

High performance liquid chromatography (HPLC) was utilized to quantify lipid classes (TAG, PE, and PC) and confirm results from the TLC procedure. A YMC-Pack PVA-SIL-NP column (polyvinyl alcohol phase, 150 mm x 4.6 mm ID, 5 µm particle size) with evaporative light scattering detector (ELSD) was used. The detector was held at 42 °C with N_2 at 3.5 barr. Specific methods (following Jones et al. 2012) were developed to quantify TAG and PL classes. The TAG method employed hexane:ethyl acetate (98.8:1.2) and isopropanol:methanol:water (3:3:1) as mobile phases, while the PL method used ethyl acetate with 0.1 % acetic acid, in addition to isopropanol:MeOH:water (3:3:1) (Tables10 and 11). TL samples were prepared to a concentration of 0.05 mg mL^{-1} in HPLC grade 1:1 hexane:2-propanol. Only TAG was determined in liver tissue; all three lipid classes were determined in the muscle tissue. All values are expressed on a wet-weight basis.

Table 10. High performance liquid chromatography (HPLC) solvents and gradient program for triacylglycerols (TAG) profile separation.

Time (min)	Solvent A (%)	Solvent B (%)	Flow rate (mL/min)
1.5	100	0.0	1.5
2	60	40	1.5
6	25	75	1.5
7	100	0.0	1.5
15	100	0.0	1.5

Solvent A: hexane: ethyl acetate (98.8:1.2),
Solvent B: isopropanol: methanol: water (3:3:1) with 0.1 % acetic acid and *0.005% triethylamine.

Table 11. High performance liquid chromatography (HPLC) solvents and gradient program for full phospholipid (PL) profile separation

Time (min)	Solvent A (%)	Solvent B (%)	Solvent C (%)	Flow rate (mL/min)
8	0.0	100	0.0	1.5
9	0.0	50	50	1.5
15	0.0	15	85	1.5
20	0.0	0.0	100	1.3
29	0.0	100	0.0	1.0
31	0.0	100	0.0	1.0

Solvent A: hexane: ethyl acetate (98.8:1.2),
Solvent B: isopropanol: methanol: water (3:3:1) with 0.1 % acetic acid and *0.005% triethylamine.
Solvent C: ethyl acetate with 0.1% acetic acid

3.2.3 Statistical analysis

All statistical analyses were carried out with IBM® SPSS Statistics (version 25). Outliers were first identified and removed using the Dixon's Q test. TL (mg g^{-1}) and lipid class data (mg g^{-1}) were transformed by taking the natural logarithm. Proportional data (lipid class and FA) were transformed by dividing each value by the sample geometric mean and then taking its natural logarithm (Filsmozer et al., 2009). The Shapiro-Wilk test was then used to test for a normal distribution using an adjusted p-value, specific to each dataset (for TL and TAG p-value: 0.017, PE/PC p-value: 0.006, and for FA data p-value: 0.003; Bonferroni adjustment) to compensate for multiple testing. Once all datasets were normally distributed, ANOVA was used to compare the mean lipid data of initial samples in November to those in late winter. If ANOVA indicated a significant result, pairwise *post hoc* (Tukey) tests were then performed. Statistical analysis was done at a pond level and reported as such throughout the paper.

3.3 Results

3.3.1 Total muscle and liver lipid

Mean muscle total lipid (TL; mg g^{-1} tissue) concentration of fish sampled in March was significantly higher (39 and 51 mg g^{-1}) than in November (16.2 mg g^{-1}) (ANOVA, *F*(2, 12) = 42.905, $p < 0.017$; Table 12). Mean liver total lipid concentration, by contrast, showed a lower trend in samples taken from fish in March in comparison to samples taken in November, but the difference was not statistically significant (ANOVA, *F*(2, 12) = 1.941, $p > 0.017$; Table 12).

Table 12. Mean (± SD; n=5) total lipid concentrations (mg g^{-1} tissue determined on a wet-weight basis) in November 2016 and March 2017 taken from liver and white muscle tissue of young-of-the-year striped bass (*Morone saxatilis*) held in two freshwater ponds.

Sampling Event	Liver Tissue	Muscle Tissue
November	236 ± 9^{a}	16 ± 2^{a}
March (Lower Pond)	201 ± 33^{a}	51 ± 9^{b}
March (Middle Pond)	213 ± 24^{a}	39 ± 9^{b}

Within a column, values with different superscripts are significantly different ($p < 0.017$).

3.3.2 Lipid classes

Liver triacylglycerols (TAG) concentrations (mg g^{-1}) exhibited no change overwinter, with means remaining between 182 and 202 mg g^{-1} (Table 13). The proportion of TAG among the total lipids (TL) in the liver, however, was significantly higher at the end of the winter with 77 % in November to > 90% in March (ANOVA, *F*(2, 10) = 12.680, $p < 0.006$; Table 13), as near the end of the winter (March sampling), TAG levels were almost the only component of TL (95 and 93 % in Lower and Middle pond, respectively).

Mean muscle TAG was higher in the winter when compared with initial samples, as we observed 4 mg g^{-1} of TAG in muscle tissue in November and 21 mg g^{-1} tissue (Lower pond) and 14 mg g^{-1} tissue in March in the two ponds (Middle pond) (ANOVA, *F*(2, 12) = 28.984, $p < 0.006$) at the end of the study. Muscle TAG also represented a higher proportion of the TL extracted from the muscle samples in the winter; 25 % of TL in initial samples, compared to between 35 and 41 % in March (ANOVA, $F(2, 12) = 13.460$, $p < 0.006$; Table 13).

Table 13. Mean (± SD) liver and white muscle tissue triacylglycerols (TAG) concentrations (mg g^{-1} determined on a wet-weight basis) and TAG proportions (%) of total lipid of young-of-the-year striped bass (*Morone saxatilis*) in November 2016 and March 2017.

	TAG (Liver)		**TAG (Muscle)**	
Sampling Event	**mg g^{-1}**	**Mass % TL**	**mg g^{-1}**	**Mass % TL**
November	182 ± 9^{a}	77 ± 3^{a}	4 ± 1^{a}	25 ± 5^{a}
March (Lower Pond)	191 ± 35^{a}	95 ± 4^{b}	21 ± 6^{b}	41 ± 4^{b}
March (Middle Pond)	202 ± 16^{a}	93 ± 3^{b}	14 ± 5^{b}	35 ± 5^{b}

Within a column, values with different superscripts are significantly different ($p < 0.017$).
N=5 for all samples except for Initial TAG liver (both concentration and proportion) and Middle pond TAG liver (proportions only), for which n=4.

Muscle PE masses were significantly higher in the white muscle when comparing initial results to those overwinter (ANOVA, $F(2, 12) = 21.588$, $p < 0.006$). The initial mean concentration from muscle tissue in November was 2 mg g^{-1}, whereas concentrations were 4 mg g^{-1} (Lower Pond) and 3 mg g^{-1} (Middle Pond) in March (Table 14). However, the proportion of PE in TL of the muscle tissue remained constant for all samples analyzed (ANOVA, $F(2, 12) = 2.637$, $p > 0.006$; Table 14) with 11 % in November, 8 % in the March low pond, and 8 % in March Middle pond. Muscle phosphatidylcholine (PC) masses and proportions remained unchanged in the muscle tissue when comparing initial to overwinter samples (mass: ANOVA, $F(2, 12) = 1.514$, $p > 0.006$; proportion: ANOVA, $F(2, 12) = 2.589$, $p > 0.006$; Table 14).

Table 14. Mean (± SD; n=5) white muscle tissue phosphatidylethanolamine (PE) and phosphatidylcholine (PC) concentrations (mg g^{-1} determined on a wet-weight basis) and proportions (%) of total lipid (TL) in November 2016 and March 2017 taken from white muscle tissue of young-of-the-year striped bass (*Morone saxatilis*).

	PE Muscle Tissue		**PC Muscle Tissue**	
Sampling Event	**mg g^{-1}**	**Mass % TL**	**mg g^{-1}**	**Mass % TL**
November	2 ±0.4^a	11 ±2^a	1 ±1^a	4 ±2^a
March (Lower Pond)	4 ±0.3^b	8 ±1^a	2 ±0.2^a	3 ±1^a
March (Middle Pond)	3 ±0.7^b	8 ±1^a	1 ±0.2^a	4 ±1^a

Within a column, values with different superscripts are significantly different ($p < 0.006$)

3.3.3 Fatty acid profiles for liver and muscle TAG

Six fatty acids found in liver TAG, when comparing initial and March samples, were significantly different (Figure 14; complete FA profiles for all analyzed lipid classes appear in Appendix F, Tables F1-F4). Most notable were the proportions of both 16:1n-7 (ANOVA, $F(2, 12) = 99.541$, $p < 0.003$) and 18:2n-6 (ANOVA, $F(2, 12) = 96.720$, $p < 0.003$) that were higher in the winter samples. Specifically, 16:1n-7, accounted for ~6 % of the FA profile in the initial samples, whereas it was ~10 (Lower pond) and ~9 % (Middle pond) in March. The proportion of 18:2n-6 was also high in the overwinter samples as it represented ~4% in the initial samples and ~6 % (Lower pond) and ~8 % (Middle pond) in liver tissue taken from animals in March (ANOVA, $F(2, 12) = 96.720$, $p < 0.003$). 18:0 concentrations in liver samples were significantly different between November and March (18:0; ANOVA, $F(2, 12) = 90.669$, $p < 0.003$), with the mean proportion being ~4 % (initial) and ~2 % in both ponds overwinter.

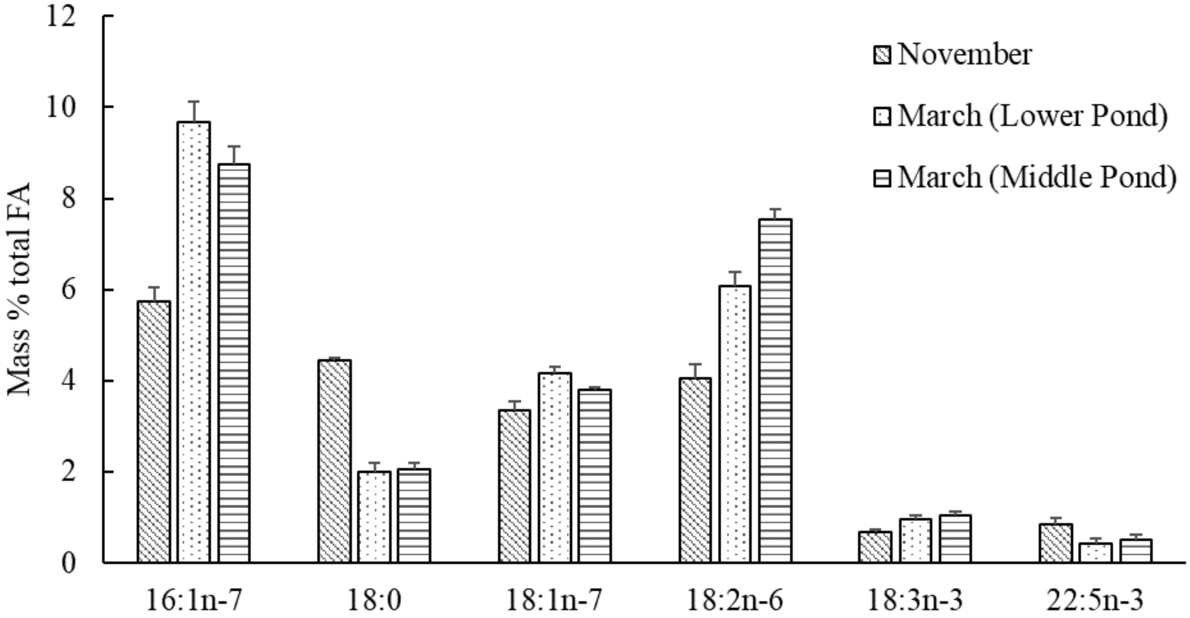

Figure 14. Liver triacylglycerol (TAG) fatty acid (FA) proportions (mass % of total FA identified; mean ± SD; n=5) that exhibited significant differences in striped bass (*Morone saxatilis*) between November (initial) 2016 and March 2017 sampling events.

Muscle TAG fatty acids showed little difference between the initial and final sampling events (Figure 15). 18:1n-9 stood out as one of the only fatty acids in muscle tissue to differ significantly between November to March in TAG with proportions of the FA profile being ~29 % in the initial samples and ~32 % (Lower pond) and ~33 % (Middle pond) in March (Figure 15; ANOVA, $F(2, 12) = 27.298$, $p < 0.003$). In addition, there was significantly less 18:2n-6 over the winter (ANOVA, $F(2, 12) = 126.681$, $p < 0.003$) in TAG muscle samples as proportions were ~6 % in November compared to ~5 % (Lower pond) and ~4 % (Middle pond) in March (Figure 15).

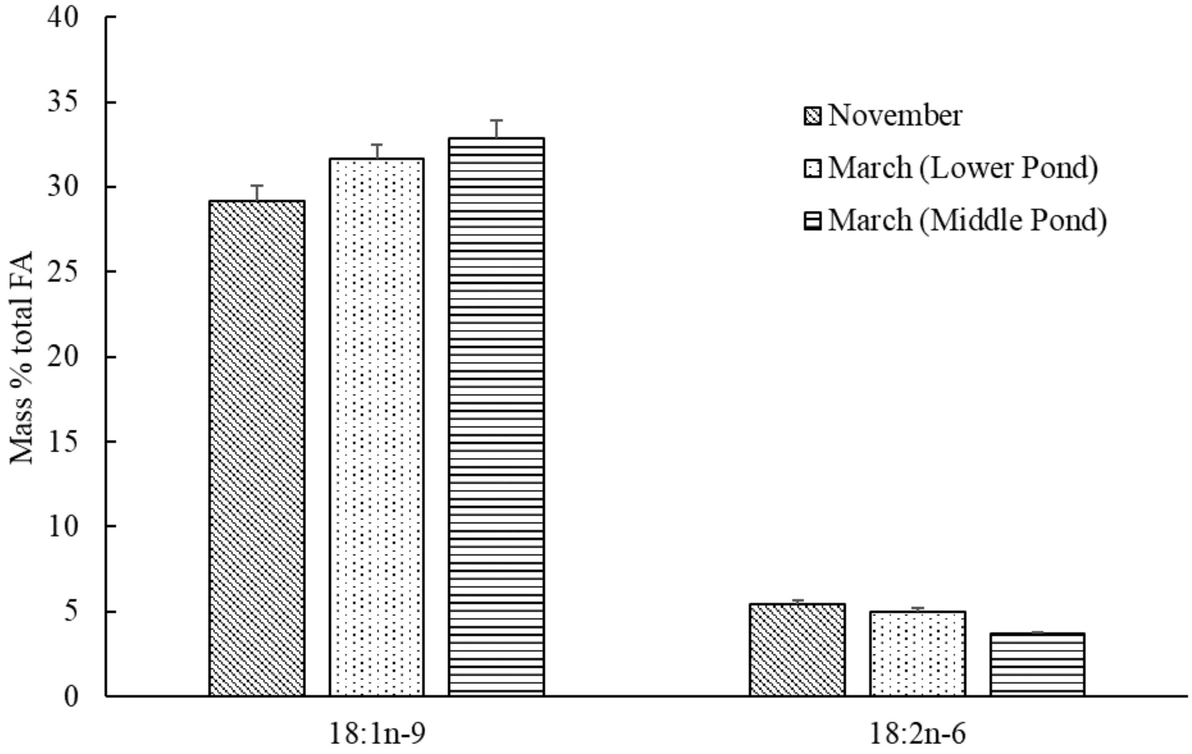

Figure 15. Muscle triacylglycerol (TAG) fatty acid (FA) proportions (mass % total FA identified; mean ± SD; n=5) that exhibited significant differences in striped bass (*Morone saxatilis*) in November (initial) 2016 and March 2017 sampling events.

3.3.4 Fatty acid profiles in phospholipids (PE and PC classes) in muscle and liver

Relatively large differences were evident in the FA profiles of samples taken in November and in March in the phospholipid classes (PE and PC) in both muscle and liver tissue compared to the differences in TAG. Six FA in both PE and PC were significantly different in March relative to samples taken in November: 18:2n-6, 20:2n-6 and 22:6n-3 in PE, 22:5n-3 in PC, as well as 22:6n-3 in both PE and PC (Figures 16 and 17).

The largest difference in muscle PE (Figure 16) occurred in 16:0 (ANOVA, *F*(2, 12) = 24.430, $p < 0.003$), 18:0 (ANOVA, *F*(2, 12) = 22.466, $p < 0.003$), 20:1n-9 (ANOVA, *F*(2, 12) = 25.108, $p < 0.003$) and 18:2n-6 (ANOVA, *F*(2, 12) = 45.319, $p < 0.003$). Of the FA in Figure 16, all FA except for 18:0 and 22:6n-3 were present in higher proportions in the winter samples. For 18:0 proportions were significantly greater in November (~10 %) compared to ~6 % and ~7 % in March (Lower and Middle ponds, respectively). Proportions of 22:6n-3 were also lower in the March samples, as the value for initial samples was 33.5% and 26.7% (Lower pond) and 26.6% (Middle pond) in March (ANOVA, *F*(2, 12) = 22.171, $p < 0.003$).

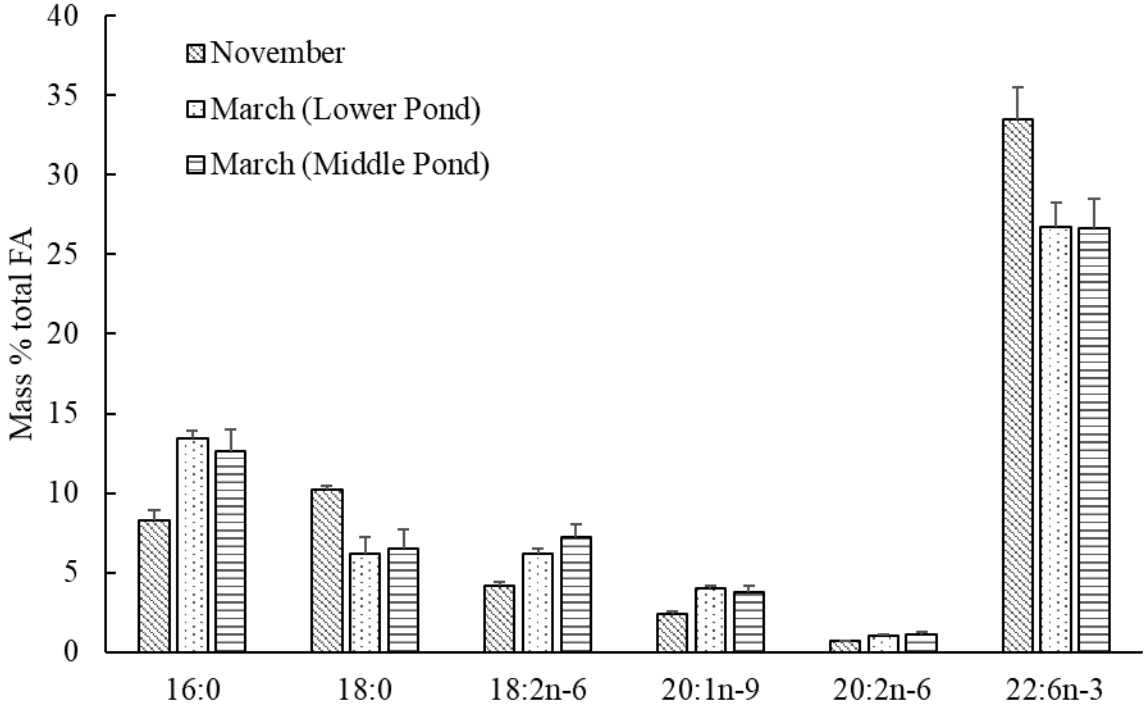

Figure 16. Muscle phosphatidylethanolamine (PE) fatty acid (FA) proportions (mass % total FA identified; mean ± SD; n=5) that exhibited significant differences in striped bass (*Morone saxatilis*) in November (initial) 2016 and March 2017 sampling events.

Significant variation in the composition of muscle PC (Figure 17) can be seen in six individual fatty acids, including 14:0 (ANOVA, *F*(2, 12) = 23.162, $p < 0.003$), 16:0 (ANOVA, *F*(2, 12) = 37.831, $p < 0.003$), 18:1n-9 (ANOVA *F*(2, 12) = 40.385, $p < 0.003$), 18:1n-7 (ANOVA, *F*(2, 12) = 22.947, $p < 0.003$), 22:5n-3 (ANOVA, *F*(2, 12) = 15.542, $p < 0.003$), and 22:6n-3 (ANOVA, *F*(2, 12) = 17.151, $p < 0.003$). Notable differences were greater proportions in 16:0 (~22 % initial to ~32 % in the Lower Pond and ~ 33 % in the Middle pond) and 22:5n-3 (from ~2 % to ~3 % and ~2 %, in the Lower and Middle ponds, respectively) in March fish. Lower proportions of 18:1n-9 and 22:6n-3 were observed in the initial fish (18:1n-9: ~15 % and 22:6n-3: ~24 %) compared to those sampled in March (18:1n-9: ~9 % Lower pond and ~10 % Middle pond; 22:6n-3: ~18 % Lower pond and ~16 % Middle pond).

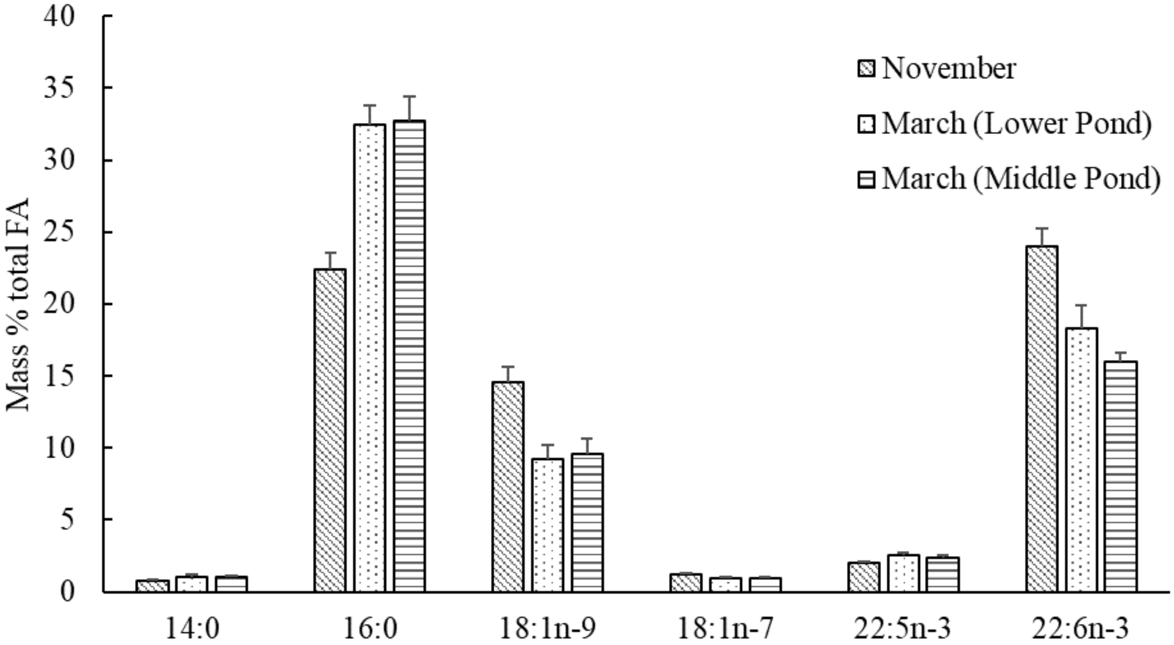

Figure 17. Muscle phosphatidylcholine (PC) fatty acid (FA) proportions (mass % total FA identified; mean ± SD; n=5) that exhibited differences in striped bass (*Morone saxatilis*) between November (initial) 2016 and March 2017 sampling events.

3.4 Discussion

This trial consisted of a combination of two stressors, thermal acclimation and reliance on endogenous reserves, that a temperate YOY striped bass will encounter during their first winter. Although in an aquaculture setting, the fish included in this trial were exposed to a natural winter environment as food was not offered, therefore considered scarce, and temperature, as well as oxygen, were not manipulated for the entirety of the winter. It has been suggested that a combination of stressors, as opposed to a single one, is the cause of large mortality events in YOY temperate fish (Schultz and Conover, 1999;

Hurst, 2007). In the context of both of the stresses highlighted in this paper, lipids may play a role in survival; however, different classes of lipids fill specific adaptation niches, as discussed below.

3.4.1 Thermal acclimation overwinter

In the context of thermal acclimation, the typical role of phospholipids (PL) is to maintain fluidity and structure of an organism's cells. It is common to observe an increase in PE concentrations in tissues, such as the brain and liver of fish (Dey et al. 1993; Farkas et al. 2001; Reynolds et al., 2014), as exhibited by the overwintering YOY striped bass. An increase of conic shaped PE in comparison to cylindrical shaped PC is thought to be one of the ways cellular membranes succeed in increasing fluidity during thermal changes, or periods of cold temperatures (Dey et al., 1993; Buda et al., 1994; Farkas et al., 2001; Reynolds et al., 2014). Thus, these higher concentrations of PE likely point to a particular thermal acclimation mechanism utilized at the time of sampling by YOY striped bass in our trial.

The proportions of 18:0 and 22:6 in PE and 18:1 and 22:6 in PC were lower in March when compared to initial November samples (Figures 16 and 17). The general reaction of PL to a temperature reduction is to increase these FA, as seen in various warm and cold adapted fish species (Dey et al., 1993; Farkas et al., 2001). Reconstruction of the lipid structures in phospholipid bilayers occurs in colder temperatures as an adaptation to maintain cell fluidity (Dey et al., 1993; Liu et al., 2019). In many FW and marine fish, the phospholipid classes, including PE and PC, contained higher proportions of specific monoenic and polyenic FA after adaptation to colder water temperatures ranging from approximately 5-10 °C (Farkas et al., 2001). This increase in monoenic/polyenic species (such as 18:1/22:6) seems to be a universal response of phospholipid remodelling of liver, brain, and gill samples of multiple cold water (5-10 ºC) adapted FW and marine fish species when compared to warm water (20-27 ºC) adapted species (Day et al., 1993; Farkas et al., 2001). This phenomenon was explored by Buda et al. (1994), as they observed an accumulation of monounsaturated/polyunsaturated fatty acids in brain phospholipids of carp (*Cyprinus carpio*) when acclimatized to 5 ºC water (Buda et al., 1994). In that work, Buda et al. (1994) presented evidence that this observed change was due to the direct effect of temperature on the phospholipids, since the fish had previously ceased feeding during the trial as temperatures decreased to below 10 °C. Although these studies suggest that an increase in monoenic/polyenic FA species occurs in colder temperatures, it is difficult to make comparisons with the present work since we only examined the effect of temperature on the FA profiles in muscle tissue.

Even so, the results of the muscle phospholipid FA provided an interesting finding as we observed the opposite of what the literature suggests will happen to fish phospholipids (PL) during cold-water adaptation. The lower proportion of both 22:6 and 18:0 in PE and 22:6 and 18:1 in PC overwinter does not seem like a coincidence. It appears those FA are being selectively removed; however, the reason for this remains unclear. We also observed a significant increase in 16:0, a saturated FA, in both PL classes in the muscle tissue (Figure 16 and 17). Multiple components including cholesterol, proteins, and the composition of the phospholipid class, can all impact the fluidity of a cellular membrane (Bell et al., 1986); therefore, an increase in saturated FA in the muscle could indicate that another component, or lipid class, is assuming the role of regulating membrane fluidity.

Variations in muscle PL and FA were not the only apparent adaptations we observed in overwintering fish in response to thermal change. We also observed higher proportions of TAG in fish overwinter (Table 13) that may be attributed to a change in another lipid class. Cholesterol (CHOL) is known to be of use in thermal adaptation purposes in cellular membranes and could be another component regulating fluidity. Multiple studies support the decrease of CHOL during periods of colder temperatures (Wodtke, 1978; Sørensen, 1993; Aaronson et al., 1982; Crokett, 1998; Reynolds et al., 2014). Of these, Crokett (1998) explains in her review that there are three responses of CHOL to temperature in ectothermic organisms, the first of which is a rise in CHOL levels with increasing temperature. Moreover, Sørensen (1993) determined that the amount of CHOL in the plasma membrane of flounder (*Platichthys flesus*) acclimated to 2.5 °C was higher than those acclimated to warmer water (20 °C). These papers indicate that lower concentrations of CHOL are expected in tissues at colder temperatures as a way to maintain membrane fluidity. If this is the case in YOY striped bass thermal change adaptation, an increase in muscle TAG could be explained by the decrease in the proportion of muscle CHOL. However, an increase in CHOL would not explain the greater TAG concentration (mg g^{-1}) in muscle of fish sampled in March that were also observed (Table 13).

Alternatively, it may be that this higher concentration in muscle TAG in March samples is an actual response to thermal stress and the decrease in temperature. A rise in TAG was observed in the muscle tissue of temperate eelpout (*Zosrces viviparous*) in relation to a decrease in PL and CHOL during a laboratory study comparing various changes in lipid concentrations in liver and muscle at different temperatures (Brodte et al., 2008). This accumulation of TAG was at its highest in *Z. viviparous* muscle tissue at 4 °C compared to fish held at 6, 12, and 18 °C (Brodte et al., 2008). The increase was attributed to a shift to creation of storage lipids, or TAG, as water temperatures decrease (Brodte et al., 2008). However, in that study, fish were being fed for the duration of the four-month study and it is therefore impossible to eliminate the possibility that that increase in TAG was facilitated by the consumption of dietary lipids. Observed higher concentrations of muscle TAG seems counterintuitive but may be filling some unknown role in adaptation to temperature.

3.4.2 Dependency on endogenous lipid reserves overwinter

Fish were not fed during the length of the overwinter trial and at the time of sampling fish had been without offered food for 131 days. From previous laboratory trials and observations, striped bass reared at the Dal-AC following the same procedures as this work show little interest in offered feed once temperatures are reduced to 5 °C (Duston et al., unpub. data). Nonetheless, there is evidence of Hudson River striped bass YOY cohorts feeding at a reduced rate throughout the months of five consecutive winters in temperatures ranging from 2 to 10.4 °C (Hurst and Conover, 2001). Striped bass from this population typically consume benthic invertebrates (predominantly *Gammarus sp*.) and small fish prey during the winter (Hurst and Conover, 2001). Therefore, while unlikely, we cannot eliminate the possibility of opportunistic food consumption throughout the winter even though at the time of sampling, guts were thought to be empty.

During times of prolonged food deprivation or scarcity, energetic demands of an organism are satisfied by the oxidation of lipids, predominantly triacylglycerols (TAG) (Adams, 1999). Our findings, with higher TAG proportions in the liver and higher concentrations in the muscle of overwintering striped bass (Table 13), are at odds with much prior research that indicates that various temperate species, including Atlantic silverside, rainbow trout (*Oncorhynchus mykiss*), and white bass (*Morone chrysops)* experience a winter deficit, or decrease in energy reserves throughout the winter (Schultz and Conover, 1997; Biro et al., 2004; Eckmayer and Margraf, 2004). Striped bass have also demonstrated this winter deficit. Hurst and Conover (2003) report that Hudson River YOY striped bass experienced a loss of up to 21 % of their total energy content in addition to 50 % depletion of neutral lipid (storage lipids/TAG) over several winters (1993-1998) when winter collected samples were compared to pre-winter samples.

We were able to identify one study that supports an increase of total hepatic (liver) lipid in striped bass when acclimated to colder temperatures. Specifically, Stone and Sidell (1981) determined that the TL in the liver was much higher in fingerling striped bass from the Hudson River population when acclimated to 5 °C compared to 25 °C, with TL making up 40 % and 25 % of the liver dry weight, respectively. While difficult to compare with our results since we did not present the proportion of TL in the liver as dry weights, we did observe significantly higher proportions of TAG in the liver tissue of fish sampled in March. Carbohydrates of fingerling striped bass from the Hudson River were determined to be 7 % of dry weight (liver) when acclimated to 5 °C, whereas at 25 °C carbohydrates represented 18 % of dry weight of the tissue (Stone and Sidell, 1981). This led to the conclusion that at colder temperatures, carbohydrates function as the primary source of energy in the liver as opposed to lipids (Stone and Sidell, 1981). Therefore, the YOY striped bass in the present work may have been sparing TAG and catabolizing carbohydrates in their place for energy requirements during periods of cold temperatures.

The significantly higher concentrations of TL we found in the muscle in March can be the result of two possible changes in the tissue: 1) a proportionately greater reduction in another component in the muscle; or 2) a real increase in lipid. A slight increase in the proportion of muscle lipid relative to total tissue mass was observed in starved adult carp (*Cyprinus carpio*) overwinter placed in monitoring net cages (Lake Kasumingaura, Japan) (Takeuchi et al., 1987). This was the result of a decrease in muscle protein when fish were stocked in lake water with winter (water) temperatures that ranged from 2.2-11.5 °C for a period of 128 days from November to March. Lipid concentrations in the visceral fat also decreased in these fish; therefore, the authors concluded that adult carp predominantly utilize muscle protein and visceral lipid as energy sources during winter starvation compared to muscle lipid (Takeuchi et al., 1987), which could explain the increase in muscle lipid proportions in the YOY striped bass that we observe. However, a decrease in water content in the tissue could also have the same effect. We did not determine the proximate composition of either tissue, so are unable to confirm which component might have decreased to cause the increase in lipid. The possibility of the higher concentrations of TL after the winter in the YOY striped bass being due to a real increase in lipid deposition in the tissue seems unlikely. Fish that are able to feed overwinter are able to maintain their supply of lipid reserves and even show an increase in growth (Kooka et al., 2009). However, we assume fish were not feeding for the duration of our overwinter trial, and thus, there is no source of additional TAG and TL to deposit in muscle.

Of the six fatty acids that demonstrated significant changes in the liver tissue over the winter, the increases in proportions of 18:2n-6 and 18:3n-3 are among the most interesting. Several species of marine fish, including red and black sea bream (*Pagnus major* and *Spondyliosoma cantharus*), opaleye (*Girella nigricans*) and striped mullet (*Mugil cephalus*) (Yamada et al., 1980), have only a limited ability to convert 18:3n-3 into

22:6n-3 during starvation. Hixon et al. (2014) also discussed the very limited ability of the marine Atlantic cod (*Gadus morhua*) to desaturate and elongate 18:3n-3 when fed experimental diets containing high proportions of n-3 FA. In general, marine fish have very low ability to convert 18:3 to long chain polyunsaturated fatty acid (LC PUFA) due to potential low activity or deficiencies of 5-desaturase and therefore require the inclusion of fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in their diets (Watanabe, 1982; Lall et al., 2000; Tocher, 2003). Indeed, Webster and Lovell (1990) reported that striped bass larvae seemed incapable of elongation and desaturation of FA chains into long chain essential FA when fed a diet of brine shrimp containing high levels of linolenic acid (18:2n-6). In contrast, FW species are able to meet their LC PUFA requirements by elongation and desaturation of dietary C18 fatty acids, specifically 18:3n-3 and 18:2n-6, to form 20:5n-3 (EPA), 22:6n-3 (DHA) and 20:4n-6 (Lall et al., 2000; Tocher, 2003, 2010). It may be that although typically classified as FW fish, YOY striped bass have lipid metabolism more similar to marine fish. This slight to non-existent ability to transform 18 C FA into essential FA (20:5n-n and 22:6n-3) amongst marine species could support the observed high level of 18 C FA in the liver of YOY striped bass, explained by the retention or accumulation of essential 18 C FA in the face of starvation. Dietary requirements for striped bass in culture are not very well known; however, studies investigating hybrid striped bass *(Morone chrysops x Morone saxitilis)* have indicated that their nutritional needs reflect those of marine fishes (Webster and Lovell, 1990; Clawson and Lovell, 1992; Nematipour and Gatlin, 1992, 1993; Barry, 2017). Thus, they should have little capacity to use those precursors to form long chain essential FA, leading to an accumulation of 18:2n-6 and 18:3n-3, as seen in March samples (Figure 14). Further, Barry and Trushenski (2019) have recently reported that hybrid striped bass diets should include n-3 and n-6 LC PUFA in order to make sure essential fatty acid requirements are met, since they are unable to do so with only C18 PUFA. This finding is important to consider when formulating diets for striped bass, to ensure they are provided with the appropriate essential FA. Future research is needed to know the full extent of dietary FA required by striped bass and trials monitoring their ability to convert C18 into the necessary LC PUFA is a step towards accomplishing this.

3.5 Conclusions

YOY striped bass were unique in their utilization of lipid reserves in the muscle and liver when held overwinter in FW ponds. Fish in the current work demonstrated higher proportions of TAG in the liver and muscle during the winter, suggesting they must be relying on other sources for energy and cellular membrane structure, such as CHOL, during the winter months. The observed higher concentrations overwinter in liver and muscle TAG is a possible adaptation process by which YOY striped bass combat winter temperatures, although it is unclear how this adaptation might be of benefit. PE and PC FA did not respond as expected to thermal adaptation. Perhaps a way forward is to determine the tissues that striped bass YOY rely on for energy sources during the winter, as our results are inconclusive.

Further investigation into the overwinter adaptations of Shubenacadie River population derived YOY striped bass is necessary. If lipids were not being used as the primary source of energy, then another constituent of the liver and muscle tissue must have been; however, we were unable to identify the constituent. Proximate composition analysis of energy storage tissues, such as liver, muscle, and visceral fat, of YOY striped bass will be necessary in order to determine the mechanisms these fish employ to survive the stresses of winter conditions.

CHAPTER 4 Conclusion

This Chapter ties together the findings of both overwinter survival trials presented in Chapter 2 with the results of the analytical lipid analysis work reported on in Chapter 3 to summarize the state of knowledge regarding YOY striped bass culture requirements and sources of mortality during their first winter. In addition, this chapter also presents some of the limitations of the research conducted, and points to future research that could be conducted next to improve our understanding of YOY striped bass culture requirements during their first winter. In addressing the above, this Chapter also considers the larger question of the potential role of striped bass culture in NS's aquaculture industry.

4.1 Research background and objectives

If striped bass are to become a more important part of NS's aquaculture sector in the future, high rates of YOY overwinter mortality when placed in a potential commercial grow-out setting, such as FW ponds, need to be prevented. Previous attempts to determine the viability of rearing striped bass for aquaculture in Canada have been reported (Cairns, 1999). More recent efforts led by researchers based in Dalhousie's Faculty of Agriculture and their industry collaborator based at NRFF, have encountered high rates of mortality amongst YOY fish when they have been placed into FW pond-based culture settings in the fall of their first year. It is in this context that this thesis set out to:

1) Better quantify the influence of pre-winter diet and body size on overwinter survival rates of YOY striped bass;
2) Gain knowledge on whether pond environment has an impact on winter survival through the use of various ponds at both NRFF and other locations;

3) Include the timing of sampling as an aspect of the experimental design to estimate more accurately the time of death of YOY striped bass in FW ponds;
4) Determine the extent to which YOY striped bass were exhausting their lipid reserves prior to the end of the winter season in FW ponds; and
5) Evaluate the concentration of TAG, PC, and PE in muscle tissue and TAG in the liver of YOY striped bass along with the FA profiles of each lipid class to determine the differences in each in fish collected in late fall (November) and mid-winter (March).

4.2 Main findings

Major results from the two consecutive overwinter trials undertaken and reported on in Chapter 2 are:

1) Rearing YOY striped bass on a tailored diet did not significantly impact survival when compared to the commercial diet, as no difference was observed between survival rates of striped bass reared on either diet;
2) Size-dependent mortality was only observed in one of the two winters, indicating that size may not be the only, or most influential, cause of mortality in YOY striped bass during the winter; and
3) Pond environment appeared to have an impact on winter survival as mortality rates were at times significantly different between rearing ponds. However, measured parameters such as temperature and DO remained adequate for the survival of YOY striped bass in ponds used in both overwinter trials.

As reported in Chapter 3, major results of the analyses of lipid content from muscle and liver tissue taken from pre-winter (November) and overwinter (March) YOY striped bass, along with the fatty acid profiles of phospholipid classes found in the muscle tissues of YOY striped bass exposed to winter rearing conditions include:

1) Energy requirements of YOY striped bass did not seem to be met by the catabolism of TAG during the winter, as seen by significantly higher concentrations and proportions of this lipid class in the muscle and higher proportions in the liver tissue of animals later in the winter than at the onset of winter;
2) Concentrations of structural lipid classes of PL, such as PE in the muscle tissue taken from YOY striped bass prior to winter (November) were found to be significantly higher than in tissues taken from animals that had survived late into the winter.
3) The proportions of specific FA in the muscle PL, such as 18:0 and 22:6 in PE and 18:1 and 22:6 in PC, were lower in March when compared to initial November samples.

4.2.1 Synthesis of main findings

Although many tested experimental factors yielded inconclusive results, those indicated above do offer insight into the possible adaptations and environmental requirements of YOY striped bass in the winter, which are discussed below.

There was no significant difference between the number of fish surviving the winter having been reared on the commercial diet compared to the tailored diet; however, more fish from the commercial diet treatment did survive the winter and this indicates that diet had no real impact on the survival of YOY striped bass in our trials. Although the number of survivors in the 2017/18 trial was quite low, this result suggests that YOY striped bass are able to survive on a diet that is more specific to salmonid species. That YOY striped bass were able to survive on a diet that was formulated to replicate a typical salmonid diet is not entirely surprising as striped bass have been, and are, regularly reared on commercial salmonid feeds (Cairns, 1999; J. Duston unpub. data). For example, at the Dal-AC, and once transferred to NRFF in the spring (ca. 1 year of age), striped bass are generally fed a low fat commercially available salmonid diet, such as EWOS Vita (14 % crude fat) or Corey Aquasea (between 16-18 % crude fat) and seem to thrive. The dietary requirements of striped bass in culture, and in particular the requirements of juvenile striped bass, are not well described in the literature. However, there is indirect evidence that suggests that their dietary needs may be similar to marine fish species based on efforts to understand the dietary requirements of hybrid striped bass *(Morone chrysops x Morone saxatilis*) in culture (Webster and Lovell, 1990; Clawson and Lovell, 1992; Nematipour and Gatlin, 1992, 1993; Barry, 2017; Barry and Trushenski, 2019; see Chapter 3, section *3.4.2*). Specifically, it has been reported that hybrid striped bass are unable to meet the LC PUFA demand with only a supply of C18 fatty acids, or PUFA, and instead need to acquire essential fatty acid requirements through their diet (see Chapter 3, section *3.4.2;* Barry and Trushenski, 2019), unlike salmonid species. Though the dietary requirements of hybrid striped bass align more closely to those of marine fish, the instances of relatively high rates of survival of some of the YOY fish in the two trials has practical implications for future potential striped bass farmers who all will have ready access to a variety of commercially prepared salmonid feeds.

It can also be concluded that YOY fish within our study did not seem to be dying during the winter due to exhaustion of lipid reserves, as has been previously described as a possible cause of mortality seen in YOY temperate fish species (see Chapter 1, section *1.7.1*). Energetic lipid (TAG) concentrations, as well as the proportion that TAG represented of TL, were higher in muscle tissue taken from fish in the late winter than in November. Though differences in the concentration of TAG in liver tissue were not as marked (see Table 13), the proportion of TL that was comprised of TAG in the liver tissue was higher in fish sampled in the late winter when compared to those sampled in November. Though not formally quantified, the amount of visceral fat present in the abdomens of fish sampled throughout the winter was considerable, possibly indicating that the YOY striped bass were not mobilizing energy from these reserves. Body size is also related to the discussion of lipid reserves since larger fish were also dying during the winters of the two trials. This is particularly evident in results of the 2017/18 trial (see Table 7) and suggests that starvation is not the main cause of mortality since larger fish tend to have larger reserves when heading into winter (Post and Parkinson, 2001; see Chapter 1, section *1.7.1*).

The proportions of 18:0 and 22:6 in PE and 18:1 and 22:6 in PC were lower in March when compared to initial November samples, which is the opposite of what is expected in fish overwinter (see Chapter 3; Dey et al., 1993, Farkas et al., 2001). There was also a significant increase in 16:0, which is a saturated FA, in both PE and PC of the muscle tissue. It is possible that another component of the muscle, such as cholesterol or proteins, were impacting cellular fluidity (Bell et al., 1986); therefore, an increase in saturated FA in the muscle could indicate that another component, or lipid class, is assuming the role of regulating membrane fluidity during the winter.

Pond environment had an effect on survival, something that was particularly evident in the 2016/17 trial, which led to the inclusion of more ponds into the experimental design in 2017/18. Despite the initial indication that there was a pond effect on survival from

2016/17, when results of all trials are considered, the effect of the pond environment is inconclusive for two reasons. First, survival results obtained from the same pond across both trials are markedly different from one another. This was particularly evident from trials conducted in the Middle pond located at NRFF. In 2016/17, survival rate for all fish at the end of the winter (April) in this pond was ca. 86 %. The following year, there was zero survival in this same pond in April. Survival in general at NRFF was much lower over the second winter trial, as only 5 % of fish sampled were alive during the last sampling event. The difference in survival between these two years makes it difficult to conclude that the source of mortality was indeed something about the pond environment, especially when temperature, DO concentrations, and other measured water quality parameters all appeared to remain within what is thought to be acceptable to YOY striped bass (see Chapter 2). Second, the addition of two other ponds (Dixon and Faulkner) did not provide clear evidence that other locations can support the survival of YOY striped bass throughout the winter. Striped bass placed into the Faulkner pond experienced near 100 % mortality as of January of 2018, whereas striped bass placed in the Dixon pond had high survival rates as of March 2018. However, one month later (April 2018), survival of striped bass that remained in the Dixon pond (all had been reared on the diet formulated to mimic a commercial feed) was extremely low, with only two fish still alive. It is unclear if this low survival is due to particularly harsh winter conditions, or possible stress experienced as a result of sampling that occurred in March or based on some completely unidentified source of mortality. Though the specific mechanism(s) of mortality that could arise from an attribute of the pond environment remains elusive, more research into possible overwinter sites for the culture of striped bass should be undertaken along with expanding the suite of characteristics of the pond environment(s) that are monitored to more fully understand the limitations of pond culture for this species.

4.3 Limitations

Certain questions could not be fully explored due to errors in experimental design. Most notably, we were unable to investigate the impact of varying proportions of saturated and unsaturated fat inclusion in pre-winter diets in 2016/17, as the diets formulated and fed to fish in the fall of 2016 were subsequently found to not differ significantly in this regard. Prior work by Kelly and Kohler (1999) had identified a positive effect on the survival of juvenile striped bass when subjected to rapid, or acute, temperature decreases when fish were fed diets with elevated levels of unsaturated fats.

The impact of individual size on overwinter mortality was another question that could not be fully investigated, especially during the 2016/17 overwinter trial. Over the fall, the YOY striped bass grew very uniformly in size while being reared in the lab prior to their transfer to NRFF, making the grading into two distinct size classes (small and large) impossible. Instead we compared the FL of individual survivors and mortalities during all sampling events and found that there was a size-dependent mortality trend (longer FL individuals survived longer than their shorter counterparts) during the 2016/17 trial (Figure 11). It should be noted that some of the individual fish length data underlying this finding have uncertainties associated with them. Some mortalities collected from cages were severely decomposed making accurate measurement of FL difficult. In addition, caudal fins on some individuals were partially eroded at the time of sampling, forcing the estimation of FL rather than a more accurate measurement.

Most of the overwinter experimental work on YOY striped bass conducted to date by researchers at the Dal-AC has used ponds located at NRFF whose characteristics may present unrecognized challenges to overwinter YOY survival. On the one hand, DO concentration and temperature data (see Figures 8, 9, 12 and 13) from these ponds all appear to remain within levels required for survival. In addition, commercial grow-out of age 1+ striped bass in these ponds has been successful with low levels of mortality reported for these older animals and informal overwintering experiments with salmonids (brook trout and Arctic char) also result in low levels of mortality. However, persistent high rates of overwinter mortality amongst YOY striped bass in some or all ponds used for trials at NRFF suggest that one or more characteristics of the ponds' environment may be a source of mortality. Recognizing this, two new ponds located away from NRFF were incorporated into the 2017/18 overwinter experimental work. Though survival in these ponds was also generally poor, additional sites should be considered in future work in order to more fully understand the environmental requirements of YOY striped bass in the winter.

In this regard, it should be noted that all ponds included in this thesis work were FW, providing no insight into whether a certain level of salinity may be beneficial to YOY striped bass during the winter. The specific salinity sought out by YOY striped bass in the wild during winter is unclear, predominantly because the overwinter location of Shubenacadie River striped bass in unknown. It is possible though that the preferred winter habitat of YOY striped bass is in estuarine or marine waters (Bradford and Cook, 2004; Bradford et al., 2014). An important next step (discussed further below) would be to investigate the role that salinity plays in overwinter survival amongst cultured YOY striped bass derived from the Shubenacadie River population.

The final noteworthy limitations to this research are the lack of either whole body, or muscle and liver tissue specific proximate analysis conducted alongside the lipid analysis and presenting results on a wet-weight basis. Without the information provided by proximate composition of tissue samples used for detailed lipid analysis taken from animals in November and March of the 2016/17 trial, I was unable to assess absolute levels of TL along with other major constituents of these tissues. This made the interpretation of some of the results of the lipid analyses more challenging. For example, I observed higher concentration of TL in the muscle tissue of fish sampled in March (2017) than in tissues of fish sampled in November (2016), but am unable to conclude whether this was due to an actual increase in lipid within the tissue, or a decrease in another component. Having a better grasp of why certain lipid changes are occurring during the winter will provide better information into the mechanisms YOY striped bass use to survive the winter.

When taking these limitations into account, the work presented in this thesis adds to the current state of knowledge on overwinter sources of mortality in YOY striped bass, but much more work remains to be undertaken. We have gained the understanding that size may not be the most critical requirement for survival during the first winter, and instead other factors seem to play an important role, though what these factors may be remains elusive. It is also possible that conditions that vary as a result of either the duration or severity of winter could be important as rates of mortality were often highest late in the winters in the trials conducted (see Figure 10; Tables 7 and 9). Finally, the identification of future research directions based on results of the work undertaken is an important outcome of this thesis research, as it highlights a path forward towards understanding the overwinter process of YOY striped bass.

4.4 Future research recommendations

It is clear that further research is required to understand the main source, or sources, of overwinter mortality in YOY striped bass placed in a pond culture environment and how these sources of mortality might be mitigated. Without this knowledge, it will remain challenging to grow the production of this species in this particular setting in the NS aquaculture industry. Below I identify and briefly discuss three main directions of possible future research that could be undertaken to advance understanding of overwinter mortality in YOY striped bass in a pond culture environment. They are 1) the salinity of water in the overwinter environment, 2) the use of visceral fat during the winter, and 3) better understanding the effect of body size of the striped bass before entering the overwintering environment on survival.

4.4.1 Salinity

Studies have indicated that the level of salinity in a YOY striped bass' environment can affect overwinter survival and that levels of salinity, such as 15 ppt, can positively influence a fish's osmoregulatory abilities when subjected to a thermal decline (see Chapter 1, section *1.7.3*; Hurst and Conover, 2002). For example, survival rates of Hudson River striped bass held in tanks with salinities ranging from 5 to 30 ppt were higher than those held in FW when subjected to temperatures decreases from 7 °C to -1 and -2 °C (Hurst and Conover, 2002). Since YOY striped bass in our studies were kept in a FW environment for the entirety of the winter, their osmoregulatory ability could have been negatively impacted and been a potential cause, or contributor to mortality. Further, the possibility of a link between osmoregulation and metabolic demand exists. Hurst and Conover (2002) suggest that osmoregulating in FW can place a higher metabolic demand on individuals. This is significant due to the relationship between body size and size of somatic energy reserves of YOY fish entering the winter since the size of these energy reserves increases with body size.

The potential role of salinity on survival of YOY striped bass is also indicated as a potentially important factor from what little is known from the life history of the wild Shubenacadie River population from which the animals in culture in NS, and in the present work, are derived. As mentioned, while adult striped bass from the Shubenacadie River are known to migrate to, and overwinter within the FW environment of Grand Lake, YOY fish have not been documented in this environment. It is speculated that they remain in areas that are estuarine, or marine (Bradford and Cook, 2004; Bradford et al., 2014), suggesting that a saline environment may be important in the survival of these younger animals. Multiple ideas come to mind to assess the potential of salinity on overwinter survival of YOY striped bass. Laboratory trials could be conducted in which small groups of fish are held in tanks with different levels of salinity, ranging between 0 and 35 ppt (average salinity of ocean water) while temperature profiles are varied over the experiment to mimic aquatic winter conditions in NS. Osmoregulatory stress could be measured by determining the osmolality of blood plasma samples collected from fish held at different salinities and temperatures. Alternatively, if a suitable pond site can be located that has a natural level of salinity, is also reasonably accessible from Truro, and can be permitted for temporary introduction of striped bass, the experimental overwinter trials could be conducted there. However, such sites may prove to be difficult to identify and secure access to. Finally, determining the overwinter habitat of wild YOY striped bass native to the Shubenacadie River would also be of value in understanding the preferences of these fish during the winter, and which could then be used to inform future experimental work with this species.

4.4.2 Use of visceral fat during the winter

Although we did not analyze the visceral fat when taking samples from fish either before, during, or after the overwinter trials, we did observe the presence of visceral fat within the body cavity of the fish during each sampling event where live fish were euthanized and dissected. It may be that YOY striped bass rely on the energy reserves present in this area of the body, more so than they do lipids stored in the liver and muscle, but to date this has not been assessed. In gizzard shad (*Dorosoma cepedianum*), liver tissue is thought to perhaps be an "emergency energy store" (Fetzer et al., 2011, p. 1467) tissue that is only drawn upon during times of extreme need, while visceral fat serves as the main source of TAG supporting energetic needs (White et al., 1987; Fetzer et al., 2011). Fetzer et al. (2011) investigated the adaptations of gizzard shad during starvation and cold temperature stress and concluded that fish were perhaps unable to access their energy reserves in the visceral tissue. In response to this, gizzard shad included in the Fetzer et al. (2011) trial needed to turn to their emergency reserves in the liver and as a result were functioning as if they had exhausted their visceral fat-based energy reserves (Fetzer et al., 2011). In light of this, it is possible that the conditions in the ponds in which our trials were conducted were not cold, or dire, enough in the later winter to require YOY striped bass to draw upon their liver-based lipid reserves, and for this reason we saw no significant difference in this tissue's TAG concentrations overwinter. Instead, up until the point when we sampled tissue of fish in March of 2017, they may have been primarily utilizing their visceral fat energy reserves to survive.

Moreover, Arctic char and brook trout were also placed in experimental cages in NRFF's Lower and Middle ponds from December 2016 to April 2017. Although these fish were left until the ice thawed in April, all fish (n=15 per species in each pond) were alive at the time of sampling. Though liver and muscle tissues were not analyzed for their TL or lipid class content, we observed an exhaustion of all visceral fat within the body cavities of these fish. This depletion of visceral fat reserves suggests that salmonids and striped bass might respond to overwinter stresses (food deprivation and cold-water thermal stress) in different ways, as visceral fat remained in the body cavity of YOY striped bass overwinter when sampled in March. If liver and muscle tissues are the last resorts used overwinter by YOY striped bass to meet energetic demands, and we only sampled survivors, it is possible that the TAG in these tissues had not yet started to be metabolized by fish sampled in March of 2017. In the future, it would be beneficial to sample fish as they were nearing death in order to determine if moribund fish were using alternative sources of energy reserves. While this was not possible in our trials as fish could not be observed through the ice covering the ponds, it is something that could be incorporated into a laboratory study, since fish are more easily monitored and accessed.

4.4.3 Pre-winter size requirements

Although not a part of my research, earlier work in the lab at the Dal-AC found that YOY striped bass exposed to temperature conditions that mimicked FW pond winter conditions experience very low rates of mortality (J. Duston unpub. data). However, it is unclear whether these lab-based findings are good analogs for winter conditions in wilder, less controlled, environments such as those found in the ponds. A more robust look into the impacts of size dependent mortality in a captive setting could be beneficial in order to determine the minimum size (FL) requirement for YOY striped bass being placed in pond settings prior to winter to survive. As discussed in Chapter 2, the FL of wild striped bass at the onset of winter can vary between year classes (Bradford et al., 2001; Douglas et al., 2003; Duston et al., 2018). Indeed, even when comparing our two trials, larger size as determined by FL was not a sure indicator of winter survival. However, if striped bass are to be incorporated in the aquaculture industry of NS, a size threshold that meets limited mortality during the winter year after year is required to make the venture worthwhile.

4.5 Final conclusions

Within this thesis, I have not identified the source, or sources, of mortality experienced by YOY striped bass in FW ponds. Nevertheless, I have been able to further the understanding of experimental factors that seemingly do not contribute to overwinter mortality. Most notably, the temperature and DO concentrations that YOY striped bass experienced in our trials were not factors that impacted mortality. Further, I was able to provide some valuable data for both temperature and DO tolerances for Shubenacadie River striped bass of this year class. Additionally, we determined that feeding a diet tailored to striped bass' dietary requirements does not appear to influence survival. This finding is of importance, especially if the culture of this species is to develop in the future, as farmers would be able obtain acceptable feed that is already commercially available.

Future areas of research were also highlighted through the synthesis of both the 2016/17 and 2017/18 trials that will assist in answering the questions of whether striped bass are a viable species to include in the aquaculture industry of NS. Of these, the most pressing are understanding the role salinity plays in overwinter survival of YOY Shubenacadie River striped bass, determining whether visceral fat is an important energy storage area for winter metabolic demands, and identifying a minimum FL threshold that needs to be met for repeatable high levels of overwinter survival while in an aquaculture setting.

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APPENDIX A – Bathymetric maps of all North River Fish Farms Ltd. ponds

Bathymetric data was collected for all three of the ponds located at NRFF (45º29’53” N, 63º12’31” W). The Lower and Middle ponds were surveyed on August 29, 2017 (Figures A2 and A3), whereas the Upper pond was surveyed on June 18, 2018 (Figure A1). Methods for collecting the data were the same for both dates and included the use of a depth sensor in addition to a weighted down rope. This rope was attached to a weight, which allowed it to sink straight down to the bottom. When pulled taught, the depth was able to be recorded at the surface as the rope contained marked measurements at every meter and 10 cm.

The collection of all measurements and circumference waypoints was done from a kayak since it was impossible to walk around the entirety of all three ponds. We began by collecting the circumference waypoints by using a GPS device approximately every 5 meters. The numbers of these waypoints were recorded as circumference data only. Next, sticks with high visibility tape were placed in the ground at approximately every 10 meters on one end of the pond. Sticks were then placed on the other side of the pond (typically from a kayak) in an approximate straight line to create transects. Along these transects, depth measurements were taken multiple times, usually every 5 m or so. A waypoint was taken for each recorded measurement. At times, depth was taken with both the sensor and rope in order to confirm measurements. Finally, depth recorded near the edges of the ponds and in very shallow areas were taken only with the rope since the depth sensor was unable to read these depths. Once data was collected and compiled, maps were created using ArcGIS by both Jennifer Strang (GIS Analyst, GIS Centre, Dalhousie University) and Caitlin Cunningham (Interdisciplinary PhD candidate, Dalhousie University).

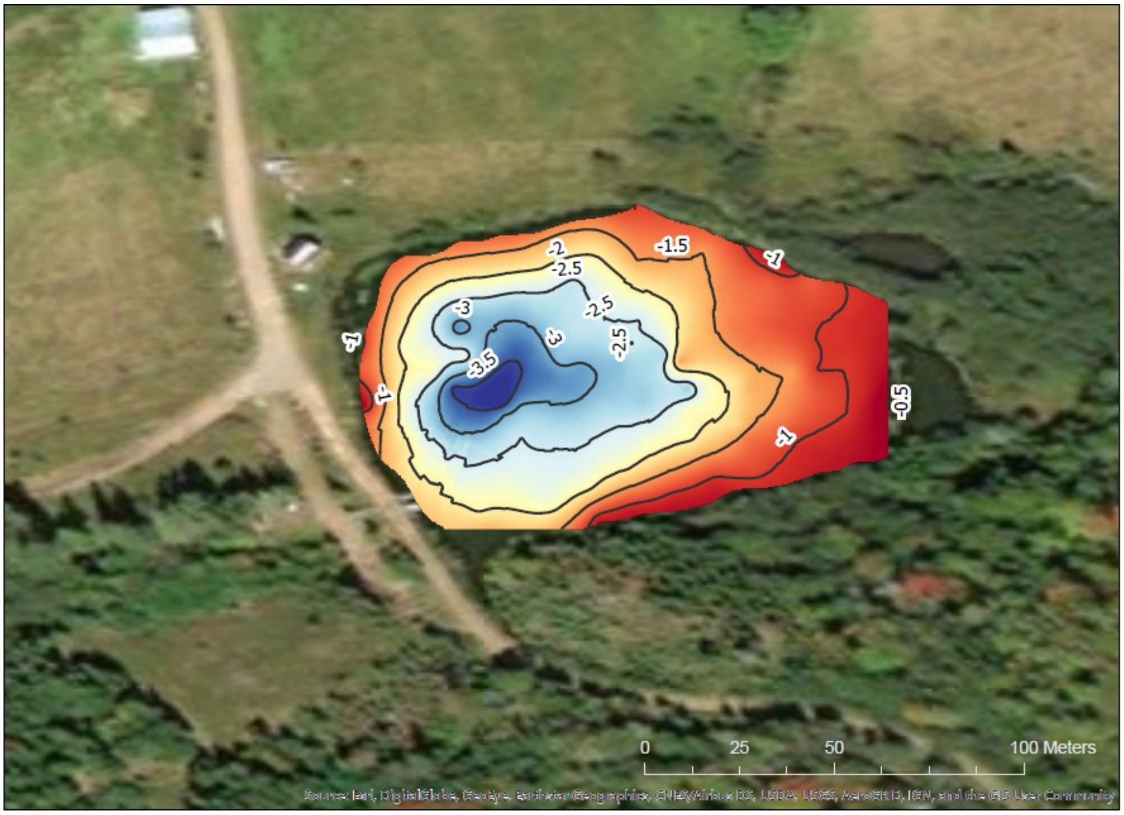

Figure A1. Bathymetric map of the Upper pond at North River Fish Farms Ltd. Measurements (m) were taken on June 18, 2018.

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure A2. Bathymetric map of the Middle pond at North River Fish Farms Ltd. Measurements (m) were taken on August 29, 2017.

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure A3. Bathymetric map of the Lower pond at North River Fish Farms Ltd. Measurements (m) were taken on August 29, 2017.

APPENDIX B – Proportions of fatty acids included in the 2016/17 trial diets

Diets used in the pre-winter feeding period of the 2016/17 trial were analyzed at the Marine Lipids Lab at Dalhousie University on October 20th, 2016 to determine the inclusion (%) of fatty acids present in the diets. The ratios of fish oil:poultry fat in the three diets were as follows: (Diet 1) 7% fish oil:1% poultry fat, (Diet 2) 4% fish oil: 4% poultry fat and (Diet 3) 1% fish oil: 7% poultry fat.

Table B1. Fatty acid inclusion (%) in all diets used during the pre-winter feeding period from September 20 to the end of October 2016 prior to transferring young-of-the-year striped bass (*Morone saxatilis*) to freshwater ponds at North River Fish Farms Ltd. for the duration of the winter.

Fatty Acid	**Diet 1**	**Diet 2**	**Diet 3**
12:0	0.10	0.09	0.07
13:0	0.03	0.02	0.01
i-14:0	0.03	0.02	0.02
14:0	6.30	4.49	2.71
14:1n-9	0.09	0.07	0.06
14:1n-7	0.02	0.03	0.02
14:1n-5	0.06	0.09	0.11
i-15:0	0.14	0.11	0.07
ai-15:0	0.04	0.03	0.02
15:0	0.40	0.31	0.22
i16:0	0.06	0.05	0.04
16:0	18.15	19.11	19.76
16:1n-11	0.28	0.24	0.17
16:1n-9	0.21	0.26	0.30
16:1n-7	7.25	6.17	5.00
16:1n-5	0.15	0.12	0.09
17:1(a)	0.03	0.03	0.03
i-17:0	0.13	0.10	0.07
16:2n-6	0.14	0.09	0.05
ai-17:0	0.11	0.11	0.11
17:1(b)	0.14	0.11	0.08

16:2n-4	0.87	0.59	0.30
17:0	0.36	0.30	0.25
16:3n-4	0.97	0.66	0.33
17:1	0.18	0.18	0.16
16:4n-3	0.06	0.04	0.03
16:4n-1	0.77	0.55	0.32
18:0	3.39	3.84	4.25
18:1n-13	0.03	0.04	0.04
18:1n-11	0.06	0.08	0.08
18:1n-9	17.34	22.71	28.38
18:1n-7	2.58	2.39	2.21
18:1n-5	0.08	0.07	0.08
18:2n-7	0.07	0.05	0.07
18:2n-6	10.50	13.93	17.57
18:2n-4	0.23	0.16	0.09
18:3n-6	0.18	0.15	0.14
18:3n-4	0.26	0.18	0.11
18:3n-3	1.95	2.02	2.15
18:3n-1	0.10	0.07	0.04
18:4n-3	1.14	0.83	0.50
18:4n-1	0.18	0.15	0.07
20:0	0.27	0.25	0.22
20:1n-11	0.11	0.11	0.10
20:1n-9	0.94	0.81	0.67
20:1n-7	0.16	0.12	0.08
20:2n-9	0.00	0.00	0.00
20:2n-6	0.17	0.17	0.15
20:3n-6	0.19	0.17	0.14
20:4n-6	0.89	0.74	0.58
20:3n-3	0.06	0.06	0.04
20:4n-3	0.57	0.40	0.22
20:5n-3	9.61	6.94	4.19
22:0	0.19	0.17	0.15
22:1n-11	0.57	0.53	0.43
22:1n-9	0.17	0.16	0.13
22:1n-7	0.06	0.05	0.03

22:2n-6	0.01	0.00	0.01
21:5n-3	0.40	0.28	0.16
23:0	0.05	0.04	0.03
22:4n-6	0.15	0.13	0.10
22:5n-6	0.28	0.22	0.16
22:4n-3	0.05	0.04	0.02
22:5n-3	1.56	1.13	0.68
24:0	0.16	0.14	0.13
22:6n-3	7.67	6.42	5.10
24:1n-9	0.34	0.28	0.25

APPENDIX C – Proximate composition of the 2017/18 trial diets

Table C1. Proximate composition proportions (%) of moisture, fat, protein, and other of both the commercial and tailored diets fed to young-of-the-year striped bass (*Morone saxatilis*) during the 2017/18 overwinter trial from September to November 2017. Samples from pellets of two different sizes (2 and 3 mm) were taken as feed was pelleted into two sizes to accommodate fish growth. Analysis was accomplished at the Dalhousie Agricultural Campus Nutrition Laboratory on October 16, 2017.

Diet treatment	**% Moisture**	**% Fat**	**% Protein**	**% Other**
Tailored 2mm	4.95	18.21	46.28	30.56
Tailored 3mm	5.33	18.08	46.49	30.10
Commercial 2mm	8.67	17.38	48.08	25.87
Commercial 3mm	6.79	17.53	48.38	27.30

APPENDIX D – Proportions of fatty acids included in the 2017/18 trial diets

Diets used during the 2017/18 trial were analyzed by the Marine Lipids Lab at Dalhousie University on October 16, 2017 to determine the proportion of inclusion (%) of fatty acids present in the diets. Both diets were made into two pellet sizes, 2 mm and 3 mm, and the fatty acid inclusion for both are listed below.

Table D1. Complete fatty acid inclusion (%) in both the tailored (2 mm and 3 mm) and commercial (2 mm and 3 mm) diets used during pre-winter feeding period from September 14 to October 31, 2017 prior to the transfer of young-of-the-year striped bass (*Morone saxatilis*) to freshwater ponds for the duration of the winter.

Fatty acid	**Tailored 2mm**	**Tailored 3mm**	**Commercial 2mm**	**Commercial 3mm**
12:0	0.15	0.14	0.06	0.06
13:0	0.06	0.06	0.02	0.02
i-14:0	0.06	0.06	0.02	0.02
14:0	8.33	8.02	2.64	2.66
14:1n-9	0.11	0.10	0.03	0.03
14:1n-7	0.04	0.04	0.01	0.01
14:1n-5	0.06	0.06	0.03	0.03
i-15:0	0.25	0.24	0.08	0.08
ai-15:0	0.07	0.07	0.03	0.03
15:0	0.64	0.62	0.22	0.22
i16:0	0.08	0.08	0.02	0.02
16:0	20.76	20.50	12.03	11.95
16:1n-11	0.40	0.39	0.12	0.12
16:1n-9	0.24	0.24	0.14	0.14
16:1n-7	8.82	8.57	3.26	3.28
16:1n-5	0.18	0.17	0.07	0.07
17:1(a)	0.02	0.03	0.00	0.00
i-17:0	0.21	0.20	0.06	0.05
16:2n-6	0.19	0.18	0.05	0.05
ai-17:0	0.11	0.10	0.07	0.07
17:1(b)	0.21	0.19	0.08	0.07
16:2n-4	1.23	1.19	0.38	0.39

17:0	0.52	0.50	0.21	0.21
16:3n-4	1.48	1.42	0.46	0.47
17:1	0.17	0.14	0.10	0.10
16:4n-3	0.12	0.11	0.04	0.04
16:4n-1	1.30	1.25	0.40	0.40
18:0	4.05	3.99	2.92	2.89
18:1n-13	0.04	0.03	0.01	0.01
18:1n-11	0.05	0.04	0.02	0.02
18:1n-9	10.10	11.29	38.83	38.85
18:1n-7	2.67	2.65	2.57	2.54
18:1n-5	0.10	0.09	0.06	0.05
18:2d5,1	0.13	0.10	0.03	0.03
18:2n-7	0.06	0.05	0.06	0.07
18:2n-6	5.16	5.77	18.79	18.66
18:2n-4	0.32	0.30	0.11	0.11
18:3n-6	0.23	0.21	0.08	0.08
18:3n-4	0.28	0.25	0.09	0.09
18:3n-3	1.32	1.45	5.07	5.07
18:3n-1	0.08	0.08	0.02	0.02
18:4n-3	1.97	1.92	0.61	0.62
18:4n-1	0.17	0.16	0.05	0.05
20:0	0.30	0.32	0.46	0.44
20:1n-11	0.12	0.13	0.07	0.06
20:1n-9	0.83	0.86	0.90	0.89
20:1n-7	0.22	0.22	0.08	0.08
20:2n-9	0.06	0.05	0.02	0.02
20:2n-6	0.16	0.15	0.10	0.11
20:3n-6	0.17	0.20	0.08	0.06
20:4n-6	1.09	1.06	0.41	0.42
20:3n-3	0.11	0.08	0.03	0.03
20:4n-3	0.70	0.66	0.22	0.22
20:5n-3	10.92	10.64	3.30	3.41
22:0	0.15	0.15	0.26	0.25
22:1n-11	0.52	0.56	0.19	0.19
22:1n-9	0.14	0.14	0.07	0.07
22:1n-7	0.09	0.09	0.04	0.04

22:2n-6	0.03	0.03	0.02	0.02
21:5n-3	0.53	0.51	0.16	0.16
22:4n-6	0.16	0.16	0.07	0.08
22:5n-6	0.35	0.34	0.10	0.11
22:4n-3	0.08	0.08	0.03	0.02
22:5n-3	1.56	1.53	0.49	0.50
24:0	0.00	0.00	0.00	0.00
22:6n-3	8.75	8.59	2.74	2.86
24:1n-9	0.37	0.36	0.18	0.18

APPENDIX E – Complete water quality parameter tables for all ponds analyzed by AGAT Laboratories during the 2017/18 trial

Table E1. Water quality parameters for all sampling events during the 2017/18 overwinter trial in the Upper pond of North River Fish Farms Ltd.

Parameter	**Unit**	**RD**	**11/22/201**	**02/10/201**	**03/03/201**	**04/10/201**
pH			7.18	6.57	6.88	6.97
Reactive Silica as SiO2	mg/L	0.5	4.8	2.2	2.0	2.6
Chloride	mg/L	1	4.00	4.00	3.00	3.00
Fluoride	mg/L	0.12	<0.12	<0.12	<0.12	<0.12
Sulphate	mg/L	2	3.00	<2	2.00	2.00
Alkalinity	mg/L	5	20.00	7.00	8.00	10.00
True Color	TCU	5	35.00	19.00	7.00	36.00
Turbidity	NTU	0.1	5.6	11.9	4.1	6.8
Electrical Conductivity	umho/c	1	52.00	37.00	39.00	40.00
Nitrate + Nitrite as N	mg/L	0.05	0.10	0.13	0.08	0.11
Nitrate as N	mg/L	0.05	0.10	0.13	0.08	0.11
Nitrite as N	mg/L	0.05	<0.05	<0.05	<0.05	<0.05
Ammonia as N	mg/L	0.03	0.03	0.05	<0.03	0.05
Total Organic Carbon	mg/L	0.5	6.3	5.0	3.8	5.4
Ortho-Phosphate as P	mg/L	0.01	<0.01	0.02	<0.01	0.01
Total Sodium	mg/L	0.1	2.5	2.6	2.5	2.2
Total Potassium	mg/L	0.1	0.7	0.9	0.7	0.6
Total Calcium	mg/L	0.1	4.2	3.3	3.7	3.5
Total Magnesium	mg/L	0.1	1.3	1.1	1.2	1.1
Bicarb. Alkalinity (CaCO3)	mg/L	5	20.00	7.00	8.00	10.00
Carb. Alkalinity (CaCO3)	mg/L	10	<10	<10	<10	<10
Hydroxide	mg/L	5	<5	<5	<5	<5
Calculated TDS	mg/L	1	29.00	18.00	19.00	19.00
Hardness	mg/L		15.8	12.8	14.2	13.3
Langelier Index (@20C)	NA		-2.43	-3.58	-3.16	-3.00
Langelier Index (@ 4C)	NA		-2.75	-3.90	-3.48	-3.32
Saturation pH (@ 20C)	NA		9.61	10.1	10.0	9.97
Saturation pH (@ 4C)	NA		9.93	10.5	10.4	10.3
Anion Sum	me/L		0.58	0.26	0.29	0.33
Cation sum	me/L		0.47	0.45	0.44	0.41
% Difference/ Ion Balance (NS)	%		10.4	26.4	20.7	10.7
Total Aluminum	ug/L	5	132.00	346.00	194.00	210.00

Total Antimony	ug/L	2	<2	<2	<2	<2
Total Arsenic	ug/L	2	<2	<2	<2	<2
Total Barium	ug/L	5	8.00	8.00	7.00	7.00
Total Beryllium	ug/L	2	<2	<2	<2	<2
Total Bismuth	ug/L	2	<2	<2	<2	<2
Total Boron	ug/L	5	<5	<5	<5	<5
Total Cadmium	ug/L	0.01	<0.017	0.021	0.017	<0.017
Total Chromium	ug/L	1	<1	<1	<1	<1
Total Cobalt	ug/L	1	<1	<1	<1	<1
Total Copper	ug/L	1	2.00	<1	<1	<1
Total Iron	ug/L	50	317.00	420.00	297.00	258.00
Total Lead	ug/L	0.5	<0.5	<0.5	<0.5	<0.5
Total Manganese	ug/L	2	43.00	56.00	60.00	43.00
Total Molybdenum	ug/L	2	<2	<2	<2	<2
Total Nickel	ug/L	2	<2	<2	<2	<2
Total Phosphorous	mg/L	0.02	0.05	0.05	0.05	0.03
Total Selenium	ug/L	1	1.00	<1	<1	<1
Total Silver	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Strontium	ug/L	5	9.00	8.00	8.00	8.00
Total Thallium	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Tin	ug/L	2	<2	<2	<2	<2
Total Titanium	ug/L	2	5.00	10.00	6.00	6.00
Total Uranium	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Vanadium	ug/L	2	<2	<2	<2	<2
Total Zinc	ug/L	5	<5	<5	<5	<5

Table E2. Water quality parameters for all sampling events during the 2017/18 overwinter trial in the Middle pond of North River Fish Farms Ltd.

Parameter	**Unit**	**RDL**	**11/22/201**	**02/10/201**	**03/03/201**	**04/10/201**
pH			7.12	6.54	6.71	6.94
Reactive Silica as SiO2	mg/L	0.5	3.8	3.0	2.3	2.5
Chloride	mg/L	1	4.00	4.00	4.00	3.00
Fluoride	mg/L	0.12	<0.12	<0.12	<0.12	<0.12
Sulphate	mg/L	2	3.00	2.00	2.00	2.00
Alkalinity	mg/L	5	14.00	7.00	7.00	8.00
True Color	TCU	5	38.00	23.00	10.00	36.00
Turbidity	NTU	0.1	3.3	17.3	5.5	5.3
Electrical Conductivity	umho/c	1	46.00	39.00	39.00	37.00
Nitrate + Nitrite as N	mg/L	0.05	0.18	0.16	0.13	0.17
Nitrate as N	mg/L	0.05	0.18	0.16	0.13	0.17
Nitrite as N	mg/L	0.05	<0.05	<0.05	<0.05	<0.05
Ammonia as N	mg/L	0.03	0.11	0.08	0.06	0.05
Total Organic Carbon	mg/L	0.5	6.4	5.1	4.1	5.1
Ortho-Phosphate as P	mg/L	0.01	<0.01	0.02	<0.01	0.02
Total Sodium	mg/L	0.1	3.0	2.5	2.6	2.4
Total Potassium	mg/L	0.1	1.0	0.8	0.7	0.6
Total Calcium	mg/L	0.1	3.9	3.0	3.2	3.3
Total Magnesium	mg/L	0.1	2.2	1.0	1.0	1.1
Bicarb. Alkalinity (CaCO3)	mg/L	5	14.00	7.00	7.00	8.00
Carb. Alkalinity (CaCO3)	mg/L	10	<10	<10	<10	<10
Hydroxide	mg/L	5	<5	<5	<5	<5
Calculated TDS	mg/L	1	27.00	19.00	19.00	19.00
Hardness	mg/L		18.8	11.6	12.1	12.8
Langelier Index(@20C)	NA		-2.67	-3.65	-3.45	-3.15
Langelier Index (@ 4C)	NA		-2.99	-3.97	-3.77	-3.47
Saturation pH (@ 20C)	NA		9.79	10.2	10.2	10.1
Saturation pH (@ 4C)	NA		10.1	10.5	10.5	10.4
Anion Sum	me/L		0.47	0.31	0.30	0.30
Cation sum	me/L		0.57	0.45	0.42	0.41
% Difference/ Ion Balance (NS)	%		10.1	18.8	16.1	15.8
Total Aluminum	ug/L	5	120.00	518.00	245.00	193.00
Total Antimony	ug/L	2	<2	<2	<2	<2
Total Arsenic	ug/L	2	3.00	<2	<2	<2
Total Barium	ug/L	5	26.00	20.00	20.00	16.00

Total Beryllium	ug/L	2	<2	<2	<2	<2
Total Bismuth	ug/L	2	<2	<2	<2	<2
Total Boron	ug/L	5	15.00	<5	<5	<5
Total Cadmium	ug/L	0.01 –	<0.017	<0.017	<0.017	<0.017
Total Chromium	ug/L	1	<1	<1	<1	<1
Total Cobalt	ug/L	1	<1	<1	<1	<1
Total Copper	ug/L	1	2.00	1.00	2.00	1.00
Total Iron	ug/L	50	505.00	543.00	356.00	241.00
Total Lead	ug/L	0.5	1.0	<0.5	<0.5	<0.5
Total Manganese	ug/L	2	62.00	77.00	80.00	52.00
Total Molybdenum	ug/L	2	<2	<2	<2	<2
Total Nickel	ug/L	2	<2	<2	<2	<2
Total Phosphorous	mg/L	0.02	0.07	0.04	0.04	0.03
Total Selenium	ug/L	1	2.00	<1	<1	<1
Total Silver	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Strontium	ug/L	5	15.00	9.00	9.00	9.00
Total Thallium	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Tin	ug/L	2	<2	<2	<2	<2
Total Titanium	ug/L	2	5.00	19.00	7.00	5.00
Total Uranium	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Vanadium	ug/L	2	3.00	<2	<2	<2
Total Zinc	ug/L	5	<5	5.00	<5	7.00

Table E3. Water quality parameters for all sampling events during the 2017/18 overwinter trial in the Lower pond of North River Fish Farms Ltd.

Parameter	Unit	RD	11/22/201	02/10/201	03/03/201	04/10/201
pH			7.11	6.59	6.96	7.06
Reactive Silica as SiO2	mg/L	0.5	3.4	2.5	3.2	2.4
Chloride	mg/L	1	3.00	4.00	19.00	6.00
Fluoride	mg/L	0.12	<0.12	<0.12	<0.12	<0.12
Sulphate	mg/L	2	2.00	<2	5.00	3.00
Alkalinity	mg/L	5	16.00	6.00	16.00	8.00
True Color	TCU	5	67.00	22.00	7.00	39.00
Turbidity	NTU	0.1	14.5	9.8	13.9	7.2
Electrical Conductivity	umho/c	1	46.00	35.00	125.00	37.00
Nitrate + Nitrite as N	mg/L	0.05	0.17	0.14	0.44	0.11
Nitrate as N	mg/L	0.05	0.17	0.14	0.44	0.11
Nitrite as N	mg/L	0.05	<0.05	<0.05	<0.05	<0.05
Ammonia as N	mg/L	0.03	0.05	0.06	<0.03	0.09
Total Organic Carbon	mg/L	0.5	6.9	5.4	3.4	4.8
Ortho-Phosphate as P	mg/L	0.01	<0.01	0.02	<0.01	0.02
Total Sodium	mg/L	0.1	2.9	2.5	13.9	2.3
Total Potassium	mg/L	0.1	0.6	0.7	1.1	0.6
Total Calcium	mg/L	0.1	3.2	3.0	7.7	2.9
Total Magnesium	mg/L	0.1	1.0	0.9	1.5	1.0
Bicarb. Alkalinity (CaCO3)	mg/L	5	16.00	6.00	16.00	8.00
Carb. Alkalinity (CaCO3)	mg/L	10	<10	<10	<10	<10
Hydroxide	mg/L	5	<5	<5	<5	<5
Calculated TDS	mg/L	1	25.00	16.00	61.00	22.00
Hardness	mg/L		12.1	11.2	25.4	11.4
Langelier Index (@20C)	NA		-2.71	-3.66	-2.52	-3.10
Langelier Index (@ 4C)	NA		-3.03	-3.98	-2.84	-3.42
Saturation pH (@ 20C)	NA		9.82	10.3	9.48	10.2
Saturation pH (@ 4C)	NA		10.1	10.6	9.80	10.5
Anion Sum	me/L		0.46	0.24	0.99	0.40
Cation sum	me/L		0.48	0.40	1.24	0.39
% Difference/ Ion Balance (NS)	%		2.1	24.7	11.0	1.4
Total Aluminum	ug/L	5	221.00	244.00	667.00	207.00
Total Antimony	ug/L	2	<2	<2	<2	<2
Total Arsenic	ug/L	2	2.00	<2	<2	<2
Total Barium	ug/L	5	32.00	19.00	30.00	19.00

Total Beryllium	ug/L	2	<2	<2	<2	<2
Total Bismuth	ug/L	2	<2	<2	<2	<2
Total Boron	ug/L	5	7.00	<5	8.00	<5
Total Cadmium	ug/L	0.01	<0.017	<0.017	<0.017	<0.017
Total Chromium	ug/L	1	<1	<1	1.00	<1
Total Cobalt	ug/L	1	<1	<1	<1	<1
Total Copper	ug/L	1	3.00	2.00	<1	1.00
Total Iron	ug/L	50	1,610.00	427.00	542.00	357.00
Total Lead	ug/L	0.5	<0.5	<0.5	<0.5	<0.5
Total Manganese	ug/L	2	242.00	122.00	59.00	116.00
Total Molybdenum	ug/L	2	<2	<2	<2	<2
Total Nickel	ug/L	2	<2	<2	<2	<2
Total Phosphorous	mg/L	0.02	0.09	0.04	0.04	0.04
Total Selenium	ug/L	1	1.00	<1	<1	<1
Total Silver	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Strontium	ug/L	5	10.00	12.00	30.00	9.00
Total Thallium	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Tin	ug/L	2	<2	<2	<2	<2
Total Titanium	ug/L	2	7.00	7.00	12.00	5.00
Total Uranium	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Vanadium	ug/L	2	3.00	<2	<2	<2
Total Zinc	ug/L	5	<5	<5	<5	<5

Table E4. Water quality parameters for all sampling events during the 2017/18 overwinter trial in the Dixon pond.

Parameter	Unit	RD	11/22/201	02/02/201	03/03/201	04/10/201
pH			7.50	7.35	6.75	7.57
Reactive Silica as SiO2	mg/L	0.5	7.0	2.4	2.1	2.0
Chloride	mg/L	1	76.00	52	4.00	34.00
Fluoride	mg/L	0.12	<0.12	<0.12	<0.12	<0.12
Sulphate	mg/L	2	13.00	11	2.00	8.00
Alkalinity	mg/L	5	48.00	42	7.00	30.00
True Color	TCU	5	<5	5	11.00	19.00
Turbidity	NTU	0.1	1.6	3.1	5.5	3.8
Electrical Conductivity	umho/c	1	377.00	288	39.00	191.00
Nitrate + Nitrite as N	mg/L	0.05	<0.05	0.29	0.14	0.22
Nitrate as N	mg/L	0.05	<0.05	0.29	0.14	0.22
Nitrite as N	mg/L	0.05	<0.05	<0.05	<0.05	<0.05
Ammonia as N	mg/L	0.03	<0.03	<0.03	0.08	0.04
Total Organic Carbon	mg/L	0.5	1.4	3.2	4.2	0.9
Ortho-Phosphate as P	mg/L	0.01	<0.01	0.02	<0.01	0.01
Total Sodium	mg/L	0.1	40.5	35.2	2.5	20.4
Total Potassium	mg/L	0.1	1.7	1.6	0.7	1.1
Total Calcium	mg/L	0.1	21.0	15.5	3.1	10.8
Total Magnesium	mg/L	0.1	4.2	3.1	1.0	2.4
Bicarb. Alkalinity (CaCO3)	mg/L	5	48.00	42	7.00	30.00
Carb. Alkalinity (CaCO3)	mg/L	10	<10	<10	<10	<10
Hydroxide	mg/L	5	<5	<5	<5	<5
Calculated TDS	mg/L	1	185.00	145	19.00	96.00
Hardness	mg/L		69.7	51.5	11.9	36.9
Langelier Index (@20C)	NA		-1.11	-1.44	-3.43	-1.50
Langelier Index (@ 4C)	NA		-1.43	-1.76	-3.75	-1.82
Saturation pH (@ 20C)	NA		8.61	8.79	10.2	9.07
Saturation pH (@ 4C)	NA		8.93	9.11	10.5	9.39
Anion Sum	me/L		3.37	2.56	0.30	1.74
Cation sum	me/L		3.21	2.61	0.41	1.67
% Difference/ Ion Balance (NS)	%		2.5	1.1	14.6	2.2
Total Aluminum	ug/L	5	34.00	34	186.00	70.00
Total Antimony	ug/L	2	<2	<2	<2	<2
Total Arsenic	ug/L	2	<2	<2	<2	<2
Total Barium	ug/L	5	77.00	46	22.00	38.00

Total Beryllium	ug/L	2	<2	<2	<2	<2
Total Bismuth	ug/L	2	<2	<2	<2	<2
Total Boron	ug/L	5	15.00	17	<5	11.00
Total Cadmium	ug/L	0.01 –	<0.017	0.022	<0.017	<0.017
Total Chromium	ug/L	1	<1	1	<1	<1
Total Cobalt	ug/L	1	<1	<1	<1	<1
Total Copper	ug/L	1	3.00	1	2.00	<1
Total Iron	ug/L	50	132.00	154	365.00	121.00
Total Lead	ug/L	0.5	<0.5	1.4	<0.5	<0.5
Total Manganese	ug/L	2	15.00	46	156.00	25.00
Total Molybdenum	ug/L	2	<2	<2	<2	<2
Total Nickel	ug/L	2	<2	<2	<2	<2
Total Phosphorous	mg/L	0.02	0.04	0.03	0.03	0.03
Total Selenium	ug/L	1	1.00	<1	<1	<1
Total Silver	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Strontium	ug/L	5	74.00	63	10.00	43.00
Total Thallium	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Tin	ug/L	2	<2	<2	<2	<2
Total Titanium	ug/L	2	<2	<2	5.00	<2
Total Uranium	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Vanadium	ug/L	2	<2	<2	<2	<2
Total Zinc	ug/L	5	<5	<5	<5	<5

Table E5. Water quality parameters for all sampling events during the 2017/18 overwinter trial in the Faulkner pond.

Parameter	Unit	RD	11/22/201	02/02/201	03/02/201	04/10/201
pH			7.54	7.47	7.45	7.67
Reactive Silica as SiO2	mg/L	0.5	11.0	2.3	6.4	8.0
Chloride	mg/L	1	34.00	29	38.00	37.00
Fluoride	mg/L	0.12	<0.12	<0.12	<0.12	<0.12
Sulphate	mg/L	2	6.00	6	6.00	6.00
Alkalinity	mg/L	5	61.00	52	51.00	51.00
True Color	TCU	5	<5	7	<5	15.00
Turbidity	NTU	0.1	1.1	2	1.6	2.0
Electrical Conductivity	umho/c	1	244.00	231	253.00	240.00
Nitrate + Nitrite as N	mg/L	0.05	0.64	0.83	0.71	0.81
Nitrate as N	mg/L	0.05	0.59	0.83	0.71	0.81
Nitrite as N	mg/L	0.05	0.05	<0.05	<0.05	<0.05
Ammonia as N	mg/L	0.03	0.10	<0.03	<0.03	0.06
Total Organic Carbon	mg/L	0.5	<0.5	0.7	1.9	<0.5
Ortho-Phosphate as P	mg/L	0.01	<0.01	0.03	0.01	0.01
Total Sodium	mg/L	0.1	24.3	20.6	23.1	19.0
Total Potassium	mg/L	0.1	1.3	1.1	1.4	1.2
Total Calcium	mg/L	0.1	16.6	20.2	22.4	20.7
Total Magnesium	mg/L	0.1	3.6	4.3	5.3	4.9
Bicarb. Alkalinity (CaCO3)	mg/L	5	61.00	52	51.00	51.00
Carb. Alkalinity (CaCO3)	mg/L	10	<10	<10	<10	<10
Hydroxide	mg/L	5	<5	<5	<5	<5
Calculated TDS	mg/L	1	126.00	116	130.00	124.00
Hardness	mg/L		56.3	68.1	77.8	71.9
Langelier Index (@20C)	NA		-1.05	-1.1	-1.09	-0.90
Langelier Index (@ 4C)	NA		-1.37	-1.42	-1.41	-1.22
Saturation pH (@ 20C)	NA		8.59	8.57	8.54	8.57
Saturation pH (@ 4C)	NA		8.91	8.89	8.86	8.89
Anion Sum	me/L		2.35	2.04	2.27	2.25
Cation sum	me/L		2.23	2.29	2.60	2.33
% Difference/ Ion Balance (NS)	%		2.6	5.8	6.9	1.8
Total Aluminum	ug/L	5	13.00	10	23.00	164.00
Total Antimony	ug/L	2	<2	<2	<2	<2
Total Arsenic	ug/L	2	<2	<2	<2	<2
Total Barium	ug/L	5	35.00	28	34.00	33.00

Total Beryllium	ug/L	2	<2	<2	<2	<2
Total Bismuth	ug/L	2	<2	<2	<2	<2
Total Boron	ug/L	5	6.00	7	9.00	8.00
Total Cadmium	ug/L	0.01	<0.017	<0.017	<0.017	<0.017
Total Chromium	ug/L	1	<1	<1	<1	<1
Total Cobalt	ug/L	1	<1	<1	<1	<1
Total Copper	ug/L	1	3.00	<1	<1	<1
Total Iron	ug/L	50	126.00	111	129.00	265.00
Total Lead	ug/L	0.5	<0.5	<0.5	<0.5	0.5
Total Manganese	ug/L	2	20.00	21	27.00	42.00
Total Molybdenum	ug/L	2	<2	<2	<2	<2
Total Nickel	ug/L	2	<2	<2	<2	<2
Total Phosphorous	mg/L	0.02	0.05	0.03	0.04	0.04
Total Selenium	ug/L	1	1.00	<1	<1	<1
Total Silver	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Strontium	ug/L	5	83.00	106	125.00	106.00
Total Thallium	ug/L	0.1	<0.1	<0.1	<0.1	<0.1
Total Tin	ug/L	2	<2	<2	<2	<2
Total Titanium	ug/L	2	<2	<2	<2	4.00
Total Uranium	ug/L	0.1	<0.1	0.1	0.1	0.2
Total Vanadium	ug/L	2	<2	<2	<2	<2
Total Zinc	ug/L	5	<5	<5	<5	<5

APPENDIX F – Fatty acid profiles of all lipid classes analyzed during the 2016/17 overwinter trial

Table F1. Proportions (%) of individual fatty acids from liver triacylglycerols (TAG) included in statistical analysis. Samples were collected from young-of-the-year striped bass (*Morone saxatilis*) from the Diet 1 treatment (7% fish oil and 1 % poultry fat) in November 2016 (initial samples) and March 2017 (overwinter samples) as part of the 2016/17 overwinter trial taking place in the Lower and Middle ponds at North River Fish Farms Ltd.

Sample ID #	**Fatty acid proportions (%)**												
	14:0	**16:0**	**16:1n-7**	**18:0**	**18:1n-9**	**18:1n-7**	**18:2n-6**	**18:3n-3**	**20:1n-9**	**20:2n-6**	**20:5n-3**	**22:5n-3**	**22:6n-3**
6 – Low. L	1.99	13.07	10.38	1.79	39.44	4.02	5.9	0.96	7.99	1.17	2.04	0.55	2.81
7 – Low. L	1.99	12.01	9.93	1.79	41.97	4.10	6.24	0.96	8.19	1.17	1.78	0.35	1.90
8 – Low. L	2.29	13.17	9.38	2.04	38.61	4.00	6.55	1.09	6.77	1.02	2.23	0.47	2.27
9 – Low. L	1.89	12.65	9.57	2.27	39.11	4.28	5.70	0.93	7.17	1.03	2.04	0.48	2.53
10 – Low. L	2.02	12.00	9.09	2.16	40.44	4.36	6.00	0.88	8.76	1.17	1.44	0.30	1.36
16 – Mid. L	1.92	14.75	8.36	2.03	39.45	3.84	7.28	0.93	6.27	1.06	1.34	0.40	1.82
17 – Mid. L	1.83	13.50	8.56	2.21	39.95	3.69	7.55	1.08	6.44	1.24	1.86	0.55	3.00
18 – Mid. L	1.75	11.95	9.43	1.77	37.96	3.78	7.56	1.09	7.44	1.44	1.78	0.67	3.68
19 – Mid. L	1.63	12.31	8.90	2.12	41.56	3.84	7.28	1.00	5.81	0.98	1.59	0.54	3.07
20 – Mid. L	1.81	12.66	8.54	2.09	39.90	3.775	7.955	1.12	6.975	1.44	1.65	0.42	1.995
1 – Initial L TAG	1.87	19.62	5.67	4.46	41.41	2.97	4.32	0.62	7.02	0.89	1.55	0.64	3.13
2 – Initial L TAG	2.08	15.49	5.41	4.43	39.83	3.48	4.05	0.71	8.01	0.98	2.46	1.06	4.52
3 – Initial L TAG	2.16	16.62	6.29	4.48	40.71	3.24	3.44	0.60	9.42	0.89	1.86	0.75	3.10
4 – Initial L TAG	2.24	16.70	5.57	4.53	40.19	3.55	4.04	0.68	8.56	1.05	2.05	0.84	3.28
5 – Initial L TAG	2.27	15.67	5.76	4.31	39.35	3.47	4.33	0.74	8.62	1.14	2.41	0.95	3.73

*Low. L: Lower pond liver TAG samples; Mid. L: Middle pond liver TAG samples; Initial L TAG: initial sampling event liver TAG samples.

Table F2. Proportions (%) of individual fatty acids from muscle triacylglycerols (TAG) included in statistical analysis. Samples were collected from young-of-the-year striped bass (*Morone saxatilis*) from the Diet 1 treatment (7% fish oil and 1 % poultry fat) in November 2016 (initial samples) and March 2017 (overwinter samples) as part of the 2016/17 overwinter trial taking place in the Lower and Middle ponds at North River Fish Farms Ltd.

Sample ID #	**Fatty acid proportions (%)**												
	14:0	**16:0**	**16:1n-7**	**18:0**	**18:1n-9**	**18:1n-7**	**18:2n-6**	**18:3n-3**	**20:1n-9**	**20:2n-6**	**20:5n-3**	**22:5n-3**	**22:6n-3**
1 – Low. M	4.08	18.20	7.11	2.99	30.94	2.51	7.18	1.18	3.33	0.49	4.83	1.20	4.61
2 – Low. M	3.89	18.54	7.06	3.09	32.69	2.45	6.78	1.09	3.60	0.46	4.80	1.17	4.74
3 – Low. M	4.06	18.15	6.82	2.89	32.18	2.52	7.25	1.18	3.25	0.44	5.03	1.33	5.31
4 – Low. M	4.17	18.30	7.16	2.73	30.59	2.62	7.49	1.20	3.37	0.51	5.29	1.31	5.28
5 – Low. M	3.73	18.21	6.92	2.86	32.06	2.56	6.89	1.07	3.69	0.50	5.01	1.34	5.78
11 – Mid. M	3.23	18.03	6.31	2.72	31.86	2.32	8.55	1.17	3.11	0.52	3.71	0.94	3.82
12 – Mid. M	3.18	17.91	6.55	2.96	33.94	2.40	8.78	1.18	3.44	0.58	3.78	0.97	4.19
13 – Mid. M	3.23	17.73	6.32	2.98	31.99	2.36	8.68	1.18	3.60	0.59	3.70	0.96	4.12
14 – Mid. M	3.05	18.15	6.34	3.24	34.36	2.35	8.31	1.13	3.03	0.48	3.70	1.09	4.58
15 – Mid. M	2.92	16.94	6.10	2.96	32.28	2.41	8.63	1.16	3.27	0.58	3.61	0.99	3.89
1 – Initial M TAG	4.47	19.46	6.95	3.22	28.47	2.65	7.70	1.35	3.17	0.54	5.67	1.29	5.30
2 – Initial M TAG	4.11	19.84	6.89	3.45	30.83	2.56	6.89	1.21	3.47	0.51	5.12	1.17	4.69
3 – Initial M TAG	4.26	19.30	6.94	3.29	29.46	2.60	7.31	1.23	3.84	0.48	5.26	1.19	5.05
4 – Initial M TAG	4.47	19.46	6.95	3.22	28.47	2.65	7.70	1.35	3.17	0.54	5.67	1.29	5.30
5 – Initial M TAG	4.34	19.16	6.98	3.17	28.76	2.63	7.60	1.33	3.46	0.56	5.59	1.29	5.32

*Low. M: Lower pond muscle TAG samples; Mid. M: Middle pond muscle TAG samples; Initial M TAG: initial sampling event muscle TAG samples.

Table F3. Proportions (%) of individual fatty acids from muscle phosphatidylethanolamine (PE) included in statistical analysis. Samples were collected from young-of-the-year striped bass (*Morone saxatilis)* from the Diet 1 treatment (7% fish oil and 1 % poultry fat) in November 2016 (initial samples) and March 2017 (overwinter samples) as part of the 2016/17 overwinter trial taking place in the Lower and Middle ponds at North River Fish Farms Ltd.

Sample ID #	**Fatty acid proportions (%)**														
	14:0	**16:0**	**16:1n-7**	**18:0**	**18:1n-9**	**18:1n-7**	**18:2n-6**	**18:3n-3**	**20:1n-9**	**20:2n-6**	**20:4n-6**	**20:5n-3**	**22:5n-6**	**22:5n-3**	**22:6n-3**
1 – Low. M	0.76	12.92	1.88	6.93	11.08	3.39	5.67	0.62	4.00	1.01	2.90	12.53	0.79	3.06	27.11
2 – Low. M	0.98	14.07	2.25	5.35	12.78	3.58	6.13	0.67	4.17	0.97	2.35	12.56	0.69	2.77	23.70
3 – Low. M	0.60	14.03	1.52	5.36	10.86	3.99	6.59	0.65	3.65	1.07	2.39	11.91	0.87	3.15	28.42
4 – Low. M	0.77	13.17	1.82	5.48	11.13	3.65	6.28	0.65	4.09	1.05	2.46	13.11	0.82	2.83	26.98
5 – Low. M	0.63	13.10	1.75	7.84	11.03	3.65	6.42	0.56	4.14	1.12	2.67	11.07	0.83	3.15	27.28
11 – Mid. M	0.39	11.52	1.38	4.50	9.57	3.40	7.17	0.62	4.02	1.13	2.74	13.90	0.73	2.68	28.12
12 – Mid. M	0.71	14.89	1.71	7.96	11.35	2.64	6.34	0.58	3.07	0.85	3.15	12.92	0.72	2.63	26.02
13 – Mid. M	0.57	11.43	1.65	6.71	10.73	3.21	7.32	0.65	4.32	1.19	2.79	11.98	0.82	2.73	26.08
14 – Mid. M	0.53	13.37	1.70	6.00	12.17	4.01	8.64	0.70	3.82	1.20	2.61	11.65	0.72	2.88	23.72
15 – Mid. M	0.34	12.07	1.00	7.44	8.71	3.07	6.83	0.54	3.57	1.15	3.02	12.98	0.78	2.80	29.22
1 – Initial PE	0.46	9.43	1.58	9.88	11.63	2.82	4.65	0.58	2.24	0.69	2.20	10.37	0.73	2.52	30.41
2 – Initial PE	0.37	8.27	1.40	10.02	10.31	2.31	4.19	0.62	2.58	0.71	2.40	11.32	0.81	2.96	32.11
3 – Initial PE	0.31	7.57	1.30	9.94	9.61	2.55	3.99	0.54	2.66	0.71	2.36	10.90	0.81	2.87	34.35
4 – Initial PE	0.33	8.28	1.17	10.56	8.65	2.24	4.07	0.52	2.16	0.70	2.23	10.33	0.85	3.04	34.72
5 – Initial PE	0.31	7.77	1.12	10.45	8.80	2.19	3.73	0.53	2.35	0.72	2.24	10.70	0.83	2.86	36.06

*Low. M: Lower pond muscle PE samples; Mid. M: Middle pond muscle PE samples; Initial PE: initial sampling event muscle PE samples.

Table F4. Proportions (%) of individual fatty acids from muscle phosphatidylcholine (PC) included in statistical analysis. Samples were collected from young-of-the-year striped bass (*Morone saxatilis*) from the Diet 1 treatment (7% fish oil and 1 % poultry fat) in November 2016 (initial samples) and March 2017 (overwinter samples) as part of the 2016/17 overwinter trial taking place in the Lower and Middle ponds at North River Fish Farms Ltd.

Sample ID #	**Fatty acid proportions (%)**													
	14:0	**16:0**	**16:1n-7**	**18:0**	**18:1n-9**	**18:1n-7**	**18:2n-6**	**18:3n-3**	**20:1n-9**	**20:4n-6**	**20:5n-3**	**22:5n-6**	**22:5n-3**	**22:6n-3**
1 – Low. M	1.24	32.55	2.46	3.32	10.37	1.06	5.64	0.57	1.18	1.98	15.98	0.58	2.31	15.92
2 – Low. M	1.19	29.81	2.31	4.07	10.48	1.04	5.35	0.65	1.13	2.01	16.81	0.63	2.65	16.77
3 – Low. M	0.83	33.14	1.64	2.77	8.64	0.84	4.87	0.52	0.72	1.98	17.31	0.69	2.64	19.61
4 – Low. M	1.01	33.01	1.86	2.97	8.17	0.89	5.43	0.61	0.94	1.74	15.84	0.74	2.62	20.03
5 – Low. M	0.87	33.62	1.67	3.36	8.39	0.94	4.98	0.44	0.95	1.95	16.73	0.69	2.60	18.96
11 – Mid. M	1.09	34.51	2.46	2.27	9.04	0.83	8.44	0.73	0.85	1.59	14.83	0.60	2.54	15.62
12 – Mid. M	1.12	30.08	2.55	4.16	11.65	1.14	8.08	0.68	1.34	1.94	13.41	0.58	2.18	16.53
13 – Mid. M	1.10	34.31	2.42	2.78	9.61	0.83	8.51	0.68	1.12	1.73	14.78	0.57	2.34	15.01
14 – Mid. M	0.97	31.50	2.00	3.79	9.18	0.89	7.68	0.66	0.77	2.44	16.74	0.61	2.61	16.33
15 – Mid. M	0.95	33.27	1.95	4.36	8.36	0.84	8.40	0.65	0.83	2.20	14.35	0.61	2.35	16.58
1 – Initial PC	0.80	22.78	2.51	4.42	16.34	1.25	6.14	0.66	0.93	1.95	13.40	0.71	1.85	21.57
2 – Initial PC	0.60	20.78	2.32	4.09	15.10	1.20	5.88	0.62	0.99	2.22	14.79	0.80	2.02	24.33
3 – Initial PC	0.69	21.61	2.29	3.76	14.18	1.17	5.58	0.65	0.91	2.22	14.43	0.76	2.04	25.28
4 – Initial PC	0.82	23.56	2.37	3.98	13.87	1.33	6.41	0.74	1.01	1.86	12.45	0.75	2.09	23.56
5 – Initial PC	0.72	23.45	2.14	3.61	13.17	1.16	5.73	0.67	0.91	2.07	14.15	0.74	1.98	25.03

*Low. M: Lower pond muscle PC samples; Mid. M: Middle pond muscle PC samples; Initial PC: initial sampling event muscle PC samples.