TOXICITY OF A BRINE EFFLUENT ON EARLY LIFE-STAGE AND JUVENILE STRIPED BASS (*MORONE SAXATILIS*)

by

Hayden Matthew Breau

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
April 2020

“God had to have somebody willing to ride the ruts at double speed to get the hay in ahead of the rain clouds and yet stop in mid-field and race to help when he sees the first smoke from a neighbor's place. So, God made a farmer.”

-Paul Harvey

Dedication

This thesis is dedicated to my grandparents Allison “Tony” DeLeavey and Edith DeLeavey both of whom highly valued education, agriculture and kindness. Without their influence this document would not likely exist.

Table of Contents

List of Tables

List of Figures

Abstract

The striped bass of the Shubenacadie River, in Nova Scotia, Canada are an important part of the Bay of Fundy population which is considered endangered by COSEWIC. The estuary is key striped bass nursery habitat and the site of the planned controlled release of a brine effluent created via formation of natural gas storage caverns. Therefore, the toxicity of the brine was quantified on the sensitive early life stages including eggs, larvae, and juveniles. The median lethal concentration of brine on striped bass ranged between 30.9-65.7 ppt, which is above a regulatory mandated 28 ppt maximum threshold for the release of brine. Threshold-observable-effect concentrations were also more than this threshold value 30.8-59.7 ppt excluding 2 conditions. Changes in salinity tolerance were related to ontogenetic development, relating to behaviour and physiology. These data suggest the current plan to release brine poses little risk to the bass.

Keywords: striped bass, toxicology, brine, salinity tolerance, Alton gas, median lethal concentration, Shubenacadie river.

List of Abbreviations and Symbols Used

AC	Alton Core
BC	British Columbia
BW	Body Weight
CTD	Conductivity, Temperature, Depth
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
Dal-AC	Dalhousie Agriculture Campus
DFO	Department of Fisheries and Oceans
DPH	Days Post Hatch
EJ	Early Juvenile
ETM	Estuarine Turbidity Maximum
FFL	First-Feeding Larvae
FL	Fork length
FW	Fresh Water
IO	Instant Ocean
LC_{50}	Median Lethal Concentration
LJ	Large Juvenile
LL	Large Larvae
LOEC	Lowest Observable Effects Concentration
MATC	Maximum Acceptable Toxicant Concentration
NB	New Brunswick
NGO	Non-Governmental Organization
NOEL	No Observable Effect Level
NS	Nova Scotia
NSE	Nova Scotia Environment
NTU	Nephelometric Turbidity Units
PPT	Parts Per thousand
rkm	River Kilometer(s)
RW	River Water
TDS	Total Dissolved Solids
TL	Total Length
TOEC	Threshold-Observed-Effect-Concentration
TRC	Total Residual Chlorine
WW	Well Water
YOY	Young of the Year
YSL	Yolk Sac Larvae

Acknowledgments

I wish to acknowledge first Alton Natural Gas Storage LP., for providing financial means to complete this research, four years of the Shubenacadie River, four years of learning and broadening my horizons.

I am grateful to my committee members, Drs. Janine Gray, and Stefanie Colombo for many lessons and chats which all have helped me to improve as a scientist. And also a great thanks to my supervisor Jim Duston, who continued to push me to improve, adapt and overcome for the duration of this project, even on days where I would not let him get a word in edgewise, which is quite frequently might I add. I'd also like to thank Juan Manriquez-Hernandez for assistance, guidance and chats the helped me become a better researcher and improve this document. The Aquaculture centre staff; Audrie-Jo McConkey, Scott Jeffrey and Paul MacIsaac who were pivotal in the completion of this project through assistance, guidance or life chats, which kept me going in the right direction.

I'd like to thank my parents Julie and Curtis, without their support throughout this winding road, I'd likely not have been able to pull it off. My siblings Hunter and Holden for modern housing solutions and my friends Julia Beaton and Lilly Burgoyne for sticking around for 4 years of me whining about having to someday buckle up and write this thesis and holding me slightly accountable for writing promises. And to all the other friends who kept bricks in the walls when things got tough. To all the people I interacted with that led me to this point, thank you.

Chapter 1: Introduction

1.0 The Alton Gas Project, Shubenacadie River and its Population of Striped Bass

Estuaries are vulnerable aquatic habitats that are at risk from anthropogenic activities (Barbier et al., 2011). Their sensitivity is due to complex and diverse trophic chains which can be easily disrupted by pollutants from both freshwater and marine origins (Kennish, 1994). Estuaries in many parts of the world continue to be developed for tourism, shipping centres, and natural resources, all of which pose a threat to fish production, nursery habitat, and loss of filtering capacity due to drainage of complex marshes (Barbier et al., 2011; EPA, 2018). In the Inner Bay of Fundy, by contrast, the highest tides in the world have restricted shoreline development, and the rich turbid waters are largely protected from human interference by the fast currents and vast mud flats. The Shubenacadie River has no shipping lanes, only hosting a small inland fishery, and some tourism founded around canoeing and tidal-bore rafting, but is vulnerable, nevertheless. Rising sea levels have the potential to displace coastlines and wetlands, while changing tidal intrusion into estuaries (Schneider and Chen, 1980). Altered rain patterns can lead to decreased periods of salinity and temperature, followed by drought, which will raise temperatures and increase salt intrusion into estuaries. Climate change will impact rainfall by changing global water cycles; areas that currently experience frequent rainfall will receive more frequent and severe precipitation, and dry areas will become more likely to experience drought (NASA, 2019). To curb the progress of climate change and reduce emissions, it is key to invest in infrastructure to make clean energy sources readily available. In Nova Scotia, 52 % of electricity is generated via the burning of coal, and petroleum coke (Nova Scotia Power, 2019). The use of natural gas as a substitute for coal, reduces emissions by 33 % when

producing heat and by 50 % when producing electricity (IEA, 2019). Therefore, carbon dioxide emissions in Nova Scotia could be greatly reduced if there is a shift to green energy and natural gas. Developing infrastructure, such as gas storage caverns, can therefore aide the Maritime provinces transition to cleaner energy sources. When developing energy infrastructure there is almost always the potential for environmental impact, which needs to be carefully assessed.

The Shubenacadie River Estuary, located in Nova Scotia, Canada is an example of an 'essential estuary' as it plays a key role in the production of diadromous, euryhaline fish species. Moreover, it is the sole known nursery habitat for the Bay of Fundy striped bass (*Morone saxatilis*). Emerging evidence suggest that the Saint John River in New Brunswick may provide viable spawning and nursery habitat for striped bass (Bradford et al., 2015; Leblanc et al., 2018). The Shubenacadie River is the planned site of industrial development by an oil and gas company, Alton Gas, a sub-division of Altagas. This development will pump river water 10 km to dissolve underground deposits of halite (mostly NaCl) to form caverns for the storage of natural gas. This process will create a hypersaline brine effluent that will be released in a controlled manner into the Shubenacadie River. This release will occur 25 rkm from the estuary mouth, at the same site as water uptake (Alton Gas, 2015). This location will be referred to in this thesis as the "Main Site" henceforth. Despite full environmental approval by the Nova Scotia Department of Environment and Department of Fisheries and Oceans of the project in 2007 and associated ecological research on the Shubenacadie River and its ecosystem since 2008, the release of brine has not yet commenced. Environmental concerns were expressed from groups such as non-governmental organizations and First Nations, as there was

concern that the brine may harm the estuary ecosystem. This was an influence leading to the requirement of acute toxicity testing of the brine (The Chronicle Herald, 2019). The fear the brine will cause harm to the estuarine ecosystem is the principal driver leading Federal and Provincial regulators to require acute toxicity testing of the brine. That decision, in the fall of 2015, led to this research project.

The Stewiacke-Shubenacadie River stock of striped bass is important, as at the start of this project it was considered the sole successful spawning population in the Bay of Fundy (COSEWIC, 2012; Bradford et al., 2015). Despite its 'endangered' status, the Shubenacadie-Stewiacke system population has high abundance, following highly successful recruitment years in the late 1990's, leading to "no less than 15,000 breeding adults" striped bass being present in the estuary in 2002, the date of the most recent stock assessment from DFO (Department of Fisheries and Oceans; Douglas et al., 2003). The Stewiacke-Shubenacadie River population and Bay of Fundy designable unit are discussed in greater detail in section 1.4.

Due to the biological importance of the Shubenacadie River population of striped bass and its cultural significance to the First Nations, ensuring protection of this ecosystem is imperative (COSEWIC, 2004). Establishing the acute toxicity of the hypersaline brine produced by the Alton Gas Project, the main objective of this thesis, has helped to define the risk to sensitive early life stages of striped bass. Determining the concentration of salt which is lethal to 50 % of a test sample (LC_{50}) and the concentration of salt that has no measurable effect on survival or threshold-observed-effect-concentration (LC_0; TOEC) over a 1 h exposure, for a range of life-history stages from eggs through to juveniles, will help regulators devise appropriate protection for the estuary.

Tests to determine the LC_{50} of a 1 h exposure to a hypersaline brine are described in the present study for six life stages of striped bass: eggs, yolk-sac larvae, 5-10 dph (days post hatch, the number of days after hatching) larvae, 11-20 dph larvae, 30 mm FL juveniles, and 120 mm FL juveniles. After 2016 tests, 5-10 dph larvae are referred to as first-feeding larvae, 11-20 dph larvae are referred to as large larvae, and 30 mm FL juveniles are referred to as either 20-60 mm juveniles or early juveniles as the methods and terminology developed over time. This is due to the late acquisition of salt core in 2017 that led 30 mm juveniles to be reclassified as 20-60 mm juveniles and continues to be reclassified under more easily understood names. Quantifying the salinity tolerance of a range of developmental stages can provide insight of the ontogenetic change in the osmoregulatory ability of striped bass. The larval stages of euryhaline teleosts are the most sensitive, as they lack the ability to control drinking rates, and lack fully functional osmoregulatory capabilities (Varsamos et al., 2001). Within the turbulent estuary, larval striped bass are unable to dictate their own position, as they have limited swimming ability. Water velocity at the Main Site ranges from 50 cm/s at low tide to a maximum of 160 cm/s during the flood tide, on a spring tide (Duston et al., 2018). Larvae 6.0 to 6.9 mm total length can only swim against water speeds of 1.7 cm/s; 7.0 to 7.9 mm larvae only swim against currents of 2.1 cm/s, and 8.0 to 8.9 mm larvae only can swim against currents of 3.0 cm/s (Meng, 1993). Larger striped bass (28 to 837 g) are strong swimmers, capable of swimming 2.9 to 3.3 body lengths per second, allowing them to better control their position within the estuary (Freadman, 1979).

A portion of the research described in this thesis was published recently, in the Archives of Environmental Contamination and Toxicology **78**(1): 124-136., with myself

as second author (Manriquez-Hernandez et al., 2020). The paper presents data solely from tests conducted in 2018 on striped bass derived from eggs retrieved from the wild and only using river water as a diluent. This thesis includes additional data from 2016 and 2017 during which the experimental and statistical methods were refined, and tests included striped bass derived from eggs from both wild and domesticated broodstock, and used the combination of Instant Ocean salt and well water. Consequently, this thesis is a full record of my toxicology research activities as an MSc student.

The following sections of Chapter 1 develop the rationale for the experimental work by describing the physical characteristics of the estuary (Section 1.1), explaining the Alton Gas Project and engineering plan (Section 1.2); defining the chemical and physical composition of the salt deposit (Section 1.3); reviewing the relevant knowledge on Stewiacke-Shubenacadie River Striped bass (Section 1.4); reviewing the osmoregulatory abilities of striped bass (Section 1.5); outlining the state of knowledge on salt toxicity on striped bass (Section 1.6); a review of the use of striped bass in toxicological analysis (Section 1.7) and the objectives of this thesis (Section 1.8).

1.1 The Shubenacadie River

The Shubenacadie River flows 72 km north from Shubenacadie Grand Lake into the Inner Bay of Fundy near Maitland (Lay, 1979). The watershed is 2,800 km^2 and up to 64 km of the Shubenacadie River is influenced by the daily tidal cycle. The Stewiacke River is the main tributary of the Shubenacadie; their confluence is 27 river kilometers from the estuary mouth (27 rkm; Figure 1). The Stewiacke River headwaters are located in Round Lake, in Pictou County Nova Scotia, and flows 88 km into the Stewiacke Valley before emptying into the Shubenacadie. The lower 14 km of the Stewiacke River is influenced

by the tide, a portion of which is the principal site of striped bass spawning (Rulifson and Tull, 1999).

The incoming tide rushes into Cobequid Bay, forming a tidal bore near Maitland (Figure 1). During a single tidal cycle, about $59x10^6$ m^3 of water enters the estuary mouth near Maitland, forcing its way upriver rapidly, changing the salinity and river depth (Martec Limited, 2007). The tidal cycle is asymmetrical; the duration of the flood- and ebb-tide at Maitland is about 3 and 9 h respectively, and at the Main Site is 1.5 and 10.5h (Dalrymple et al., 1990; Duston et al., 2018). The macrotidal estuary is highly turbulent resulting in very high turbidity, often exceeding 100 NTU or 500 mg/L (Martec, 2007; Duston and Astatkie, 2012). On spring tides, the maximum tidal range is about 11 m near Maitland, and 4 m at the Main Site, with rainfall and freshwater runoff further influencing the size of the bore and water level (GOVNS, 2007). At the Main Site between low and high tide, the width of the river changes between 50 and 240m, and depth from 1.5 to 5 m (Duston et al., 2018). Water velocity of the main channel at the Main Site ranges from 0.8 to 3.2 m/s (mean 1.6 m/s) and is 20 to 40 % greater than along both the east or west bank of the river during the flood tide. The velocity of the main channel peaks 20 minutes after bore arrival and then slowly decreases for the remainder of the flood tide, characterized by a maximum velocity between 1.9 and 3.2 m/s depending on the size of the tide (Duston et al., 2018).

The salinity dynamics of the Main Site are an important reference point because of the planned discharge of brine. Salinity at the Main Site varies with the tidal cycle and the magnitude of freshwater runoff. Following heavy rainfall events (>30 mm), salinity at high tide is commonly below 2 ppt and near 0 ppt on the low tide (Figure 2). By contrast, at the

estuary mouth the typical range of salinity is between 15 and 22 ppt. When freshwater runoff is low, the salinity at the Main Site can increase from 0 to 20 ppt within 20 minutes after the arrival of the tidal bore (MacInnis, 2012). Tidal flow can extend 62 km up the Shubenacadie River on the largest tides. Water temperature in the Shubenacadie River ranges from a low of -1 °C in winter (January 24th, 2020; Conductivity, Temperature, Depth Logger) to near 25 °C during the late summer (Duston et al., 2018).

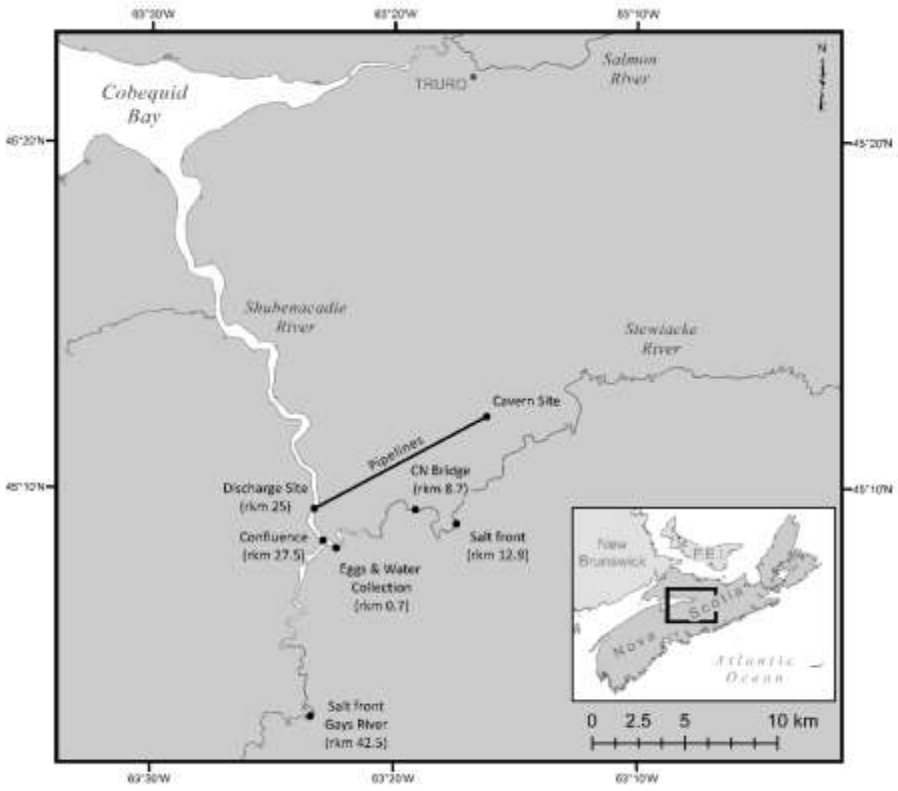


Figure 1: The Shubenacadie River System up to 43 (rkm), including the confluence of the Shubenacadie and Stewiacke River (27.5 rkm), the Main Site, indicated as the discharge site on the map and the pipeline (25 rkm; Manriquez-Hernandez et al., 2020).

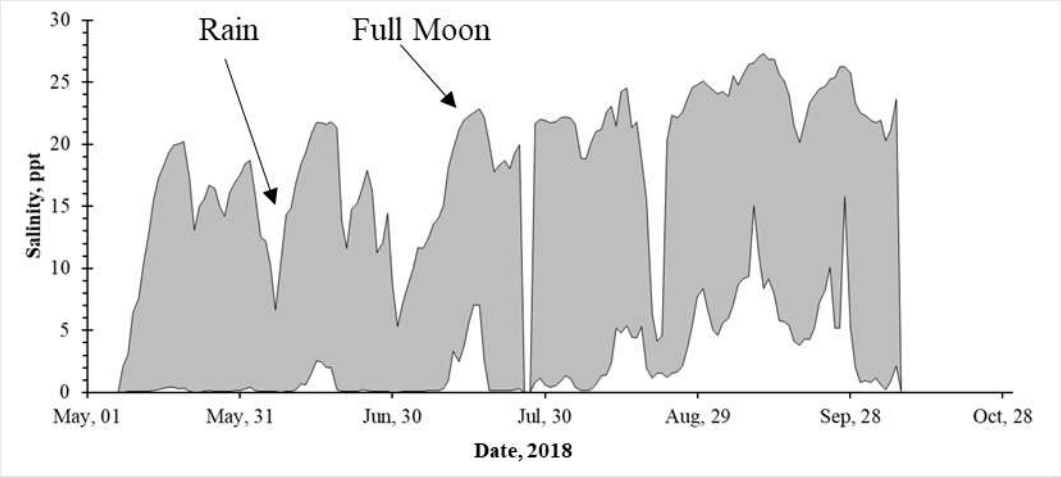


Figure 2: Salinity fluctuation of the Shubenacadie River at the Main Site (25 rkm) in 2018 from May 6 to October 8. The grey shaded area shows the daily maximum range and the white area shows the daily low salinity. The absence of data July 26 to 27 is because the CTD logger was removed from the water for service.

1.2 Alton Gas Project

Caverns for storing natural gas will be created via a process known as solution mining. This method is commonly used to dissolve and extract salt deposits for human consumption (Sanford, 1996; Khaledi et al., 2016). At the Main Site, Shubenacadie River water will be diverted into a 200 m long bypass channel (Figure 3). Water will be withdrawn through an uptake pipe behind a gabion wall, which is intended to reduce the risk of impingement of eggs, larvae, and fishes (Alton Gas, 2015). The water will be pumped 12 km, then forced 900 m underground to dissolve halite deposits to form a cavern. Once saturated with salt and sediments the water will have a salinity near 260 ppt. The brine will be pumped back to the Main Site into a holding pond to allow suspended solids to settle out. From the holding pond the brine will be released in a controlled manner into the channel during the flood and early ebb tide. The salinity of the effluent will decrease below a regulatory maximum of 28 ppt within 5m of the outfall pipe in the bypass channel (Figure 3). The

bypass channel is 200m in length and 30m in width the engineers envisaging a continuous river water flow when it is functioning (Figure 3; Alton Gas, 2016).

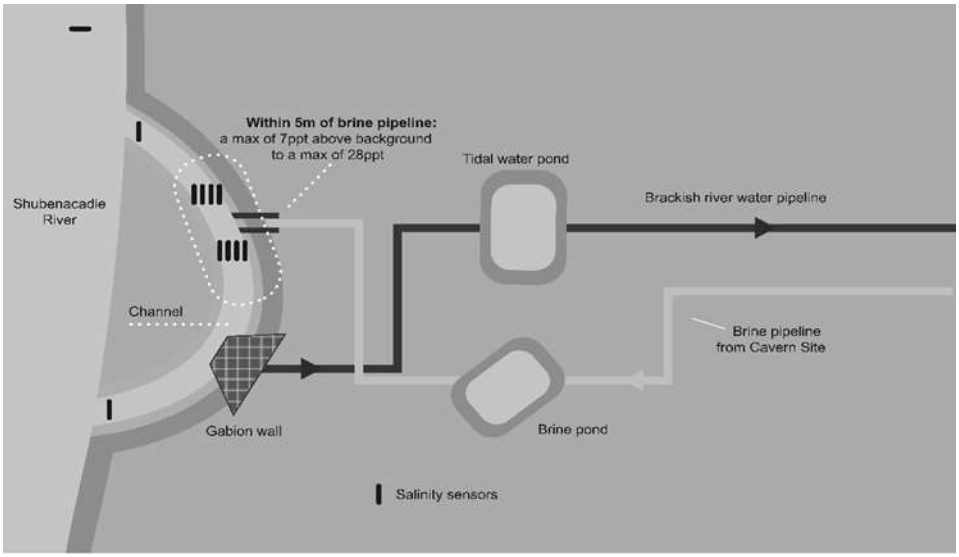


Figure 3: Brine Effluent Release Site. This diagram illustrates the bypass channel along the side of the Shubenacadie River at the Main Site. Brine will be released into the channel where the mixing will occur, this water will then flow past an array of salinity sensors and into the Shubenacadie River (Alton Gas, 2016).

For the brine toxicity tests a 1 h test period was selected due to the rapid mixing of the brine with the estuary water due to the natural high-water velocities ranging from 0.7 to 0.9 m/s within the bypass channel (Manriquez-Hernandez et al., 2020). A 1 h LC_{50} is conservative, as the potential duration of exposure to high salinity will be far shorter, nearer 5 minutes based on the 200 m bypass channel with the lower flow rate of 0.7 m/s. The bypass channel will be equipped with a series of salinity sensors to relay information to a computer control module, that will control the rate of brine release (Alton Gas, 2016). The sensors will be located 5 m on either side of the point of discharge to monitor both ebb- and flood-tide cycles. Brine release will stop any time salinity within the bypass channel exceeds 7 ppt above the background or if the river background salinity exceeds 28 ppt (Alton Gas, 2016). Additional protections will be added during the striped bass spawning

season: once the first egg is detected brining will be immediately stopped for a 24 day period, with the maximum upper limit for salinity being decreased from 28 ppt to 20 ppt within 5 m of the discharge pipe (Alton Gas, 2015; DFO, 2016; NSE, 2016).

1.3 Salt Deposit Characteristics and Brine Effluent Ionic Composition

The gas caverns will be constructed in a salt deposit known as the Shubenacadie sub-basin which has a thickness between one and two kilometers (MacNeil et al., 2018). The Shubenacadie sub-basin is an example of a Saline Giant, created by the evaporation of ancient hypersaline seas. The Shubenacadie sub-basin is a part of the Mississippian Windsor Group, which dates back 344 million years. Changes in sea-level led to the complex layering of mineral deposits, mainly that of silicate/carbonates and salts such as halite and polyhalite (Carol et al., 2016; MacNeil et al., 2018). The carbonate and silicate deposits are a result of alternating phases of colonization and extinction of shellfish (MacNeil et al., 2018). The major constituents are mixed layers of halite, anhydrite, marine carbonate, red and grey siltstone and potash salts with some geographical variation (Giles and Boehner, 2001). Due to this complex layering of minerals the composition of the salt varies with depth, suggesting the composition of the brine will vary during the cavern formation process. To investigate the potential for differences in salt composition and potentially the toxicity, the core samples used to create the brine used in the testing came from two sources. The first core samples were donated by the Nova Scotia Department of Natural Resources Core Library (Stellarton, Nova Scotia, Canada) and were used in trials conducted in 2017. The samples were drilled in June 2006, at core hole ALT 06-01; 45º 11.9'N – 63º 16.3' W approximately 500 m from the planned cavern site. They were from three different depth ranges, 560 to 564 m, 624 to 627 m and 941 to 944 m. For use in the

trials the salts were homogenized by mixing all depths to represent as close as possible the true effluent, including potential inconsistencies in the salt formation. The second set of core samples, used in all the toxicity tests conducted in 2018, were from the proposed cavern site at Alton, NS, 45º12'04.9" N, 63º16'11.6" W in summer 2014. The core number was 08-01, and the samples were from 883 to 933 m depth, the depth of the proposed cavern formation. The chemical composition of the core samples were not compared since they were from the same geographical location.

1.4 Stewiacke-Shubenacadie River Striped Bass

The striped bass (*Morone saxatilis)* is an anadromous perciforme of the family *Moronidae* (Scott and Scott, 1988). The Shubenacadie-Stewiacke River population is the only one spawning in a macrotidal estuary. Historically, three populations contributed to the Bay of Fundy stock; however, both the Annapolis River and Saint John River populations are no longer productive (Douglas et al., 2003). The status of the Bay of Fundy population may have to be revised as recent evidence indicates the Saint John River population persists and is genetically discrete from the Shubenacadie River population (Leblanc et al., 2018). The other Canadian population of striped bass, the Gulf of St. Lawrence stock, spawns in the Miramichi River system in North-Eastern New Brunswick and is genetically discrete from the Shubenacadie River population (Wirgin et al., 1993). The abundance of striped bass in the Shubenacadie remains high following highly successful recruitment years in the 1990s (Douglas et al., 2003). Recruitment success is dependent on multiple factors including temperature, rainfall, and prey availability (Rutherford and Houde, 1995). The most recent year with good recruitment in the

Shubenacadie River was 2016, with poor years following in 2017, 2018 and 2019 (Duston et al., 2018).

The Shubenacadie River estuary is fully mixed lacking stratification, whereas other important striped bass spawning and nursery habitats, like Chesapeake Bay and Miramichi River, are stratified (Rulifson and Dadswell, 1995; Duston et al., 2018). Stratified estuaries have a zone called the estuarine turbidity maximum (ETM) where freshwater meets saltwater creating a concentration of suspended particles where plankton and larval fish congregate (Schoellhamer, 2001). Salinities can be lower than 6 ppt, or near 20 to 30 ppt at the ETM (Schoellhamer, 2001; Vinh et al., 2017).

Spawning occurs in the Stewiacke River, and typically commences when the estuary warms by 11 to 20 degree days (function of time and temperature) above 12°C, from mid-May through June (Duston et al., 2018). During spawning, in the tidal freshwater, a single female, 45 cm fork length can produce upwards of 50,000 eggs, whilst females over 90 cm can produce up to 2.1 million eggs each year (Paramore, 1998; MacInnis, 2012). Eggs are transported by the ebb tide into the Shubenacadie River, where they develop and hatch within 2 days (Rulifson and Tull, 1999). The water temperature associated with the first spawning episode in the Shubenacadie River has ranged from a minimum of 12.3°C in 2010 to a maximum of 19.0°C in 2015, with the mean of 14.8 °C (Duston et al., 2018). Spawning can occur daily if water temperature is sustained but will stop if estuary cools below 12 °C, which occurred in both 2010 and 2011 (Duston et al., 2018). Upwelling currents and tidal mixing keep the semi-buoyant eggs suspended in the water column until hatch. Time from fertilization to hatch is about 44 to 48 h at 16º - 17°C (Reinert and Peterson, 2008). The emergent yolk-sac larvae are about 3 mm total length (TL) and are

vertically orientated in the water column (Hardy, 1978). Survival of newly emerged striped bass is largely dependent on temperature. At times Chesapeake Bay cools to 12°C during spring storms, this decrease in water temperature is associated with high mortality among larval striped bass (Rutherford and Houde, 1995; Secor et al., 2017). The first feeding stage is reached at about 5 days post hatch at 16°C at about 5 mm TL (Hardy, 1978). The larvae begin to actively feed if suitable prey is available, but still partly rely on endogenous energy deposits (Eldridge et al., 1983). In the absence of appropriately sized prey, striped bass larvae in the Shubenacadie River remain around 6 mm TL for three weeks (Duston et al., 2018). In the Shubenacadie River estuary the main prey at first feeding is a small harpacticoid copepod (Findlay, 2019). This is contrary to stratified estuaries such as the San Francisco Estuary, Chesapeake Bay and the Miramichi River Estuary, where striped bass prey heavily on the copepod *Eurytemora affinis*, which rarely occurs in the Shubenacadie River (Brown, 1984; Robichaud-LeBlanc et al., 1996; Martino and Houde, 2010). Larvae begin to gain pigment around 15 mm TL, and metamorphosis occurs at 25-30 mm TL (Hill et al., 1989; Cook, 2003). The characteristic stripes develop around 4 cm FL. Mean body size at the end of the first growing season can range from 4 to 12 cm TL (Duston et al., 2018). Underyearling (less than 1 year old) striped bass are broadly distributed throughout the Shubenacadie River and have been detected in Cobequid Bay (Douglas et al., 2003; Cook, 2003). To survive winter, there is evidence striped bass need to reach 10 to 12 cm FL (Hurst and Conover, 1998). Overwinter habitat for Shubenacadie striped bass includes Shubenacadie Grand Lake and potentially Minas Basin (Bradford et al., 2015; DFO, 2016; Keyser et al., 2016). The presence of 2 + year old class striped bass has been confirmed in Shubenacadie Grand lake, detected by an acoustic scanner (Bradford

et al., 2015). The overwinter habitat of young-of-year Shubenacadie striped bass has not been identified but is speculated to be in brackish water (Bradford et al., 2015). Growth of striped bass in northern climates is limited by the short growing season. Southern populations of striped bass grow and become sexually mature at an earlier age than northern populations (Setzler et al., 1980). In the Shubenacadie-Stewiacke system, age at first maturity among females is four years (Paramore, 1998). Male domestic striped bass become sexually mature at two years of age, and females at four to five years of age (Paul MacIsaac, Dalhousie University, Aquaculture-Aquarium Systems Manager, Personal Communication, 2019). In the Potomac River in Maryland, age at first maturity is two years for males and six years for females at 17 and 43 cm FL respectively (Setzler et al., 1980).

Both wild and domestic striped bass were used in the toxicity tests, ensuring a reliable supply of test animals as the exact timing of spawning is difficult to predict, paired with high levels of mortality following spawning at both the egg and larval stages. The culture methods for both are described in Chapter 2. For wild fish used in the toxicology tests, rearing from egg incubation onwards was identical to domestic larvae. Domestic striped bass used in the toxicity tests were produced by in-tank spawning of one female and three males, hence within a cohort were either full- or half-siblings. The wild stock had a greater genetic variability since they were from eggs collected from the Stewiacke River following mass spawning episodes.

1.5 Osmoregulation and Salinity Tolerance

Tolerance is the ability of an organism to cope with various environmental stressors. Each species of fish and their developmental stages live within a range of environmental

conditions where normal bodily function remains unhindered (Shelford, 1931). This theory, known as Shelford's Law of Tolerance, proposes that abiotic factors can dictate the abundance or the distribution of animals in each habitat (Shelford, 1931). The abiotic factors include salinity, temperature and pH. For this thesis, quantifying salinity tolerance is the objective as the effluent is a hypersaline brine. Fish in the Shubenacadie River estuary are all euryhaline to some degree, allowing them to tolerate the tidal fluctuations in salinity. Euryhaline fish maintain internal ionic and osmotic balance despite changing ionic balances of their surroundings (Kültz, 2015). In the Shubenacadie River, salinity dictates the location of early life stage striped bass. This includes eggs and non-feeding larvae; therefore, this distribution is not prey driven. The abundance of striped bass eggs and all larval stages are highest between 2 and 5 ppt. At salinities above 10 ppt at the Main Site the abundance of eggs and larvae decreases (Duston et al., 2018). The cause of this decline is unclear, since lab trials indicated survival was not significantly different at salinities between 2 and 20 ppt (Cook et al., 2010).

Fish must maintain an internal blood plasma concentration that is equivalent to about 10 ppt salinity. In salinities below 10 ppt they must hyper-osmoregulate, maintaining an internal ionic concentration higher than the surrounding environment (Kidder et al., 2006). In salinities above 10 ppt, by contrast, fish must hypo-osmoregulate, maintain an internal ionic concentration lower than the surrounding environment (Schmidt-Nielsen, 1983; Kidder et al., 2006). The gills are important for both respiration and osmoregulation. In freshwater, the gills are a major site of osmotic gain of water and a diffusional loss of ions. This potentially lethal dilution of the blood plasma is countered by the active uptake of ions at the gills, via specialized ionocyte cells (aka chloride cells, mitochondria-rich cells).

The excess water is excreted in substantial amounts of dilute urine (Schmidt-Nielsen, 1983; Kültz, 2015). In marine teleosts, the passive fluxes of ions and water are reversed, as the external environment has a higher ionic concentration than the body fluids (Jobling, 1995; Kültz, 2015). Therefore, to maintain ionic balance, fish must gain water via drinking and actively excrete additional ions (Madsen et al. 1994, Jobling, 1995; Edwards and Marshall 2013, Kültz 2015). The process of osmoregulation in both freshwater and saltwater environments requires the expenditure of energy to maintain homeostasis, the magnitude dependent on both the salinity and the temperature of the surrounding environment. The uptake or efflux of water must be matched by efflux via urine or by drinking behaviour (Kidder et al., 2006). Fully euryhaline fish, such as the striped bass, can achieve homeostasis in freshwater or full-strength seawater very quickly. Striped bass (260g) abruptly transferred from freshwater to seawater exhibited a very stable blood plasma osmolality of around 340 mOsmol/kg, confirming that this species can readily and rapidly adapt to increasing salinities (King and Hossler, 1991; Madsen et al., 1994). However, the early developmental stages of euryhaline teleosts are typically less tolerant to extreme lower or upper ranges of salinity compared to juveniles or adults, making it necessary for the present study to establish LC_{50} values for a broad range of developmental stages. Early juvenile striped bass (4.6 cm FL) exhibited improved ability to cope with stressors such as exercise in both brackish 10 ppt and full-strength seawater (30 ppt), when compared to those reared in freshwater (Cech et al., 1996). The euryhalinity of larval striped bass has not been well defined via measures of blood plasma osmolality (mOsm/kg) as it is difficult to collect fluids from larval fishes; possible only with a micropipette, inserted into the heart (Varsamos et al., 2001).

Osmoregulatory capabilities of the European sea bass (*Dicentrarchus labrax*), a relative of the striped bass, has been quantified more so than striped bass (Varsamos et al., 2001; Williams et al., 2012). A salinity of 60 ppt was the upper limit for a no stress response among small *D. labrax* juveniles (6.2 g) abruptly transferred from 15 ppt salinity (Jensen et al., 1998). The degree of euryhalinity of striped bass yolk-sac larvae of the Patuxent River stock increased through the larval stage. Survival of the late yolk-sac larval stage was 0% in both 0 or 33 ppt, compared to 68 and 80% survival in 5 and 11 ppt respectively, and 3.2% in 0.7 ppt (Hirai et al., 2000). Preflexion larvae (first-feeding) had an increased ability to cope with the 0.7 ppt treatment, and survival was 29.9 %. Survival of juveniles (103 dph, 4 cm TL) was 11% in 33 ppt, compared to 0 % for earlier life stages. Survival among juveniles exposed to 0.7 ppt salinity for 72h was 50 % (Hirai et al., 2000).

The number of chloride cells gradually increased through development on both the gill lamellae and the skin, suggesting that striped bass share a developmental history of anadromy that does not originate in freshwater, as anadromous freshwater fish typically only have chloride cells on the gill lamellae (Hirai et al., 2002). Similarly, the osmoregulatory capacity of *D. labrax* was dependent on body size. Four stages were identified based on the ratio between plasma osmolality and the surrounding environment. Stage one was 3.5 mm TL; stage two was 5.2 mm TL; stage three ranged from 6.6 to 14.3 mm TL, and stage 4 ranged from 17.0 to 240 mm TL (Varsamos et al., 2001). The increase in osmoregulatory capacity between stage one and stage two was hypothesized to be related to changes in drinking rates, changes between stage two and three were related to development of juvenile morphology, and, changes between stage three and stage four marked the completion of development to the juvenile stage (Varsamos et al., 2001).

Maximum osmoregulatory capability was achieved by about 63 days post hatch (Varsamos et al., 2001). The evidence above supports the hypothesis that salinity tolerance in the temperate basses increases as they develop from larvae to juveniles.

The salinity tolerance of striped bass differs between populations. The Savannah River stock is largely riverine with a relatively low salinity tolerance; salinities above 10 ppt were lethal to larvae (Reinert and Peterson, 2008). Eggs reared in 11 different salinities between 0 and 33 ppt (3 ppt increments) showed no significant difference in mortality from 0 to 15 ppt after 72 h, ranging from 44 to 52 %. In salinities above 18 ppt, survival after 72 h was greatly reduced, at 18 ppt mortality was 71%, at 21 ppt mortality was 87%, and at salinities above 24 ppt no eggs survived (Winger and Lasier, 1994). Eggs from Shubenacadie River stock, by comparison, exhibited a greater salinity tolerance, survival between 0 and 20 ppt was close to 70%, and at 30 ppt was 45% (Cook et al., 2010). Young of the year juveniles (juveniles under one year of age) of the Shubenacadie River population are widely distributed in the estuary and Cobequid Bay at 28 ppt (Duston et al., 2018). The high degree of euryhalinity of the Shubenacadie River stock of striped bass suggested they could tolerate acute exposure to hypersaline brine, but this supposition needed to be quantified.

1.6 Salinity Tolerance of Striped Bass: effect of ionic composition, and temperature

The salinity tolerance of striped bass can be affected by the ionic composition of their environment, particularly hardness, the total concentration of dissolved polyvalent metal cations. NaCl at 5 ppt was toxic to 3 and 4 week old juvenile striped bass from the McDuffie Hatchery in Georgia when [Ca^{2+}] was <1 mg/L, but at 10 mg/L Ca^{2+}, no mortality occurred (Grizzle and Mauldin, 1995). Water hardness in the Shubenacadie

River ranges from 38 to 3,630 mg/L over the tidal cycle (Manriquez-Hernandez et al., 2020), greatly exceeding the 10 mg/L required to decrease the toxicity of NaCl. Among juvenile striped bass, the mechanism of NaCl toxicity is through a rapid efflux of potassium (K^+) that is associated with increased permeability of the gills in environments with high salinity (Grizzle and Cummins, 1996). However, the addition of K^+ to the water in high salinity treatments did not decrease mortality rates; therefore, NaCl must have other mechanisms of toxicity, that have yet to be studied (Grizzle and Cummins, 1996).

Additionally, the potential effect of changing biological and environmental conditions during egg development on subsequent life stages is of concern. Deformities of fishes, including striped bass, can be caused by a multitude of factors, including fluctuations in salinity during egg development (Hickey et al., 1977; Berillis, 2015). High levels of metals and dissolved ions in brine could lead to the development of deformities (Hickey et al., 1977; Berillis, 2015). Striped bass eggs exposed to total residual chlorine (TRC) were 2% smaller after hatch when compared to control groups, this exposure also affected hatch rates among eggs, 96.9 % of eggs hatched in the control, when TRC levels exceeded 0.43 mg/L striped bass eggs did not develop past the gastrula stage, resulting in 100% mortality (Morgan and Prince, 1977). Savannah River stock striped bass were significantly larger at hatch when eggs were incubated in 3 - 12 ppt; 4.0 mm TL, compared to 3.8 mm TL when incubated in freshwater, and 3.8, 3.2 and 2.4 mm TL when incubated in 15, 18 and 21 ppt respectively (Winger and Lasier, 1994). Deformities caused by changing environmental salinity and temperature during egg development have been reported for European sea bass, Japanese eel (*Anguilla japonica*), and striped bass (Mansueti, 1958; Hickey et al., 1977; Winger and Lasier, 1994; Okamoto et al., 2009).

To justify using both 12 and 19 °C in the toxicity tests described in this thesis, it is necessary to consider the temperature range in the estuary during the spawning and larval season. Heavy rainfall, common in spring in Nova Scotia, typically is associated with a rapid drop in water temperature which poses a threat to survival of larval striped bass. In spring 2015 a heavy rainfall event dropped water temperatures in the Shubenacadie River estuary from 19 °C to 11 °C, a temperature which is detrimental to striped bass early life stages, and associated with a large decline in the abundance of larvae (Duston et al., 2018). In Chesapeake Bay, the cooling of striped bass nursery habitat to near 12 °C was considered a lethal factor for larvae (Morgan et al., 1981; Rutherford and Houde, 1995). Therefore, testing the potential effects of decreased temperature on median lethal concentration of salt brine on striped bass was an important step in this research. In the Shubenacadie River estuary, low temperature events post-spawning is limited to spring and early summer, therefore the toxicity tests at 12ºC were limited to the egg and larval stages.

In acute toxicology testing of aquatic organisms, exposure time ranges from 4 to 96 h (Environment Canada, 1990; Duke and Mount, 1991; Environment Canada, 2013). The duration of 1 h used in this thesis is conservative, considering that striped bass will not be exposed to hypersaline solutions for a long time period due to the high water flow and mixing, likely less than 5 minutes. At present, this is the first research to evaluate the toxicity (LC_{50}) of hypersaline solutions on the either striped bass or any other species inhabiting the Shubenacadie River. Therefore, the objective was to assess the toxicity of the effluent in conditions mimicking those at the Main Site on the Shubenacadie River.

Additionally, the threshold-observed-effect-concentration (TOEC) was calculated from the tests presented in this thesis. TOEC has been adopted by Environment and Climate

Change Canada as an alternative to the Maximum Acceptable Toxicant Concentration (MATC); both are calculated in the same manner and are interchangeable (EPA, 2002; ECC, 1990; EC 2005). TOEC is the geometric mean of the no observable effects concentration (NOEC) and the lowest observable effects concentration (LOEC; EPA, 2002). The geometric mean is the average of a set of products, commonly called the nth root product of n numbers. TOEC is a concentration where no effect to the biota is detected, and this concentration should not be exceeded (EPA, 2002). TOEC is better for comparison between toxicants, as LC_{50} represents concentrations which is lethal to half a test population, whereas MATC/TOEC represent the upper limit concentration limit which with no mortality. This is a useful value to help define regulations and protections for aquatic species regarding industrial effluents, hence it was included in the present study (Larson and Woltering, 1995).

Typically, reference toxicants are used when testing a new toxicant to identify potential sources of error or variability. These sources include animal health and stress, differences between year classes or cohorts, and the performance of the analyst (ETS, 2019). Instant Ocean Sea Salt (Spectrum Brands, Blacksburg, VA, USA) served as the reference toxicant for the present study, its use was approved by regulators (DFO and NSE). Instant Ocean is used in numerous commercial effluent laboratories to adjust salinity of effluents for the purpose of testing euryhaline and marine species, making it an ideal reference toxicant to use for this work (Arnold et al., 2007; Libralato et al., 2009). As per Whole Effluent Toxicity methods, the aim of evaluating the toxicity of the brine at the point of release was to use actual effluent as a toxicant and the receiving water as a diluent (EPA, 2002). This

ensured water chemistry and environmental conditions during testing were very close to those at the Main Site on the Shubenacadie River.

1.7 The Use of Striped Bass in Toxicology Analysis

Historically, striped bass have been used for toxicology and tolerance testing for various agricultural pesticides, aquaculture pesticides and bleached pulp mill effluents (Wellborn, 1969, Burton et al., 1983, Hall, 1987, Reardon and Harrell, 1990, Bailey et al., 1994). Although some of these instances are primarily for developing safe rearing protocols for striped bass aquaculture production, others evaluated the chemical threat from run-off and effluent release. For example, striped bass were used in toxicology tests more frequently where effluents were released into their habitat, such as the Potomac River, Maryland, USA and the Blackwater River, Virginia, USA (Burton et al., 1983; Hall, 1987). Tests evaluating the toxicity of pulp mill effluents typically have a 96 h duration (Burton et al., 1983; Hall, 1987). Striped bass eggs were consistently more resilient than early larvae to a wide range of toxicants including chlorine, chloride, salinity, and combinations of salinity and temperature (Morgan and Prince, 1977; Middaugh et al., 1977; Burton et al. 1983). Striped bass eggs were not affected by bleached kraft mill effluents in concentrations of 2 to 20 % volume, however, concentrations between 8 and 12 % for durations in excess of 72 h caused a 12 to 22 percent mortality rate in striped bass yolk-sac larvae (Burton et al., 1983). Larval striped bass (4 dph) were equally sensitive as some juveniles and adults of some salmonid species to seven inorganic chemicals (cadmium, copper, zinc, selenium, nickel, chromium, and, arsenic) and three organic insecticides (toxaphene, malathion, carboxyl) (Palawaski et al., 1985). Larval striped bass were more sensitive to the toxicants compared to cyprinids (fathead minnow, *Pimephales promelas*),

ictalurids (channel catfish, *Ictalurus punctatus*) and centrarchids (bluegill, *Lepomis macrochirus*; Palawaski et al., 1985). Clearly, striped bass early life stages are highly appropriate test animals for testing the toxicity of the brine effluent, both from a conservation and a sensitivity perspective.

Rainbow trout (*Oncorhynchus mykiss*) are recommended for toxicology testing for most effluents in Canada below 10 ppt salinity (Environment Canada, 1990). When the salinity of effluents exceeds 10 ppt or the release environment is either a marine or estuary habitat, other species must be selected because rainbow trout are not fully euryhaline, and the test species should be representative of the location of effluent release (Nichols et al., 2008). In the case of Alton Gas, striped bass are the logical test species as the Shubenacadie River estuary is their primary spawning and nursery habitat. Using this species was possible because the Stewiacke River provided a convenient source of high-quality eggs, and researchers at Dal-AC Truro have established the rearing protocols. Accordingly, DFO and NSE regulators stipulated in 2016 that striped bass be the test species, which led to the research presented in this thesis.

Three-spined sticklebacks (*Gasterosteus aculeatus*) are used as a test species for analysis of the toxicity of effluents above 10 ppt salinity, as required by Environment Canada (1990). Its geographic range extends across Canada, is relatively abundant and easy to catch, making it an ideal test species (Hart, 1973; Scott and Scott, 1988, Environment Canada, 1990). Moreover, the three-spined stickleback adapts well to laboratory environments, are a suitable size for the evaluation of effluents and are euryhaline making them an ideal candidate for testing any effluent that will be released into a coastal or estuarine environment (Environment Canada, 1990). In 2019,

Environment and Climate Change Canada (ECCC) required Alton Gas complete brine toxicity tests on three-spined sticklebacks. The requirement to test the toxicity of salt on sticklebacks was not expressed until completion of the striped bass testing, therefore, those tests are not a part of this thesis. The results of stickleback testing will provide regulators with a broader perspective on the risk of brine effluent to the Shubenacadie River ecosystem, adding to the data on striped bass presented in this thesis.

1.8 Thesis Objective

Determine the 1 h LC_{50} and threshold-observed-effect-concentration (TOEC) of brine produced from halite at the Alton Gas salt cavern site on six early life stages of striped bass (egg, yolk-sac larvae, 5-10 dph larvae, 10-20 dph larvae, 30 mm juveniles, 120 mm juveniles) compared to a reference toxicant, Instant Ocean, at both 12 and 19ºC. Establishing the effect of diluent water source (river water vs. well water) and the salt source (Alton Core/Salt Core vs. Instant Ocean) on the toxicity of the NaCl on striped bass was also a secondary focus of this research.

Chapter 2: General Materials and Methods

2.0 Laboratory Design, Test Equipment and Testing Conditions

In 2016 a specialized lab for toxicity testing was constructed by J. Manriquez-Hernandez and I in a wet-lab area (floor area 4.3 x 4.6m) in the Aquaculture Centre at Dalhousie University Faculty of Agriculture in Truro, Nova Scotia. The lab has a roll-up garage door which allowed water trucked in from the river to be easily pumped inside. The lab met the requirements of both the United States Environmental Protection Agency (USEPA) and regulators of the Alton Gas project (EPA, 2002; Alton Gas, 2015). Specifically, it was isolated, air temperature was controlled, ventilation provided clean air free of oil or fumes, and all vessels and tubing for delivery of air and water were non-toxic (EPA, 2002). The lab design allowed 42 test vessels (6.8 L McDonald Jars; #J30, Pentair Aquatic Ecosystems, Miami, Florida, USA) to be operated in two separate water bath systems, composed of 3 troughs (14 in each trough; 21 in each system), at either 12 or 19°C (Figure 4). Temperature was controlled by mixing three water supplies (6, 10 and 20°C freshwater) in a header tank mounted to the wall. To supply oxygen and an upwelling water current for the eggs, compressed air was delivered to the bottom of each vessel through a rubber micro-bubble diffuser attached to a 25 mL pipet (Part # 07-200-15, Fisher Scientific, Ottawa, Canada) attached to 6 to 8 cm of rubber diffuser hose. Each vessel was also equipped with a water supply to facilitate the planned dilution from the test salinity to 2 ppt followed by 96 h of observation. Light intensity was controlled by a semi-transparent black plastic mesh covering the fluorescent ceiling lights. Light intensity was 20 lux for eggs and larvae, and 300 lux for juveniles. Allowing enough light to work while minimizing stress on the larvae as they are light sensitive. The quality control standard of

each test, approved by DFO and NSE, was mortality in the control of <30 % for eggs and larvae and <10 % among juveniles. Further, the mortality of the rearing tank was required to be <10% on a monthly basis for the juvenile stages prior to testing (see Table 1 for details).

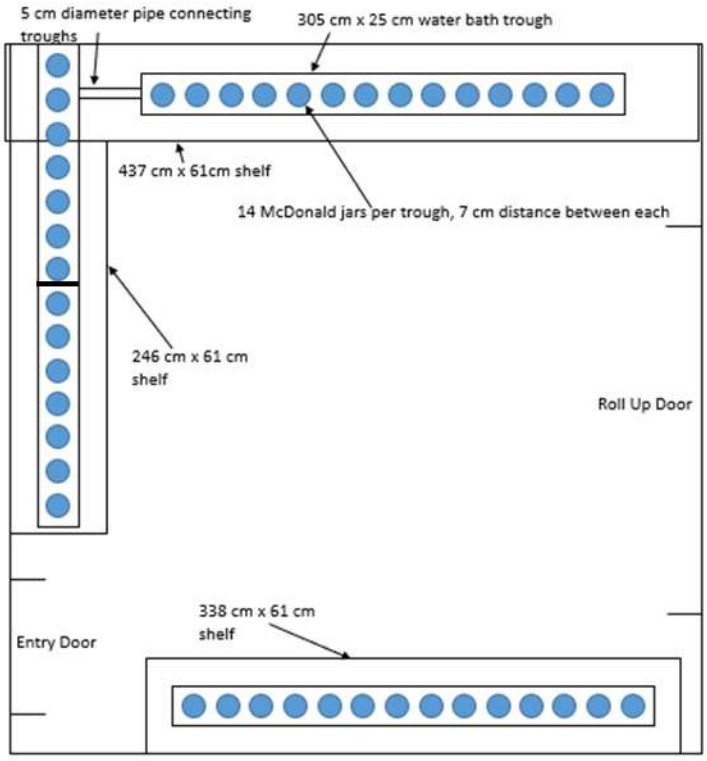


Figure 4: Plan of the Dalhousie Agriculture Aquatic Toxicology Lab showing the use of the trough system, vessels being McDonald Jars.

2.1 Egg Collection and Fish Rearing

Brine toxicity tests were conducted on both 'wild' and 'domestic' striped bass. The rearing methods were identical for both groups from the egg stage onwards. Domestic fish were derived from eggs produced from captive broodstock striped bass which themselves were reared from eggs captured from the Stewiacke River. The age of the domestic

broodstock ranged from five to eight years old. In both 2016 and 2017 each cohort of domesticated bass were either half- or full siblings produced from tank spawning of one female and three males. Wild striped bass eggs were collected in May-June 2016-18 late in the ebb-tide (<2 ppt salinity) from the Stewiacke River within 500m of the confluence with the Shubenacadie River (45°08'16.0" N 63°22'25.4" W) using a plankton net (1 mm mesh, 50 cm mouth, with a large collection bucket 15 cm in diameter; Aquatic Research Instruments, Hope, ID, USA). The eggs were carefully poured into a 10L plastic container (Rubbermaid), large debris was carefully removed, then using a plastic kitchen sifter (Home Hardware) were transferred into an insulated cooler (Coleman 12 L). Eggs were kept in suspension by gentle aeration from a small silica diffuser and a battery powered aquarium air pump. Eggs were then transported back to the lab, 30 minute transit time, and carefully transferred using a plastic sifter into an upwelling incubator with a flow-through supply of brackish water (1-5 ppt, 16-18 °C).

To induce spawning of adult domestic striped bass, thermal acclimation was needed, from 12 to 17-18 °C over a 10 day period, not exceeding 1 °C per day to minimize stress. At this point, females with oocytes at the correct stage of development received an Ovaplant (Syndel, Vancouver, Canada) pellet injected into the dorsal musculature. Each implant contained 150µg of Gonadotropin-releasing hormone (analog GnRH-a). This typically induced ovulation and oviposition in a few days. In case of failure, the female received an injection of Human Chorionic Gonadotropin or HCG (Chorulon; 350IU/kg; Fundy Vets, Murray Siding, Truro). Spawning was induced in a tank (2000L) with a single adult female and 2 to 3 males. Following spawning, fertilized eggs were collected by an

external egg collector attached to the rearing tank, then transferred to upwelling egg incubators at 16-18°C and 1 to 5 ppt salinity.

Table 1. Final environmental conditions during brine toxicology testing, and vessel type and maintenance for striped bass eggs, larvae and juveniles.

Test type	Static non-renewal
Test duration	1 h
Temperature	19 or 12 ºC
Light source	Fluorescent lamps
Light intensity	20 lux (eggs - larvae), 300 lux (juveniles)
Photoperiod	Not applicable
Test vessel size	15 mL well plate (eggs), 30 mL cup (yolk-sac and first feeding larvae), 2 L container (large larvae), 26 L tote (juveniles)
Test solution volume	10 mL (eggs), 2 L (larvae), 15 L (juveniles)
Renewal of test solutions	None
Age of test organisms	Between eggs and 155 days post hatch
Nº organisms per test vessel	20 (eggs and larvae), 10 (juveniles)
Nº replicate vessels per concentration	3
Nº organisms per concentration	60 (eggs and larvae), 30 (juveniles)
Feeding regime	No food
Test vessel cleaning	Cleaning not required
Test solution aeration	No
Dilution water	Filtered Stewiacke River, Dal-AC Domestic well water
Endpoint	Mortality
Test acceptability criterion	<30 % mortality in the control (eggs and larval stages) <10 % mortality in the control (juveniles)

Table 1 shows the guidelines and test conditions selected by DFO, NSE and Dalhousie University via which reflect USEPA guidelines. These guidelines were followed to the extent of practicality and where change was made it was reported to regulatory bodies for their approval.

Two designs of upwelling incubator were used, cone shaped (40L) and hemispherical (80L Aquabiotech, Coaticook, PQ). Time to hatch at 16-18ºC was two days for both wild and domestic eggs confirming the eggs retrieved from the estuary were at the early blastula stage when caught. Wild and domestic eggs were reared separately. At 3 days post-hatch (dph) larvae were transferred in water to a 1.5m diameter tank (40 cm water depth). Swim bladder inflation was facilitated by addition of a porcelain clay slurry (6-50, Dragonfire Pottery and Supplies, Dartmouth, NS, Canada), maintaining 150 NTU turbidity from 4 to 7 dph. Additionally, a spray of water was applied to the surface of the tank to break the surface tension (Clayton and Summerfelt, 2010). Larvae were fed Stage I *Artemia* nauplii (Aquafauna Bio-Marine, Inc., Hawthorn, CA, USA) from 6 to 12 dph. From 12 to 33 dph the larvae were fed stage II nauplii enriched with Algamac 3050 (Aquafauna Bio-Marine, Inc. Aquafauna). Larvae were reared between 2 and 5 ppt at 20°C. Age 35 dph larvae were weaned onto Gemma Micro 300ZF particulate diet then Nutra ST 0.3 (Skretting, Saint Andrews, NB, Canada). Pellet size increased with body size, all feeds were commercial salmonid diets (Nutra 0.5 and 1.0 mm, Skretting; Vita 1.5 and 2.0 mm, Ewos, Surrey, BC, Canada). Bass were reared in the 1.5m tank until about 10-12 mm TL (12-15 dph) then transferred to a small recirculation system (Aquabiotech) with six tanks (each 140L). Rearing temperature and salinity remained at 20°C and 2-5 ppt until mid-August (10g body size) when the salinity was decreased to 0 ppt, as the juveniles could then tolerate full-strength freshwater. The switch to freshwater was primarily for economical reasons since seawater had to be purchased. Photoperiod was Light: Dark 24:0 until August, then simulated natural daylength (Latitude 45 ºN). Light intensity at the water surface up to 10 dph was 0 lux, then near 30 lux after 10 dph (Light meter 840022,

Sper Scientific, Scottsdale, AZ, USA). All protocols were approved by the Faculty's Animal Care and Use Committee (ACUC 2016-52).

2.2 Toxicology Protocol

All tests followed the USEPA guidelines for measuring the acute toxicity of effluents on aquatic organisms (EPA, 2002). The initial range of test salinities was 2 ppt as a control, and six test salinities: 15, 25, 35, 45, 55 and 100 ppt, each with three replicates. These salinities were useful for range finding for each life stage, which were followed by a narrower series of salinities to quantify the LC_{50} to regulatory standards. Exposure time for the six test salinities was 1 h. After 1 h, the number of mortalities was recorded, then, in the initial year, 2016, tanks were diluted to 2 ppt, and then fish were observed for 96 h, following the original plans approved by DFO and NSE. This holding period was intended to determine if mortalities occurred in the days following exposure. The dilution and 96h observation steps were abandoned in 2017 and 2018 because they proved both unnecessary and impractical, as described further in Section 3.2. Hence, juvenile fish surviving the 1h test were placed into a recovery tank at 2 ppt. Survivors were never used in subsequent tests.

Four types of brine (>100 ppt) were produced using one of three sources of salt (Instant Ocean, Alton Core from 2006, and Alton Core from 2014) and one of two sources of dilution water (well water or river water). Combinations of brine included: Instant Ocean and well water, Instant Ocean and river water, Alton Core from 2006, and 2014 Alton Core from 880 to 933 m depth and river water. The 2014 core samples, each 1 m long and 10 cm diameter, were from Alton, Nova Scotia (Core hole 08-01, 45º12'04.9" N, 63º16'11.6" W). Water (<2 ppt) from the Stewiacke River (45°08'38.9" N 63°20'59.5" W) was

collected late in the ebb-tide using a 208 cc two inch trash pump (Red Lion, 6RLAG-2LST, Home Hardware, Canada) into a 1400 litre tank (#82124649, Global Industries, Ontario, Canada) on a ¾ ton truck (GMC 2500HD; Flint, Michigan, USA). Water was drawn from the Stewiacke River rather than the Alton Gas Site (45° 09.423 N -63° 23.133 E) on the Shubenacadie River, as site access from May 2017 onwards was permanently blocked by protestors from NGOs. The water stood in the trucking tank for 24 h to allow most of the suspended sediments to settle. It was then pumped (MD12, Danner Supreme, Islandia, NY, USA) into the lab through a one µM filter (#Bag1, Pentair Aquatic Ecosystems, Miami, Florida) into a 1000 litre insulated storage tank (Insulated Container #3201, Xactics Canada, Ontario, Canada). To create 100 ppt brine, either 28 kg of Instant Ocean Sea Salt (Spectrum Brands, Blacksburg, VA, USA) or 28 kg crushed core sample was mixed in 210 L of either river water or well water. Dissolution of the salt was achieved in 24 h by heating to 36°C (Top Light Excel 300 W, Rena, Charlotte, NC, USA) and vigorous aeration (airstone ALR23, Sweetwater, Pentair Aquatic Eco-Systems, Miami, FL, USA). The brine was then cooled to test temperature (Cyclone AE5DA, AquaLogic, San Diego, CA, USA). Measurement of the test salinities up to 70 ppt were made using a handheld meter (Pro2030, YSI, Yellow Springs, OH, USA), and above 70 ppt with a refractometer (STX-3, VeeGee, Kirkland, WA, USA), and by diluting 1:1 with distilled water and measured using a handheld meter.

2.3 Statistical Analysis

2.3.1 LC_{50} Determination

The statistical methods were recommended by the EPA (2002). The Trimmed Spearman-Karber (package "tsk") method via R 3.4.3, and R-Studio was used to analyse

the data (Hamilton et al., 1978; Stone, 2015). This program is more robust than PROC Probit in SAS or Minitab 18 as it can cope with data sets that do not fit the PROC Probit model due to data that isn't smooth or ideal. It also accommodates data sets that do not conform to the requirements of other procedures. This includes data sets where no mortality occurs in the control and 100 percent mortality occurs in the highest treatment with no mortality occurring in the other treatments. Additionally, data sets where mortality was higher in a low treatment level and lower at a high treatment level were still able to be analysed using the Trimmed Spearman-Karber method. This allowed all data to be analysed by the same statistical program. To compare paired toxicity tests within the same cohort, a difference was considered significant when the 95% confidence intervals did not overlap. Additionally, polynomial regressions of LC_{50} and body size were conducted for each combination of dilution water and salt type Sigma-Plot by Systat Software.

2.3.2 Analysis of Survival via Slope and TOEC

The highest test concentration of brine resulting in zero mortality was determined for each life stage and test condition by calculating the mean between the highest test concentration with no mortality and the concentration where mortality first occurred for each test. The slope of the survival curves for each life stage and test condition was calculated by linear regression between highest test concentration with 100 % survival and the lowest test concentration with 0 % survival, including all the intermediate points, then each pair of slopes were compared by two-sample *t*-test (SAS 9.4, Institute Inc., Cary, NC, USA). No Observed Effect Concentration (NOEC) was estimated for each test by comparing the survival of the different test concentrations with the control by an ANOVA, Proc Mixed model with Bonferroni adjustment (SAS 9.4). In cases where the normality of

the residuals was not met, the data was transformed, however due to the large number of zero values transformations could not make the data suitable. In such cases the non-parametric Kruskal-Wallis test was conducted in SAS 9.4. The Threshold Observed Effect Concentration (TOEC) was determined for each test condition, based on the geometric mean of the NOECs (EC 2005). To analyze the difference between two TOECs (geometric means), the NOECs were log transformed and a two-sample *t*-test was conducted in SAS (Zar, 2014).

2.3.3 Water Quality Analysis

The chemical composition of the dilution water (1 µm filtered estuary water) and brine samples were evaluated by Standard Water Analysis and Total Metals (AGAT Laboratories, Dartmouth, NS). Due to ion interference, not all the samples were analyzed to the same detection limit (Gros, 2013). Parameters were standardized to the lowest Reported Detection Limit possible that allowed a comparison between samples. Halite (%) of each sample was calculated from the proportion of sodium chloride relative to the total ionic composition. Salinity (ppt) of each sample was calculated from the chloride concentration (Wooster et al., 1969). The difference in the mean concentration of each parameter between brine made from either salt core or Instant Ocean was analyzed by a 2-sample *t*-test using SAS statistical software.

2.3.4 Body Size, Stage and Salinity Tolerance

To compare the effect of the type of brine (SC or IO) at 19 ºC a 2-way ANOVA (life stage and salt source) was conducted using the Proc Mixed model with Bonferroni adjustment (SAS 9.4, Institute Inc., Cary, NC, USA). Yolk-sac larvae were excluded from this analysis because only a single datum was available for the IO brine. A second 2-way ANOVA (life stage and temperature) was conducted to compare the effect of temperature (12 ºC or 19 ºC) when SC brine was used.

A linear regression model was used to analyze pooled 2016, 2017 and 2018 data sets for each water combination in relation to body length in mm. These statistical tests were conducted using Sigma Plot by Systat software.

2.3.5 Effect of Water Type on LC_{50}

Differences between the mean salinity tolerance of tests conducted using Instant Ocean and either river water or well water were analyzed using a two-way ANOVA in Minitab 18 software using Tukey's Test post-hoc.

Chapter 3: 1 h LC_{50} Toxicity Test Methodology, Results and Discussion

3.0 Introduction

The seasonal availability of striped bass eggs and larvae restricted toxicology testing to between May and September annually. The methods and results are arranged chronologically by year to allow a clear presentation of the technical challenges and the progressive refinement of the methods. The principal difficulties were encountered with the eggs and early stage larvae, because they were small, delicate, and transparent. Difficulties were compounded by the turbid river water required to be used as diluent, despite filtering it to 1 μM. The original protocol devised by J. Manriquez-Hernandez and J. Duston, approved by regulators in December 2015, proved unworkable for eggs and early larvae in tests conducted in 2016 and 2017. In 2016, testing started in July, as soon as the lab was assembled. Of the 83 tests completed over three seasons (2016-2018), only the 35 conducted in 2018 were published, since they most closely mimicked the conditions at the Main Site (Manriquez-Hernandez et al., 2020). The two parameters common to these 35 tests were the striped bass were all derived from wild eggs, and the diluent was river water. Moreover, the tests used salt from the planned cavern site (Core Sample ALT 08-01). The additional 48 tests reported here used striped bass derived from both domestic broodstock and wild stock, well water as diluent and brine made from the salt core from the Stellarton library (Core Sample 08-01). Tests using Instant Ocean brine served as an important control to the brine tests and are presented both here and in the published paper. This chapter reports the methods and results from each of the three years in sequence, followed by an overall discussion.

3.1. 2016 Methods

The laboratory was completed in July 2016, allowing one test on larvae and seven tests on juveniles that season. The methods were as devised in 2015, and approved by both DFO and NSE, but were based on no practical experience. Instant Ocean was the test toxicant since salt core was unavailable at that time, and Dal-AC well water as a diluent. Each test consisted of three replicates of each of 7 test salinities including one control for a total of 21 vessels. Test 1 was conducted in 21 McDonald jars each partly filled with 6 L of brine: a control at 2 ppt and 6 treatments at 15, 25, 35, 45, 55, and 100 ppt. Ten (10-20dph) wild striped bass larvae were added to each vessel using a small spoon. During the next hour, mortalities were assessed visually by as three observers through the clear sidewall of the jars. After the 1 h exposure the dilution phase started by running 2 ppt water into all 21 McDonald jars simultaneously at the rate of 250 mL/min. A mesh screen on the top of each jar aimed to retain the larvae, but it proved inefficient.

Trials 2 to 8, between July and November 2016, tested juveniles between 30 and 120 mm TL. The test vessels were 21 plastic bins (each 25L; Sterilite Clear View Latch 1763, 43.2 x 28.3 x 32.4 cm) partly filled with 15 L of test brine. Oxygen was maintained at 100% in each bin with compressed air and a silica diffuser (Marina 2.84 Cylindrical Air Stones, #A692, Walmart Canada). For 30 and 120 mm TL juveniles, 10 animals were transferred using a food-safe plastic sifter into each of 21 food-safe containers (2 L) containing rearing water. A small aquarium net was used to transfer the fish from the 2 L container to the 25 L test vessels. To prevent fish from escaping during the dilution phase and subsequent 96 h observation period, these vessels had a different mesh size and side

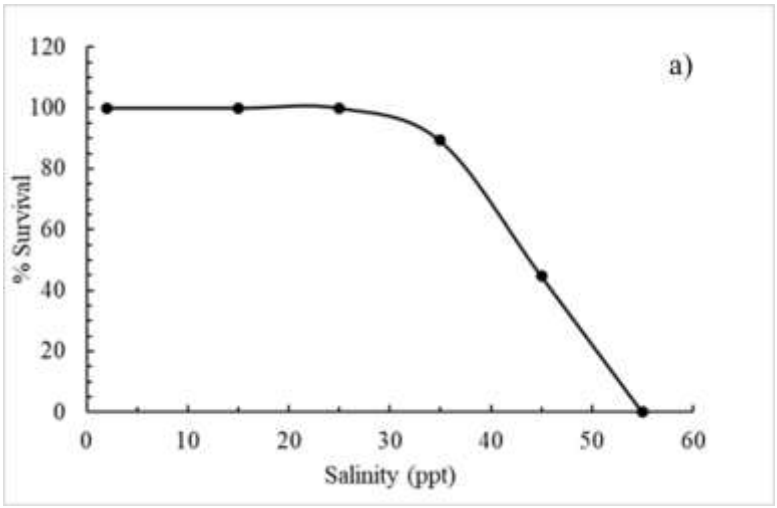
mounted overflow drains. All protocols for 2016 were approved by the local Animal Care and Use Committee (ACUC 2016-52).

3.2 2016 Results (Tests 1-8)

The McDonald jars were a suitable test vessel for use with well water and Instant Ocean brine for a 1h test, as the test specimens were visible in the clear water. The 23 mm TL larvae tested yielded a high tolerance to salinity (LC_{50}=43.4 ppt). The majority of mortality (96%) among the 2016 tests occurred during the 1 h test period. The survival curve based on mortalities after 1 h was smooth with mortality beginning at 35 ppt and 100% mortality at ≥ 55 ppt (Figure 5, a). The replicates had consistent mortality rates, except for the 35 and 45 ppt treatment level, where mortality ranged from 100 – 80% and 88 – 0% respectively (Figure 5, b). The estimated LC_{50} was 43.4 ppt, with 95% confidence limits (95% CI) between 41.3 and 45.6 ppt (Table 2). The attempt to track the survival for 96 h following dilution to 2 ppt, mimicking natural salinity patterns of the river failed. The dilution step (250 mL/min) resulted in larvae being flushed out of the McDonald jars because they could not be securely sealed. An additional confounding factor was the dilution time to reach 2 ppt ranged widely from between 10 minutes to 3 h due to the wide range of test salinities. The decision to abandon the 96 h at 2 ppt component of the salinity tolerance test was justified because there was negligible mortality. Additionally, the dilution and subsequent 96 h observation occupied the test vessels for the majority of a week, reducing the number of tests that could be completed in the short summer season. Survival among the larvae (Trial 1) retained in the jars at 2 ppt for 96 h was very high, 96%. Similarly, juveniles (Trials 2-8) exhibited 100% survival over 96 h following dilution to 2 ppt after the 1 h exposure to the test salinity. Moreover, the dilution procedure was

difficult to execute uniformly, and the 96 h holding period greatly reduced the scope for conducting trials due to the extended occupancy time.

Among juveniles, after 1h exposure, salinity tolerance (LC_{50}) increased with body size from 43 ppt for early juveniles with a FL of 46 mm to a maximum of 60.7 ppt for 123 mm FL (Trials 2 to 8; Table 2). 10-20 dph larvae (23 mm TL; Test 1) and early juveniles (46 mm TL; Test 2) had LC_{50} values of 43.4 and 43.0 ppt respectively, these values were not significantly different. Early juveniles, 33 mm FL (Test 3), had a significant increase in LC_{50} (49.0 ppt) compared to both Test 1 and Test 2. The salinity tolerance of both early juveniles 46 mm FL in 13 °C (Test 4; 54.2 ppt) and 120 mm juveniles (Test 7; 54.4 ppt) were similar based on overlap of the 95% CIs, however they were both significantly more tolerant to brine than Test 1 (10-20 dph larvae), 2 and 3 (46 and 33 mm FL early juveniles respectively). The LC_{50} values of early juveniles 63 mm FL (Test 5; 57.3 ppt) and larger juveniles 120 mm FL (Test 8; 46.8 ppt) were similar based on overlap of the 95% CIs but were significantly higher than Tests 1, 2, 3, 4 and 7. The highest LC_{50} value, 61 ppt, was exhibited by 120 mm juveniles of 120 mm FL (Test 6, Table 2). A single test (Test 4) was conducted at 13 °C, using early juveniles 46 mm in FL, yielding an LC_{50} value of 54.2 ppt.



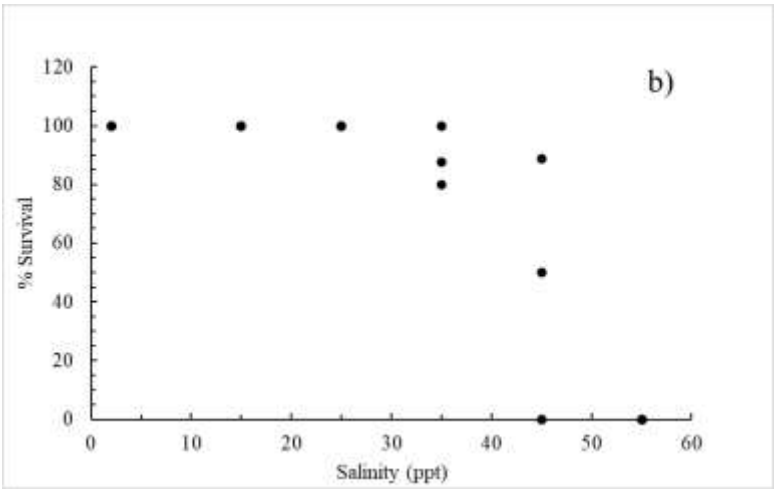


Figure 5: Trial 1: 10-20 dph striped bass larvae. a) Survival curve after 1 h exposure against test seven test salinities (ppt) with Instant Ocean brine and well water diluent at 19 °C. The LC_{50} was 43.4 ppt. b) survival among individual replicates (n=3).

Table 2. 2016 Results. Median lethal concentration (LC_{50} 1h) of Instant Ocean brine and well water on three early life stages of wild striped bass (0 to 135 days post hatch, dph) tested between 13 and 20 ºC. Length is TL for larvae and FL for juveniles. The Spearman-Karber method was used to determine lower and upper 95% confidence intervals (LCI, UCI)). Tests sharing the same letter are not significantly different, based on 95% CI overlap.

Test	Stage of Development	Length (mm)	°C	LC_{50} (ppt)	95% LCI	95% UCI
1	10-20 dph larvae	23	18	43.4^{A}	41.3	45.6
2	Early Juvenile	46	20	43.0^{A}	41.2	44.8
3	Early Juvenile	33	19	49.0^{B}	47.1	50.9
4	Early Juvenile	46	13	54.2^{C}	53.1	55.4
5	Early Juvenile	63	19	57.3^{D}	56.2	58.3
6	120 mm Juvenile	123	20	60.7^{E}	58.9	62.4
7	120 mm Juvenile	120	18	55.4^{C}	54.8	55.9
8	120 mm Juvenile	120	19	56.8^{D}	56.1	57.6

3.3 2017 Methods (Tests 9 - 42)

The dilution and 96 h observation phases were abandoned for 2017 because the 2016 tests demonstrated the dilution phase was difficult, lengthy and did not produce usable data beyond the initial exposure as 96% of all mortality occurred during the initial 1 h exposure. Both DFO and NSE approved the revised procedure based on examination of the 2016 results and associated report (Manriquez-Hernandez et al., 2017). Aeration was not used in 2017 as oxygen saturation remained >90% during all the 1 h tests. Moreover, the 'mist' of air bubbles made it difficult to see the transparent larvae and eggs. The difficulty visualizing the eggs and larvae through the wall of the McDonald jars was compounded by the relatively high turbidity of the river water diluent. Consequently, the McDonald jars were abandoned on June 7 for white plastic 2 L ice-cream containers (#QS21, Ropak, Springhill, Nova Scotia, Canada). Both eggs and larvae were relatively easy to see against the white background due to the pale green oil globule and black eyes respectively. This

method worked well for larvae, but eggs remained a problem primarily because we were unable to visually determine whether an egg was dead or alive at the end of the 1 h treatment. Another complication was the eggs floated in all treatments except 2 ppt salinity due to the high specific gravity of the brine. A suitable assay for testing eggs was not devised until 2018 (see section 3.5).

For toxicity tests on larval stages, each 2 L test vessel was partly filled with 1.5 L of brine solution, produced by either Instant Ocean or salt core, then salinity (conductivity), temperature, oxygen concentration and pH were recorded using various handheld meters (Pro2030, YSI, Yellow Springs, OH, USA; Orion 9107BNMD, Orion Star A121, Thermo Scientific, Beverly, MA, USA). Twenty larvae were counted into 21 sample cups (90 mL) by three people in about 15 minutes, each person using a small white plastic spoon. The 21 cups of larvae were then transferred into the brine test vessels within one minute. To account for the small dilution due to addition of rearing water, salinity was measured after each test, and this value was used for the LC_{50} estimation. After 1 h the trial ended and the number of dead larvae in each vessel was recorded, and survivors were euthanized. Six tests were completed during 2017 on 120 mm FL juveniles, using the identical methodology to 2016 except the dilution phase was eliminated (Section 3.1). During 2017, 34 valid tests were completed, using both Instant Ocean and Alton Core, with both river and well water. All protocols for 2017 were approved by the local Animal Care and Use Committee (ACUC 2017-44).

3.4 2017 Results

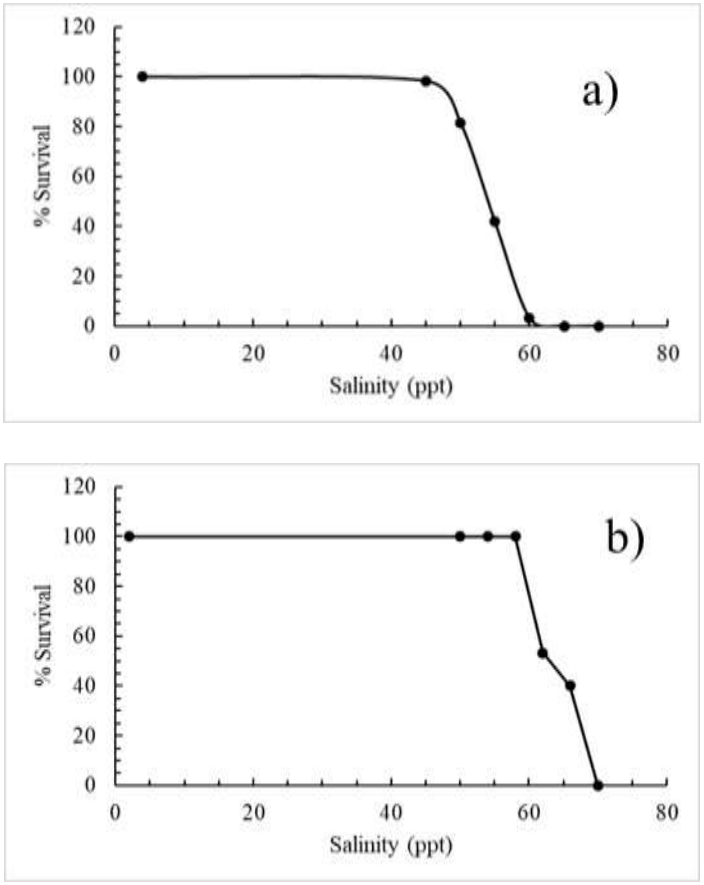


Figure 6: Survival curves from 2 tests conducted in 2017. Panel a, Test 19: yolk-sac larvae 4.0 mm total length, tested using Instant Ocean as a toxicant and well water as a diluent at 19°C. Panel b, Test 42: juveniles 124 mm in total length using Alton Core as a toxicant and river water as a diluent at 19°C .

Figure 6 shows the survival curves produced by two tests; the LC_{50} for the yolk-sac larvae (Test 19, Figure 6, a) was 53.9 ppt and for the 120 mm juveniles (Test 42, Figure 9146, b) was 63.7 ppt. The survival response curves in both tests are similar, and typical of all tests conducted in this thesis, with the difference between 100 percent survival and 0 % survival separated by only about 10 ppt (Figure 6).

Table 3. 2017 median lethal concentration (LC_{50} 1h) of Instant Ocean (IO) and well water brine on three early life stages of striped bass (0 to 17 days post hatch, dph) at 19 ºC. Size (mm) is total length. The LC_{50}, lower and upper 95% confidence intervals (95% CI), standard deviation of the LC_{50} estimate (SD), and trim level are shown. LC_{50} values sharing the same letter are not significantly different, based on 95% CI overlap.

Test	Stock	Stage	Age, dph	Size (mm)	LC_{50} (ppt)	95% CI	SD	Trim
9	Wild	YSL	4	4.0	53.9^{F}	53.0-54.9	0.5	0.1
10	Wild	5-10	5	4.5	46.3^{C}	44.7-47.9	0.8	0.1
11	Wild	5-10	8	9.1	41.4^{B}	40.4-42.3	0.5	0.3
12	Domestic	5-10	6	6.3	39.5^{A}	38.9-40.1	0.3	0.2
13	Wild	10-20	12	8.9	49.0^{DE}	48.1-50.0	0.5	0.3
14	Wild	10-20	20	11.8	59.3^{G}	58.5-60.2	0.4	0
15	Domestic	10-20	17	13.2	47.6^{CD}	46.7-48.5	0.5	0.5

In 2017, the initial seven tests used well water and Instant Ocean as the test brine (Table 3). The lowest salinity tolerance was exhibited by domestic 5-10 dph larvae, their LC_{50} was 39.5 ppt (Test 12, Table 3). Wild larvae, age 8 dph, were also relatively sensitive to the brine (Test 11; 41.4 ppt). Younger and older larvae, by comparison, were significantly more tolerant to the brine. The LC_{50} of wild larvae age 4 and 5 dph was 53.9 and 46.3 ppt respectively (Tests 9 and 10). Tests 13-15 on 10-20 dph larvae, resulted in LC_{50} estimates of 49.0 to 59.3 ppt, with the oldest larvae exhibiting the highest salinity tolerance (Test 14; Table 3).

Table 4. 2017 median lethal concentration (LC_{50} 1h) of Instant Ocean (IO) and river water brine on four early life stages of striped bass (8 to 143 days post hatch, dph) at 19 ºC. Size (mm) is total length for larvae and fork length for juveniles. The Spearman-Karber method was used to determine LC_{50}, lower and upper 95% confidence intervals (95% CI), standard deviation of the LC_{50} estimate (SD), and trim level. LC_{50} values sharing the same letter are not significantly different, based on 95% CI overlap.

Test	Stock	Stage	Age, dph	Size (mm)	LC_{50}	95% CI	SD	Trim
16	Wild	5-10	8	9.1	42.4^{B}	41.1-43.7	0.7	0.4
17	Domestic	5-10	6	6.3	35.5^{A}	33.2-37.8	1.1	0.5
18	Wild	10-20	12	8.9	48.3^{D}	47.4-49.3	0.5	0.3
19	Domestic	10-20	11	6.8	49.0^{DE}	47.8-50.1	0.6	0
20	Wild	10-20	15	10.5	46.1^{CD}	44.4-47.7	0.8	0.1
21	Domestic	10-20	13	8.5	44.7^{BC}	43.6-45.8	0.6	0
22	Wild	10-20	20	11.8	57.3^{H}	56.9-57.7	0.2	0.2
23	Domestic	10-20	17	13.2	44.7^{BC}	43.4-46.0	0.7	0
24	Domestic	10-20	20	11.4	49.8^{E}	48.9-50.8	0.5	0
25	Domestic	10-20	20	11.4	45.7^{DC}	44.7-46.8	0.5	0.5
26	Domestic	20-60	34	18.9	45.6^{DC}	44.7-46.5	0.4	0.4
27	Domestic	20-60	36	21	53.0^{FG}	51.8-54.1	0.6	0
28	Wild	20-60	43	22	60.3^{I}	58.6-62.0	0.8	0
29	Wild	20-60	52	41	51.8^{F}	50.4-53.2	0.7	0
30	Domestic	20-60	49	37	62.3^{J}	61.2-63.4	0.6	0.3
31	Wild	20-60	57	44	62.0^{IJ}	60.5-63.5	0.8	0.1
32	Domestic	120	119	121	64.7^{K}	63.8-65.5	0.4	0
33	Wild	120	130	121	64.8^{K}	63.8-65.5	0.6	0
34	Wild	120	143	123	63.9^{K}	63.5-64.3	0.2	0.2

Tests 16 to 34 in 2017 used brine made from a mix of river water and Instant Ocean salt and included four life stages: 5-10 dph, 10-20 dph, 20-60 mm, and 120 mm juveniles (Table 4, n=19 tests). The lowest LC_{50} estimate, 35.5 ppt, was exhibited by 5-10 dph

larvae 6.3 mm in TL (Test 17). Salinity tolerance increased significantly as the body size and age increased, as indicated by the progression in letter-groupings in Table 4. Accordingly, 5-10 dph larvae 9.1 mm in TL had an LC_{50} of 42.4 ppt (Test 16), and the next significant increase in LC_{50} was 46.1 ppt for 10-20 dph larvae 10.5 mm in TL (Test 20; Table 4). The highest LC_{50} values were exhibited by 120 mm juveniles yielding LC_{50} values of 64.7, 64.8, and 63.9 ppt respectively (Tests 32-34; Table 4).

Table 5: 2017 median lethal concentration (LC_{50} 1h) of Alton Core and river water brine on two early life stages of striped bass (34 to 143 days post hatch, dph) at 19 ºC. Size (mm) is total length. The Spearman-Karber method was used to determine LC_{50}, lower and upper 95% confidence intervals (95% CI), standard deviation of the LC_{50} estimate (SD), and trim level. LC_{50} values sharing the same letter are not significantly different, based on 95% CI overlap.

Test	Stock	Stage	Age, dph	Size (mm)	LC_{50}	95% CI	SD	Trim
35	Domestic	20-60	34	18.9	42.3^{A}	41.1-43.5	0.6	0.3
36	Domestic	20-60	36	21	45.4^{B}	44.2-46.5	0.6	0.3
37	Wild	20-60	43	22	58.3^{DE}	57.8-58.7	0.2	0.2
38	Wild	20-60	52	41	46.3^{BC}	44.6-48.1	0.9	0
39	Domestic	20-60	49	37	57.2^{D}	56.4-58.1	0.4	0.3
40	Domestic	120	119	121	61.0^{F}	59.7-62.3	0.7	0
41	Wild	120	130	121	65.7^{GH}	64.7-66.6	0.5	0
42	Wild	120	143	123	63.7^{G}	62.7-64.7	0.5	0

Eight tests in 2017 used brine made from river water and Alton Core (Table 5). Among these tests, the lowest LC_{50} value was exhibited by smallest fish (18.9 mm FL), 42.3 ppt (Test 35; Table 5). By comparison, juveniles that were 2 mm larger (21 mm FL) were significantly more tolerant, their LC_{50} was 45.4 ppt (Test 36; Table 5). In subsequent tests, salinity tolerance increased further with body size; the LC_{50} of bass 37 mm FL was

57.2 ppt (Test 39) compared to 61.0 ppt among juveniles 121 mm FL (Test 40). An additional test of juveniles which were 121 mm yielded a similar but statistically higher LC_{50} of 63.7 ppt (Test 42). Finally, 123 mm FL juveniles had the highest LC_{50} value for this diluent toxicant combination with an LC_{50} of 65.7 ppt (Test 41; Table 5).

3.5 2018 Methods (Tests 43-83)

For 2018, the test procedure for juveniles was the same as the previous year. The test procedure for larvae, although yielding good LC_{50} data in 2017, was improved further in 2018 to better observe the swimming behavior in response to the brine, as required by regulators. The biggest advance in methodology in 2018 was the development of a reliable method to test eggs, following repeated failures in 2017. The new procedure for eggs was adapted from Kupsco et al., (2017), utilizing tissue culture plates with six wells (Falcon, Corning Life Sciences, Oneota, NY, USA). Twenty eggs were added to each 15.5 mL well containing 10 mL of brine using a small, perforated plastic spoon that allowed excess water to drain off to avoid diluting the test solutions. Each well was stocked at 5 minutes intervals to allow time to view each well at the 1 h endpoint. After 1 h, egg survival was evaluated by quickly examining the eggs in each cell in turn under a low power dissecting scope (Leica EZ4; Germany). Dead eggs were easily identified by the cloudy appearance of the chorion, live eggs were transparent. For yolk-sac and first-feeding stage larvae, better behavioral observations were facilitated by utilizing 30 mL plastic mesh baskets (Café Cup, Spark Innovators, Fairfield, NJ, USA). Three replicate baskets were secured to the inside wall of 2 L plastic containers partially filled with 1.5 L of test brine. Twenty larvae were counted into a 90 mL specimen cup then carefully poured into each of the mesh baskets. To ensure the salinity was consistent across the three test baskets, the brine in the 2 L 'reservoir' was stirred for 5 seconds with a plastic tablespoon. After 1 h, survivors were

counted in the mesh baskets aided by hand-held LED light. Juveniles (30 mm and 120 mm) were tested in an identical method to 2017, in a 25 L bin, containing 15 L of solution for 1 h without aeration. All protocols for 2018 were approved by the local Animal Care and Use Committee (ACUC 2018-45).

3.6 2018 Results

Table 6. 2018 LC_{50} results of 1 h toxicology tests on wild caught striped bass eggs at 12.5 to 19.4°C, using either Instant Ocean or Alton Core as a toxicant and river water as a diluent. The 95 % upper and lower confidence intervals are shown. Values with different letters are significantly different based on no overlap of the 95% CI.

Test	Toxicant	Temperature °C	LC_{50}	95% LCI	95% UCI
43	Instant Ocean	19.4	65.0^{B}	61.5	68.7
44	Instant Ocean	19.3	55.2^{A}	52.9	57.5
45	Alton Core	19.2	54.2^{A}	51.0	57.4
46	Alton Core	19.2	54.3^{A}	51.3	57.2
47	Alton Core	12.5	57.4^{A}	54.6	60.3
48	Alton Core	12.5	56.4^{A}	53.2	59.5

Six tests were conducted on eggs in 2018, two using Instant Ocean and river water at about 19 °C, two using Alton Core and river water at 19 °C and two using Alton Core and river water at 12.5 °C (Table 6). Test 43, using Instant Ocean and river water at 19.4 °C gave an LC_{50} estimate of 65.0 ppt, significantly higher than the other five tests (Table 6). Tests 44 to 48 yielded similar median LC_{50} estimates, ranging from 54.2 ppt (Test 45) to 57.4 ppt (Test 47; Table 6).

Table 7. Mean median lethal concentration (LC_{50} 1h) of brine made from either salt core (SC) or Instant Ocean (IO) on six early life stages of striped bass (0 to 143 days post hatch, dph) at 19 ºC. Size (mm) is total length for larvae and fork length for juveniles. The Spearman-Karber method was used to determine LC_{50} ± 95% confidence intervals (95% CI), standard deviation of the LC_{50} estimate (SD), and trim level. Tests sharing the same letter are not significantly different, based on 95% CI overlap (Manriquez-Hernandez et al., 2020).

Test	Stage	Age, dph	Size	Salt	LC_{50}	95% CI	SD
49	Eggs	0		SC	54.2^{FGH}	51.0 - 57.4	1.6
50	Eggs	0		SC	54.3^{FGH}	51.3 - 57.2	1.5
51	Eggs	0		IO	65.0^{IJk}	61.3 - 68.7	1.9
52	Eggs	0		IO	55.2^{FGH}	52.9 - 57.5	1.2
53	YSL	3	5.0	SC	52.0^{F}	50.9 - 53.2	0.6
54	YSL	4	6.0	SC	55.0^{G}	54.7 - 55.4	0.2
55	YSL	3	5.0	IO	51.9^{F}	50.9 - 52.9	0.5
56	FFL	6	6.5	SC	41.2^{D}	40.1 - 42.4	0.6
57	FFL	6	6.5	SC	42.9^{D}	42.3 - 43.6	0.3
58	FFL	8	9.0	IO	42.4^{D}	41.1 - 43.7	0.7
59	FFL	8	6.1	IO	46.3^{E}	45.5 - 47.0	0.4
60	LL	14	7.4	SC	30.9^{A}	30.3 - 31.4	0.3
61	LL	15	10.3	SC	37.7^{C}	36.6 - 38.9	0.6
62	LL	12	9.0	IO	48.3^{E}	47.4 - 49.3	0.5
63	LL	13	7.0	IO	35.3^{B}	34.8 - 35.8	0.3
64	LL	15	11.0	IO	46.1^{E}	44.4 - 47.7	0.8
65	LL	20	12.0	IO	57.3^{H}	56.9 - 57.7	0.2
66	EJ	57	38.4	SC	54.4^{FGH}	51.1 - 57.6	1.6
67	EJ	57	38.4	SC	57.6^{H}	56.8 - 58.3	0.3
68	EJ	43	23.0	IO	60.3^{I}	58.6 - 62.0	0.8
69	EJ	52	41.0	IO	51.8^{F}	50.4 - 53.2	0.7
70	EJ	57	44.0	IO	62.0^{I}	60.5 - 63.5	0.8
71	EJ	59	41.8	IO	66.0^{Jk}	64.0 - 68.0	1.0
72	Juvenile	130	121.0	SC	65.7^{Jk}	64.7 - 66.6	0.5
73	Juvenile	131	126.5	SC	65.5^{k}	64.9 - 66.0	0.3
74	Juvenile	143	124.0	SC	63.7^{J}	62.7 - 64.7	0.5
75	Juvenile	127	129.7	IO	64.2^{J}	63.7 - 64.8	0.3
76	Juvenile	130	121.0	IO	64.8^{Jk}	63.8 - 65.9	0.6
77	Juvenile	143	124.0	IO	63.9^{J}	63.5 - 64.3	0.2

Salt core brine toxicity (LC_{50} 1h) on eggs at 19 ºC was 54.3 ± 0.0 ppt (mean ± SE; tests 49 and 50; Table 7). Yolk-sac larvae (3 - 4 dph) tolerance to SC brine was similar to

eggs, median LC_{50} was 53.5 ± 1.5 ppt with a significant difference between tests 53 and 54 (Table 7). First-feeding larvae (6 dph) were significantly more sensitive to SC brine than both eggs and yolk-sac stages, their mean LC_{50} was 42.1 ± 0.9 ppt (tests 56 and 57; Table 7). Large larvae 14 - 15 dph were the least tolerant of all developmental stages, their median LC_{50} was 34.3 ± 3.4 ppt (tests 60 and 61; Table 7). The tolerance of early juveniles to SC brine was similar to eggs and yolk-sac larvae, mean LC_{50} was 56.0 ± 1.6 ppt (tests 65 and 66; Table 7). The larger 12 cm juveniles (130 - 143 dph) were even more tolerant, the mean LC_{50} was 65.0 ± 0.6 ppt (tests 72 to 74; Table 7).

The toxicity of Instant Ocean brine at 19 ºC was not significantly different to SC brine at 19 ºC. Among eggs, LC_{50} 1h was 60.1 ppt compared to 54.3 ppt in SC brine (tests 52 and 53; Table 7). Among yolk-sac larvae (3 dph), the LC_{50} of IO brine was 51.9 ppt (test 56; Table 7), similar to the 53.5 ppt for SC brine. First-feeding larvae (8 dph) mean LC_{50} in IO brine was 44.4 ppt compared to 42.1 ppt in SC brine. Large larvae (12 - 20 dph) LC_{50} in IO brine ranged widely from 46.1 to 57.3 ppt in four tests (62 to 65; Table 7), the overall mean was 50.6 ppt. Early juveniles, 4 cm long, also exhibited significant variability in their LC_{50} values, range 51.8 to 66.0 ppt, the overall mean was 60.0 ppt (tests 68 to 71, Table 7).Among large juveniles, by comparison, the LC_{50} values were consistent, ranging from 63.9 to 64.8ppt, mean 64.3 ppt (tests 75 to 77, Table 7).

Table 8. Low temperature (12°C) median lethal concentration (LC_{50} 1 h) of salt core brine (Alton Core + river water) on early life stage striped bass (0 to 20 dph). Size (mm) is total body length. The Spearman-Karber method was used to determine LC_{50}, lower and upper 95% confidence intervals (95% CI), standard deviation of the LC_{50} estimate (SD), and trim level. Tests sharing the same letter are not significantly different, based on 95% CI overlap (Manriquez-Hernandez et al., 2020).

Test	Stage	Age, dph	Size	LC_{50}	95 % CI	SD	Trim
47	Eggs	0	N/a	57.4^{FG}	54.6-60.3	1.5	0.0
48	Eggs	0	N/a	56.4^{F}	53.2-59.5	1.6	0.0
78	Yolk-Sac Larvae	3	6.0	39.4^{ABC}	38.5-40.2	0.4	0.5
79	Yolk-sac Larvae	4	6.0	36.8^{A}	33.8-39.7	1.5	0.0
80	First-feeding larvae	7	7.0	39.7^{ABCD}	38.9-40.5	0.4	0.0
81	First-feeding larvae	7	7.0	37.8^{A}	37.0-38.6	0.4	0.5
82	Large larvae	16	10.5	38.0^{A}	36.9-39.1	0.6	0.3
83	Large larvae	20	11.0	44.8^{E}	43.7-45.9	0.6	0.5

For tests conducted at 12 ° C, median lethal concentration values for eggs were 57.4 and 56.4 ppt respectively (Tests 47, 48; Table 8). Yolk-sac larvae had a median lethal concentration ranging between 36.8 and 39.4 ppt (test 78, 79; Table 8). First feeding larvae had similar salinity tolerance with median lethal concentrations ranging from 37.8 to 39.7 ppt. Finally, large larvae, the largest life stage that might be exposed to low temperatures in the estuary, had median lethal concentrations of 38.0 and 44.8 ppt respectively (Table 8).

Comparing salinity tolerance at 12 with 19 ºC, among eggs the LC_{50} estimates were similar 56.9 ppt (tests 47 and 48; Table 8), vs. 54.3 ppt respectively (Table 7). By contrast, yolk-sac larvae (3 - 4 dph) salinity tolerance was reduced considerably at 12 ºC compared

to 19 ºC, 38.1 ppt (tests 78 and 79; Table 8) vs. 53.5 ppt (mean of tests 53-55; Table 7). First-feeding larvae (7 dph) LC_{50} was independent of temperature: 38.8 ppt at 12 ºC (tests 80 and 81; Table 8) and 42.1 ppt at 19 ºC (mean of tests 56-59; Table 7). Finally, large larvae (16 - 20 dph) exhibited significantly better survival at 12 ºC than at 19 ºC; 41.8 ppt (tests 82 and 83; Table 8) vs. a mean of 34.3 ppt for tests 60-65 (Table 7).

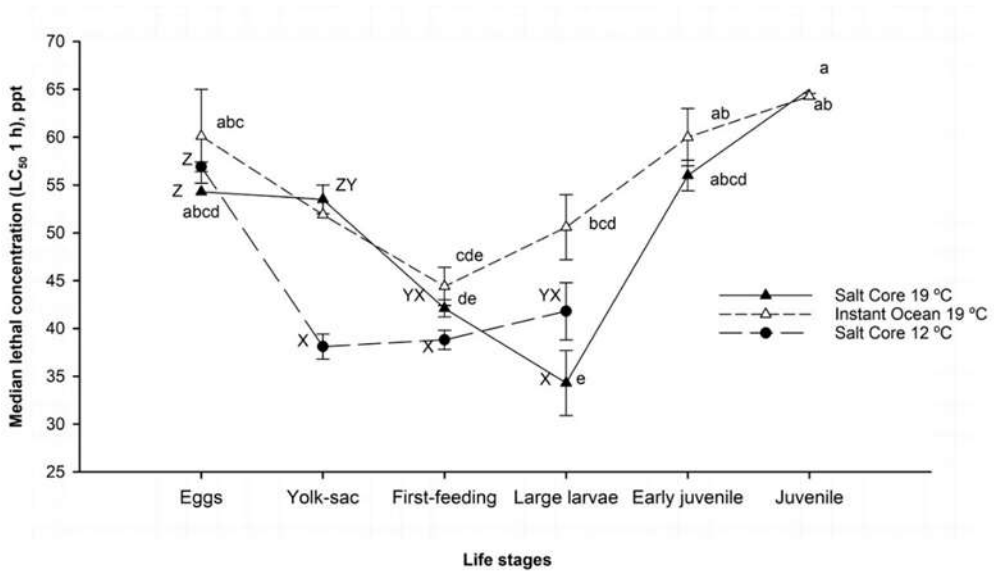


Figure 7. Median lethal concentration (LC_{50} 1h) of brine made from either salt core or Instant Ocean and river water on early stages of striped bass: eggs, yolk-sac larvae (3 - 4 days post-hatch, dph), first-feeding larvae (6 - 8 dph), large larvae (14 - 20 dph), early juvenile (43 - 57 dph) and juvenile (130 - 143 dph) exposed to 19 ºC and 12 ºC. Coordinates show the mean and standard error. At each developmental stage, lower case letters compare tests at 19ºC: Instant Ocean vs. salt core brine; upper case letters compare tests in salt core brine at 12 vs 19ºC. Means sharing the same letters are not significantly different $p>0.05$ (Manriquez-Hernandez et al., 2020).

Forty tests were completed in 2018, 35 of which are presented in Figure 7as the mean LC_{50} and standard error by stage. Both eggs and yolk-sac larvae tested in Instant Ocean and river water at 19 °C exhibited LC_{50} values that were not significantly different, despite the 8 ppt difference in the means (60 ± 4.9 vs. 52 ± 0 ppt). First-feeding larvae were significantly less tolerant to Instant Ocean brine 19ºC than the yolk-sac stage, 44 ± 2

ppt vs. 52 ± 0 ppt (Figure 7). For the large larvae, their LC_{50} was 7 ppt higher than first-feeding stage, at51 ± 3.4 ppt, but the difference was not significant (Figure 7). Through the early juvenile stage, salinity tolerance in Instant Ocean at 19 ºC increased significantly, reaching 64 ± 0.3 ppt among 120 mm juveniles (Figure 7).

LC_{50} estimates in brine made from salt core and river water at 19 °C, were similar to Instant Ocean brine for both egg and yolk-sac larvae (Figure 7). First-feeding larvae had an LC_{50} of 42 ± 0.9 ppt in salt core brine at 19ºC, which did not significantly differ from tests in Instant Ocean, and followed a similar trend presented by first-feeding larvae tested in Instant Ocean, where both the eggs and the yolk-sac larvae had significantly lower LC_{50} values. Large larvae in the salt core and river water combination at 19 °C had significantly lower LC_{50} values, (34 ± 3.4 ppt) compared to both first-feeding larvae (42 ± 0.9 ppt) and to large larvae tested in Instant Ocean and river water at 19 °C (51 ± 3.4 ppt; Figure 7). The early juvenile stage had a significant increase in LC_{50} to 56 ± 1.6 ppt, similar to the same developmental stage tested in Instant Ocean and river water at 19 °C (60 ± 3 ppt; Figure 7). 120 mm juveniles exhibited similar LC_{50} values to the early juvenile stage (65 ± 0.6 ppt) in salt core brine at 19 ºC, which was similar to the same juvenile stage tested in Instant Ocean and river water at 19 °C (64 ± 0.3 ppt; Figure 7).

Tests conducted at 12 °C using salt core and river water yielded similar LC_{50} estimates as tests at 19 ºC for egg, first-feeding larvae and large larvae (Figure 7). Yolk-sac larvae, by contrast, exhibited a markedly lower salinity tolerance at 12 ºC compared to 19 ºC (38 ± 1.3 vs. 54 ppt; Figure 7). Among first-feeding and large larvae stages, their salinity tolerance at 12 ºC was not significantly different from tests at 19 ºC (Figure 7).

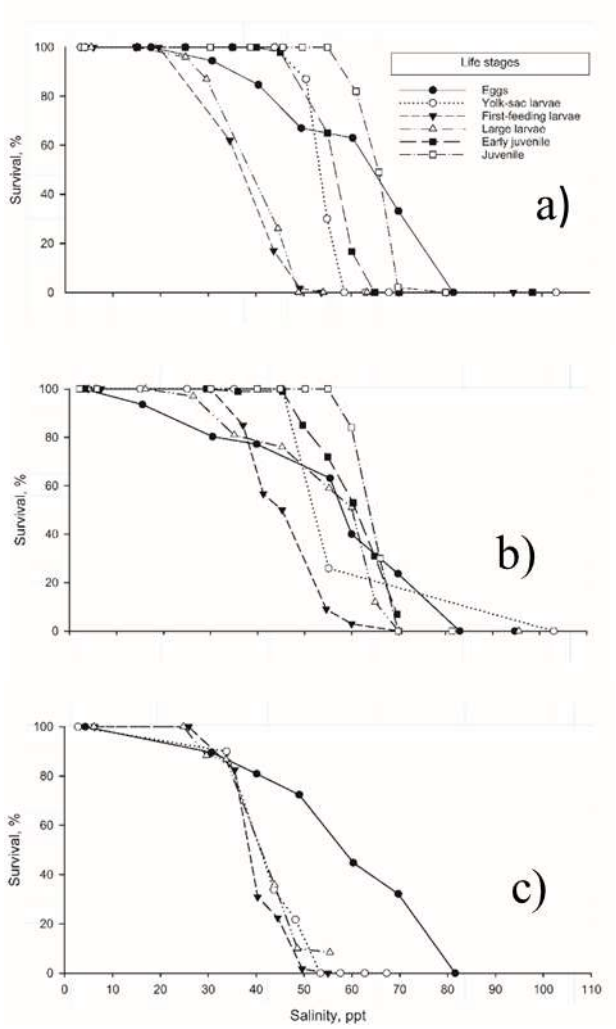


Figure 8. Mean survival of striped bass early life stages exposed for 1 h to: a) salt core (SC) brine at 19 ºC; b) Instant Ocean brine at 19 ºC; c) and SC brine at 12 ºC. The life stages were eggs, yolk-sac larvae (3 - 4 days post-hatch, dph), first-feeding larvae (6 dph), large larvae (14 - 15 dph), early juvenile (57 dph) and juvenile (130 - 143 dph; Manriquez-Hernandez et al., 2020).

Survival curves for five of the six life stages had a similar shape, the exception being eggs which exhibited a significantly lower slope in the three test conditions (Figure 8). The slope of eggs, first-feeding larvae, large larvae and juvenile did not vary between salt source or temperature ($p > 0.05$). The slope of the survival curves of yolk-sac larvae, by contrast, was significantly higher in salt core brine at 19 ºC (sudden acute mortality; Figure 8a) than at 12 ºC (Figure 8c; $p = 0.044$). Finally, the slope of the survival curve of

early juveniles was higher in IO brine 19 ºC (Figure 8b) than in SC brine 19 ºC (Figure 8a; $p = 0.038$).

Table 9. Threshold observed effect concentration (TOEC) of a brine river water combination, either derived from salt core (SC) or Instant Ocean (IO) for 1 h exposure of six early life stages of striped bass ranging from 0 to 143 days post hatch, at 19 or 12°C. TOECs sharing the same letters are not significantly different (two-sample t-test, α 0.05). TOECs without lettering are single values.

Test Condition	**Eggs**	**Yolk-Sac**	**First-Feeding**	**Large Larvae**	**Early Juveniles**	**Juvenile**
DPH	**0**	**1-4**	**5-10**	**11-20**	**57**	**130-143**
SC @ 19 °C	30.8	46.6^{ABC}	41.4^{ABC}	24.4	45.1^{BC}	59.7^{A}
IO @ 19 °C	42.1^{AB}	45.2	29.6^{ABC}	40.0^{ABC}	46.3^{AB}	58.0^{AB}
SC @ 12 °C	30.7	N/a	25.8^{C}	31.5^{BC}	---	---

The TOEC following 1 h exposure ranged from 25.8 to 59.7 ppt depending on stage of development, brine type and temperature (Table 9). In SC brine at 19 ºC, large larvae and eggs were the most vulnerable life stages (TOEC 24.4 and 30.8 ppt, respectively; Table 9). Early juveniles were significantly less tolerant than the 12 cm juveniles (TOEC 45.1 vs. 59.7 ppt, respectively; Table 9). In IO brine at 19 ºC and SC brine 12 ºC, TOEC exhibited no significant difference among the life stages (Table 9).

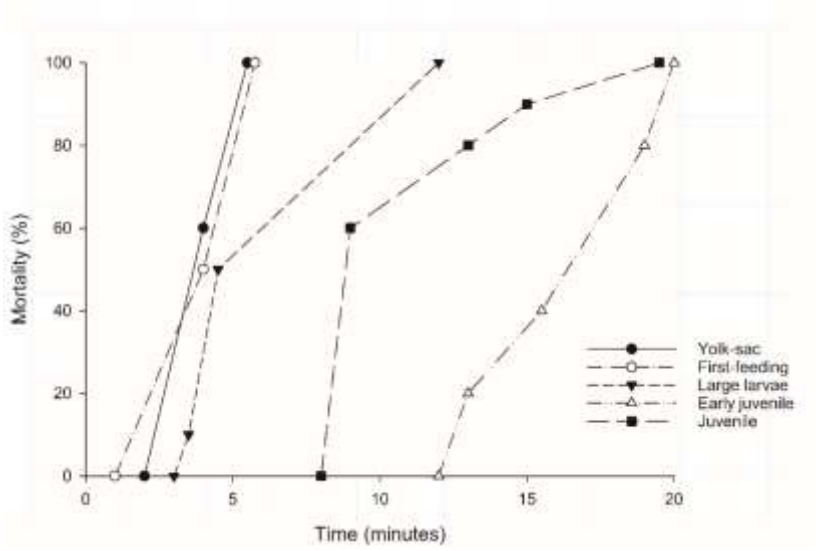


Figure 9. 2018. Time to 100% mortality for four striped bass life stages exposed to 100 ppt salt core and river water brine at 19 ºC. The large larvae stage was exposed to 100 ppt brine made from Instant Ocean and river water brine. Yolk-sac larvae (1 - 4 days post-hatch, dph), first-feeding larvae (5 - 10 dph), large larvae (11 - 20 dph), early juveniles (ca. 54 dph) and juveniles (ca. 134 dph; Manriquez-Hernandez et al., 2020).

Yolk-sac larvae (5 mm TL) exposed to 100 ppt SC brine began to die after 3 min, and 100 % mortality was reached at 5.5 min (Figure 9). First-feeding larvae (6 dph, 6.5 mm TL) reached 50 % mortality at 4 min, and 100 % at 5.75 min (Figure 9). Large larvae (13 dph, 10 mm TL, exposed to IO brine) mortality started around 3.5 min and reached 100 % at 12 min (Figure 9). Early juveniles (4 cm FL) started to die after 10 min, mortality was 20 % at 13 min, and reached 100 % at 20.5 min (Figure 9). Juveniles (12 cm FL) in 100 ppt brine started to die at 9 min and reached 100 % mortality at 19.5 min (Figure 9).

Table 10. Mean (SE) water quality parameters of the dilution river water (n = 9), salt core brine (n = 7) and Instant Ocean brine (n = 7). Salinity and halite were calculated from the parameters analyzed in the lab. RDL = Report Detection Limit. * Indicates a significant difference between the salt core brine and the Instant Ocean brine (P<0.05; 2-sample t test). Brine was prepared by dissolving 28 kg salt in 210 L filtered river water (Manriquez-Hernandez et al., 2020).

Parameter	Unit	RDL	Dilution	Salt core brine	Instant Ocean
pH			7.81 ± 0.06	7.48 ± 0.05	7.89 ± 0.03*
Hardness	mg/L		398 ± 132	2,360 ± 282	21,514 ± 979*
Total alkalinity	mg/L	5	40 ± 5	43 ± 3	201 ± 40*
Bicarbonate alkalinity (as $CaCO_3$)	mg/L	0.1	39.9 ± 4.7	43.4 ± 3.3	201.1 ± 40.1*
Carbonate alkalinity (as $CaCO_3$)	mg/L	10	< 10	< 10	< 10
Nitrite as N	mg/L	50	< 50	127 ± 5	93 ± 24
Ammonia as N	mg/L	0.03	0.17 ± 0.06	0.17 ± 0.04	1.14 ± 0.51
True color	TCU	5	26 ± 4	23 ± 7	17 ± 2
Turbidity	NTU	0.1	209.7 ± 56.5	119.4 ± 34.9	321.7 ± 116.3
Chloride	mg/L	1	894 ± 321	72,886 ± 4,974	65,571 ± 2,828
Conductivity	µS/c	1	3,335 ± 1,154	150,143 ± 9,478	137,429 ± 3,884
Calculated total dissolved [illegible]	mg/L	1	1,858 ± 646	128,571 ± 7,600	123,143 ± 3,826
Sulphate	mg/L	2,000	< 2,000	2,800 ± 790	9,746 ± 272*
Sodium	mg/L	0.1	608 ± 226	52,914 ± 2,973*	40,371 ± 1,546
Potassium	mg/L	0.1	24.0 ± 8.4	52.3 ± 12.1	1,511.4 ± 51.2*
Calcium	mg/L	0.1	46.2 ± 10.4	798.1 ± 134.8	1,428.6 ± 59.4*
Magnesium	mg/L	0.1	68.6 ± 26.0	89.0 ± 33.4	4,355.7 ± 231.0*
Calculated salinity	ppt	0.002	1.6 ± 0.6	131.7 ± 9.0	118.5 ± 5.1
Calculated halite	%		80.7 ± 4.9	93.5 ± 1.7*	81.0 ± 1.2

Table 11. Total metals in the dilution river water (n = 9), Nova Scotia Environmental Quality Standards to protect freshwater and marine pelagic aquatic life (NSE 2013), salt core brine (n = 7) and Instant Ocean brine (n = 7). All values are mean (SE). All units are µg/L. RDL = Report Detection Limit. * Indicates a significant difference between the salt core brine and the Instant Ocean brine ($p<0.05$; 2-sample t test). Brine was prepared by dissolving 28 kg salt in 210 L filtered river water (Manriquez-Hernandez et al., 2020).

Parameter	RDL	Dilution water	Fresh/Marine Standard	Salt core	Instant Ocean
Aluminum	5	3,531 ±	5 / -	3,203 ± 637	2,617 ± 1,109
Barium	5	26.1 ± 3.4	1,000 / 500	34 ± 4	134 ± 10*
Boron	5	252 ± 92	1,200 / 1,200	1,444 ± 313	15,343 ± 951*
Copper	1	5 ± 1	2 / 2	64 ± 23	73 ± 11
Iron	50	3,894 ±	300 /-	4,970 ± 860	3,832 ± 1,389
Lead	0.5	4.4 ± 0.9	1 / 2	9.2 ± 1.6	7.5 ± 3.4
Molybdenum	2	3 ± 0	73 / -	5 ± 1	10 ± 0*
Nickel	2	7 ± 2	25 / 8.3	45 ± 3	64 ± 9*
Silver	0.1	0.3 ± 0.1	0.1 / 1.5	4.0 ± 3.6	0.3 ± 0.1
Strontium	5	569 ± 163	21,000 / -	5,069 ± 608	30,857 ± 1,327*
Uranium	0.1	0.4 ± 0.1	300 / 100	1.7 ± 0.3*	0.4 ± 0.1
Vanadium	2	19 ± 3	6 / 50	468 ± 69	399 ± 40

The chemical composition of the dilution water exhibited some variation due to natural fluctuations in the tide and freshwater run-off (see Shubenacadie River estuary water, Tables 10 and 11). Nevertheless, it was consistently very hard (mean 398 mg/L), well buffered (total alkalinity 40 mg/L), oligotrophic (nitrite < 50 mg/L, ammonia 0.17 mg/L) and slightly saline (total dissolved solids (TDS) 1,858 mg/L, 1.6 ppt; Table 10). After filtration to 1 micron the water remained turbid (210 NTU); true color 26 TCU (Table 10). Total metals were highly correlated with turbidity, indicating they were bound to particulates and biologically inactive.

Salt core (SC) brine and Instant Ocean (IO) brine were similar in mean chloride concentration, conductivity, TDS and calculated salinity (Table 10). Salt core brine was

significantly higher than IO brine in calculated halite (94 vs. 81 %), sodium (53 vs. 40 mg/L) and uranium (1.7 vs. 0.4 µg/L), and significantly lower in magnesium (-98 %), potassium (-97 %), boron (-91 %), hardness (-89 %), strontium (-84 %), total alkalinity (-78 %), barium (-75 %), sulphate (-71 %), molybdenum (-51 %), calcium (-44%), nickel (-30 %) and pH (-5 %; Tables 8 and 9).

In test solutions at 19 ºC, the pH of the SC brine was significantly lower than IO brine (mean ± SE, 7.52 ± 0.10 vs. 8.07 ± 0.05), but dissolved oxygen was similar (98 ± 1 % vs. 97 ± 1 %). Tests at 12 ºC were conducted with SC brine only, the parameters were like those at 19 ºC, pH 7.64 ± 0.05 and dissolved oxygen 100 ± 1 %.

3.7 Pooled 2016, 2017 and 2018 Toxicology Data

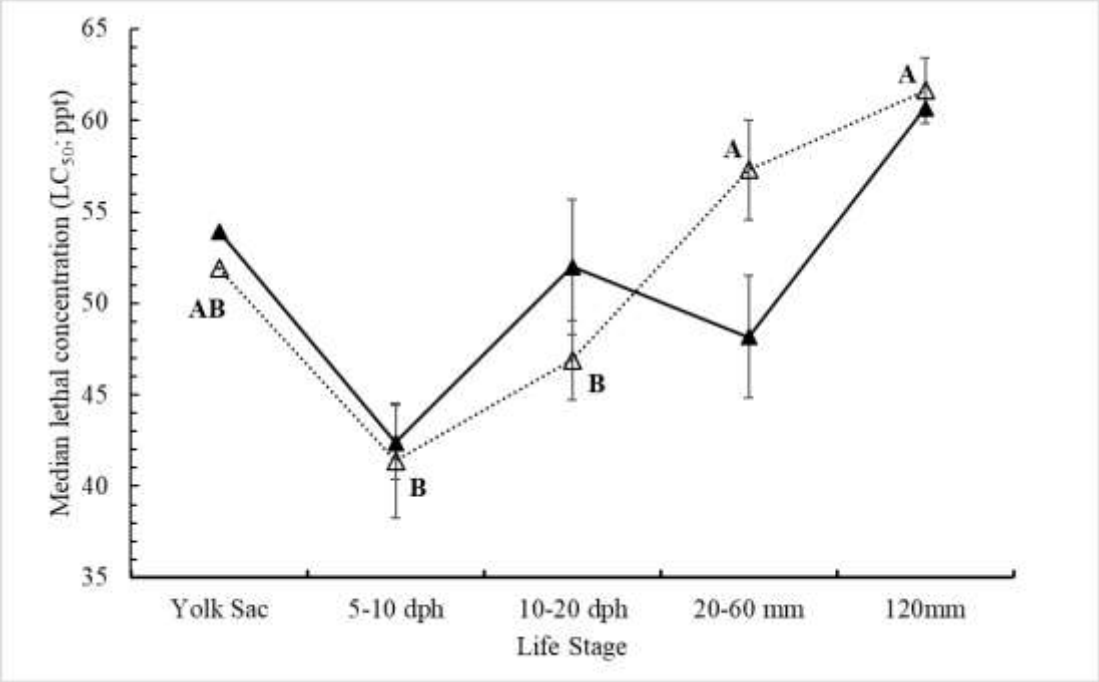


Figure 10. Median lethal concentration (LC_{50} 1h) of brine created by Instant Ocean and river water (dashed line, outlined triangle; n=25) or well water and Instant Ocean (black line, solid triangle; n=12) on early life stages of striped bass: yolk-sac larvae (3 - 4 days post-hatch, dph), first-feeding larvae (5-10 dph), large larvae (11-20 dph), early juvenile (20-60 mm) and juvenile (>120 mm) at 19 °C. This is pooled data from test conducted in 2016, 2017, and 2018 (total n=37) including tests using both domestic and wild cohorts. Mean of the LC_{50} by stage and test condition. Error bar = SE of the mean. Means with different letters are significantly different (one-way ANOVA, $p \leq 0.05$).

An effect between developmental stage and LC_{50} was not evident in tests using river water, and absent from those using well water. No significant difference between water type was found following a 2-way ANOVA (river vs. well; p=0.576). The median lethal concentration (LC_{50} 1 hr) of brine created using Instant Ocean and well water was 53.9 ppt for yolk-sac larvae, 42.4 ppt for 5-10 dph larvae), 52.0 ppt for 10-20 dph larvae, 48.2 ppt for 20-60 mm juveniles , and 60.7 ppt for 120 mm juveniles . For combinations of Instant Ocean and water collected from the Stewiacke-Shubenacadie river estuary salinity tolerance remained similar. The median lethal concentration for yolk-sac larvae was 51.9 ppt, 41.4 ppt for 5-10 dph larvae, 46.9 ppt for 10-20 dph larvae, 57.3 ppt for 20-

60 dph juveniles and 61.6 ppt for 120 mm juveniles. For yolk-sac larvae, 5-10 dph larvae and 120 mm juvenile's mean tolerance was similar, however, 10-20 dph larvae and 20-60 mm juvenile tolerance differed, with tests in well water yielding higher LC_{50} values in the 10-20 dph category and lower values in the 20-60 mm juvenile category.

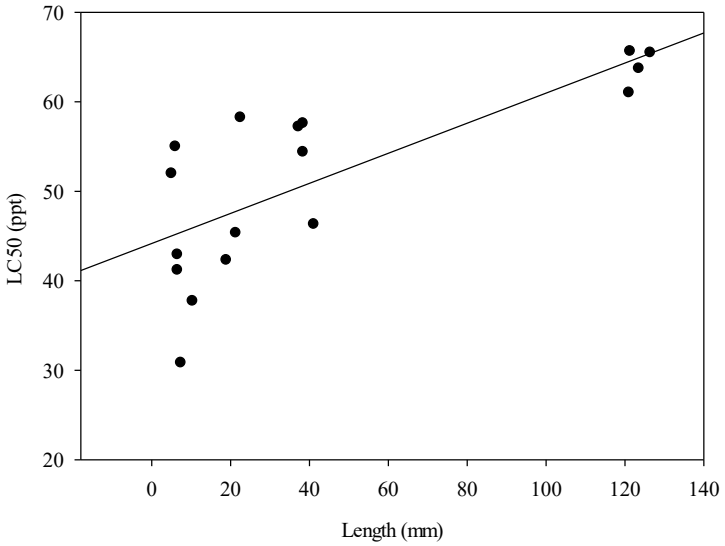


Figure 11. Salt core brine with river water as diluent relationship between salinity tolerance (LC_{50}; ppt) and body length (TL between 0 and 30 mm, and FL above 30 mm). This pooled data from 2017 and 2018 and includes both wild and domestic striped bass. The regression is represented by the equation Y = 43.720 + (0.190 * X). There is moderate correlation (R= 0.681, R^2= 0.463 R^2Adj = 0.440). The test output is highly significant, where the p-value is <0.001.

Salinity tolerance increased with body size (Figures 11, 12, 13). Exposed to salt core brine with river water as diluent, yolk-sac larvae had a mean LC_{50} of 53.5 ppt and a minimum LC_{50} of 52.0 ppt and a maximum of 55.0 ppt (Figure 11). 5-10 dph larvae had a mean LC_{50} of 42.1 ppt with the minimum determined LC_{50} being 41.2 ppt and the maximum being 42.9 ppt. 10-20 dph larvae had a mean LC_{50} of 34.3 ppt, with a minimum LC_{50} of 30.9 ppt and a maximum of 37.8 ppt. 20-60 mm juveniles had a mean LC_{50} of 51.6 ppt, with a minimum LC_{50} of 42.3 ppt and a maximum of 58.3 ppt. Lastly, 120 mm

juveniles had a mean LC_{50} of 66.7 ppt and a minimum LC_{50} of 61.0 ppt and a maximum of 65.7 ppt. The tests were conducted using river water as a diluent and Alton Core as a toxicant, showing an increasing trend in salinity tolerance and LC_{50} as body length increased.

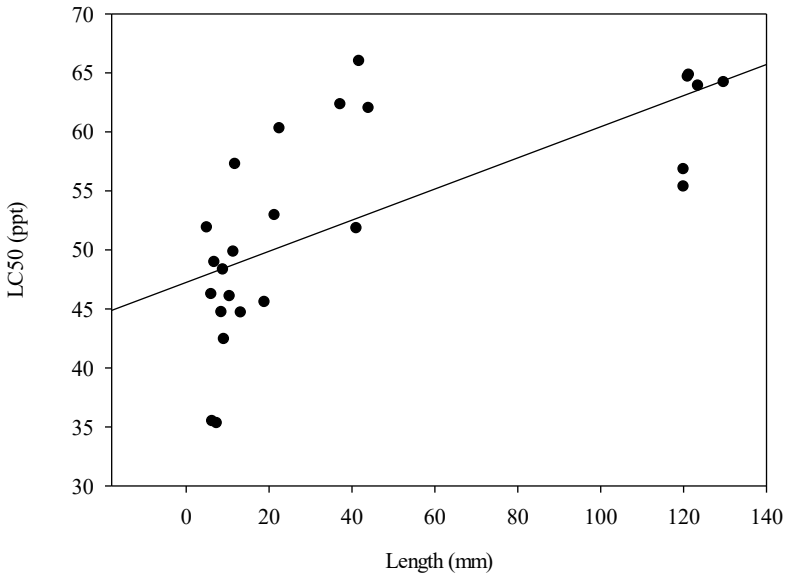


Figure 12. Instant Ocean brine with river water as diluent relationship between salinity tolerance (LC_{50}; ppt) and body length (TL between 0 and 30 mm, and FL above 30 mm). This is pooled data from 2016, 2017, and 2018 including both wild and domestic striped bass. The regression is represented by the equation Y = 44.172+ (0.168 * X). There is strong correlation (R=0.758, R^2=0.575, R^2Adj= 0.547). And the test output is highly significant, where the p-value is <0.001.

Exposed to IO brine with river water as diluent, salinity tolerance increased with body length (Figure 12). By stage there was variability in the results from individual trials. 5-10 dph larvae had a mean LC_{50} of 41.4 ppt with the minimum result being 35.5 ppt and the maximum 46.3 ppt. 10-20 dph larvae had a mean LC_{50} of 46.9 ppt and a minimum LC_{50} of 35.3 ppt and a maximum of 57.3 ppt. 20-60 mm juveniles had a mean LC_{50} of 57.3 ppt with a minimum LC_{50} of 45.6 ppt and a maximum of 66.0 ppt. Lastly, 120 mm juveniles

had a mean LC_{50} of 61.6 ppt and a minimum LC_{50} of 55.4 ppt and a maximum of 64.83 ppt.

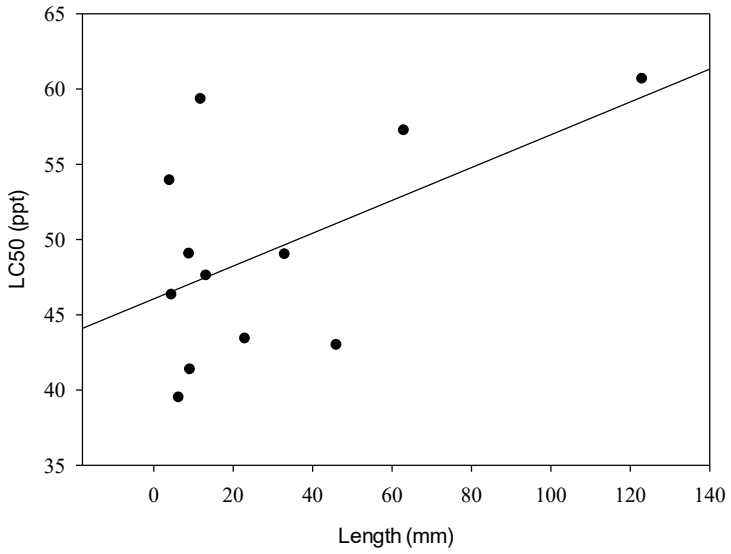


Figure 13. Instant Ocean brine with well water as diluent relationship between salinity tolerance (LC_{50}; ppt) and body length (TL between 0 and 30 mm, and FL above 30 mm). This is pooled data from 2016, 2017, and 2018 including both wild and domestic striped bass. The tests conducted using well water as a diluent and Instant Ocean as a toxicant. There is an increasing trend in salinity tolerance and LC_{50} as body length increases. The regression is represented by the equation Y = 46.063 + (0.109 * X). There is moderate correlation (R=0.534, R^2=0.285, R^2Adj= 0.213). And the test output is highly significant, where the p-value is <0.001.

Exposed to IO brine with well water as diluent, yolk-sac larvae had a mean LC_{50} of 53.9, 5-10 dph larvae had a mean LC_{50} of 42.4 ppt with a minimum LC_{50} of 39.5 ppt and a maximum LC_{50} of 46.3 ppt (Figure 13). 10-20 dph larvae had a mean LC_{50} of 52.0 ppt with a minimum result of 47.6 ppt and a maximum of 59.3 ppt. 20-60 mm juveniles had a mean LC_{50} of 48.2 ppt with a minimum LC_{50} of 43.0 ppt and a maximum of 57.3 ppt. Lastly, 120 mm juveniles had a LC_{50} of 60.7, this was a sole test (Figure 13).

Table 12. Threshold observed effect concentration of Instant Ocean and well water brine (IO+WW 19 °C) and Instant Ocean and river water brine (IO + RW 19 °C), for 1 h exposure of six early life stages of striped bass ranging from 0 to 143 days post hatch, at 19 °C. Columns marked with an * are single values. All values were not significantly different (two-sample t-test, α 0.05).

Test Condition	**Eggs**	**Yolk-Sac**	**First-Feeding**	**Large Larvae**	**Early Juveniles**	**Juvenile**
DPH/ Length		1-4	5-10	11-20	20-60 (mm)	>120 mm
IO + WW 19 °C	---	50.0*	35.0	38.1	39.2	55.0*
IO + RW 19 °C	42.1*	45.2*	29.6	40.0	46.3	58.0*

TOEC values for Instant Ocean based brines were not significantly different between developmental stage and water type (Table 12).

3.8 Discussion

To provide a measure of the threat of the planned discharge of brine into the Shubenacadie River estuary, this thesis quantified the salinity tolerance of striped bass from egg to juvenile. The threat appears to be very low given the high LC_{50} and TOEC values exhibited by all life stages. The results are compared with published literature on the development of osmoregulatory capabilities in striped bass and other euryhaline teleosts.

Striped bass eggs exhibited very high LC_{50} estimates >54 ppt, due to a highly impermeable vitelline membrane, and chloride cells which protect the embryo. Using deuterium oxide as an indicator, the permeability of the vitelline membrane of rainbow trout became immeasurably low after water hardening (Krogh and Ussing, 1937). The vitelline membrane was also impermeable to both water and salts, but oxygen could diffuse in (Krogh and Ussing, 1937). Chloride cells are present in the developing embryos of all teleosts, typically located on the yolk-sac membrane (Guggino, 1980, Katoh et al., 2000;

Rombough, 2007). Chloride cells become functional at gastrulation, the developmental stage of the eggs tested (>24hr old), maintaining the osmotic homeostasis of the extracellular fluid (Guggino, 1980; Kaneko et al., 2008). Among all striped bass stocks, spawning and water hardening occurs in freshwater, and survival is greatly reduced if they are exposed to brackish water before water hardening of the egg, salinities as low as 2 ppt could be detrimental to this vulnerable stage of egg development (Turner and Farley, 1971). Water hardening is the uptake of water into the perivitelline space, which is associated with a decrease in the permeability of the vitelline membrane, making eggs more resistant to passive transport of ions and water, while increasing their tolerance to physical disturbance (Krogh and Ussing, 1937; Suga, 1963). In the Shubenacadie River estuary, the risk of exposure of newly fertilized eggs to the brine discharge is very low. Following spawning in the Stewiacke River in freshwater, the pelagic eggs are transported down-estuary on the ebbtide at about 2 km/h (Duston unpubl. data), reaching the brine discharge site in about 3 to 4 h, by which time they are fully hardened. The water hardened eggs are still in the blastula stage, during the first ebbtide, verified by these eggs taking 48 h to hatch in incubators at Dal AC (Duston, unpublished; Turner and Farley, 1971). Due to the ~6 rkm distance between the spawning grounds on Stewiacke River and the Main Site there is a low risk of pre-gastrulation eggs being exposed to the brine in the bypass channel.

After brine release commences, any threat to striped bass eggs and larvae is further reduced since brine discharge will be stopped for 24 days after detection of the first egg, and when larvae are present the allowable upper salinity threshold 5 m from the point discharge will be 20 ppt (Alton Gas, 2015; DFO, 2016; NSE, 2016). From fertilization to hatch, about 48 h, the eggs are transported up- and down-estuary with the tide, distributed

mostly between sanities of 0.5 and 15 ppt in the main channel of the estuary (Duston et al., 2018). Despite this distribution pattern, the upper salinity tolerance appears to be greater than 15 ppt, since survival to hatch was independent of salinity up to 20 ppt and reduced significantly only at 30 ppt (Cook et al., 2010). Salinity tolerance of eggs from US stocks, by comparison, appear to be lower than Shubenacadie River stock (Lal et al., 1977; Winger and Lasier, 1994; Cook et al., 2010).

Yolk-sac larvae (3 - 4 dph, 5.5 mm TL) salinity tolerance at 19 ºC was similar to eggs since they share the same impermeable epithelium and associated chloride cells (Hirai et al., 2000). The subsequent decrease in salinity tolerance around the first-feeding stage, confirming Winger and Lasier (1994), is associated with the yolk-sac decreasing in both size and surface area, reducing its capacity for osmoregulation (Rombough, 2007). The rudimentary gills and gastrointestinal tract begin to contribute to ion and water balance at first feeding in all teleosts studied but are unable to fully compensate for the loss of yolk-sac osmoregulatory capacity (Rombough, 2007). By 11 dph, striped bass gill filaments have functional chloride cells which begin to actively excrete sodium (Na^+) and chloride (Cl^-; Hirai et al., 2002). Larval marine fish commence drinking saltwater and extract water across the intestinal wall, actively excrete Na^+ and Cl^- at the gills and magnesium (Mg^{2+}) and sulfate (SO_4^{2-}) via the primitive urinary system (Guggino, 1980; Varsamos et al., 2005; Edwards and Marshall, 2013). The changes in salinity tolerance through the larval stage reported here, was likely due the increase in density of chloride cells on the skin and gill filaments quantified from 11 to 41 dph by Hirai et al. (2002). Transformation from larva to juvenile occurs during this period, around 25 - 36 mm TL in striped bass, associated with

acquisition of definitive organs and adult morphology and structures (Otwell and Merriner, 1975; Lal et al., 1977; Hardy, 1978).

The metamorphosis from larva to juvenile can temporarily reduce osmoregulatory capacity in some species (Varsamos et al., 2005); but at its completion, the juveniles are better adapted to tolerate changes in salinity, as demonstrated by the 4 and 12 cm long striped bass exhibiting a LC_{50} 1h of 54 - 66 ppt (Figure 7), and surviving in 100 ppt SC several minutes longer than larvae (Figure 9). Similarly, survival and growth of Shubenacadie River early juveniles (6 - 9 cm FL) was independent of salinity between 1 to 30 ppt (Cook at al., 2010), and 114 dph juveniles from other stocks easily tolerated transfer to full seawater (34 ppt; Lal et al., 1977). When juvenile striped bass are transferred directly from freshwater to seawater (30 ppt), the structure of their chloride cells is modified quickly to maintain osmotic balance (King and Hossler, 1991). When striped bass 3 to 25 cm in length were rapidly transferred from freshwater to full strength seawater, there were changes in chloride cell ultrastructure within 3 h. This change in structure is thought to increase surface area to allowing increased rates of ionic exchange, potentially allowing striped bass to have greater tolerance to high salinities, compared to other euryhaline teleosts (King and Hossler, 1991). The ability of juvenile striped bass to tolerate rapid increases in salinity is due to a high abundance of gill Na^+/K^+/ATPase and $Na^+/K^+/2Cl^-$ cotransporter that are 'dormant' in freshwater, but are activated immediately when the fish are exposed to seawater (Madsen et al., 1994; Tipsmark et al., 2004). Moreover, the rapid response is facilitated by insulin-like growth factor 1 and 2 and epidermal growth factor receptors present in the gill lamellae of striped bass, are absent in other teleosts (Madsen et al., 2007; Tipsmark et al., 2007).

Differences in the ion composition between the salt core brine (SC) and the Instant Ocean brine (IO), specifically the very low potassium (K^+) and magnesium (Mg^{2+}) in the salt core, had no effect on the relative toxicity of the two brines at 19 ºC in most of the life stages (Figure 7). The independence between LC_{50} values of brine for striped bass early life stages and both the types of salt and diluent waters suggest that the salt core brine poses no specific toxicity risks to the Shubenacadie nursery habitat. The similar LC_{50} results occurred even though the concentration of some ions differed several folds. Salt core composition is dictated by the long-term oscillation of the ocean ionic make-up over the past 600 My (Horita et al., 2002; Lowenstein et al., 2003; Holt et al., 2014). There is no evidence the very low levels of either K^+ or Mg^{2+} in the SC brine would pose a threat to aquatic organisms. The K^+ concentration in the SC brine was four fold higher than the lower-lethal level to larval Gulf killifish (*Fundulus grandis*), and Mg^{2+} concentration was 30% greater than the lower-level that interfered with Na^+/K^+-ATPase activity (Fisher et al., 2013, 2015). Domestic Dal-AC well water did not contain a high level of K^+ (0.9mg/L), the use of well or river water did not affect the toxicity as the ionic composition of the tests solution was largely provided by the addition of salt, and was concentration specific. Potassium ions are essential for chloride cell function, needed for the ion exchange by the Na^+/K^+-ATPase and $Na^+/K^+/2Cl^-$ cotransporter (Fisher et al., 2013). The loss of potassium is partially due to the exchange of 3 Na^+ ions for 2 K^+ ions, therefore in saline environments, there is consistent loss of K^+ ions, as Na^+ enters the blood (Greenwall et al., 2003). Studies of the whitemouth croaker (*Micropogonias furnieri*), a euryhaline species suggest that as potassium levels of the external environment increased, the potassium levels internal of the fish decreased. This was characterized by a reduction in potassium levels from 14.39 ±

0.73 in freshwater to 6.75 ± 0.40 mmol L in 34 ppt seawater (Becker et al., 2011). Though no study evaluates the potassium level of striped bass blood, it would likely have a similar relationship, as euryhaline fish have similar adaptive mechanisms. Magnesium, and Ca^{2+} to a greater extent, serve to reduce the ionic permeability of the epithelium, their deficiency produced an osmotic shock following transfer of teleosts to hypertonic media due to a rapid influx of Na^{+} and dehydration (Lemm et al., 1993, Dolomatov et al., 2012; Fisher et al., 2015). When calcium is removed from the rearing water of goldfish (*Carassius auratus*), there was a twofold increase in the influx of sodium via the gills, confirming that calcium has a crucial role in the ionic permeability of the teleost gill (Cuthbert and Maetz, 1972). Furthermore, in rearing waters where calcium was removed, the permeability of the trout gill to water increased between 1.5 and 2 fold, further confirming the role calcium plays on osmoregulatory functions of the gill (Robertson and Hazel, 1999). Increased water permeability of the gill is thought to be a function of increased numbers of chloride cells, as water more easily crosses the gills in both saltwater and freshwater acclimatized Japanese eels as the number of chloride cells increase (Ogasawara and Hirano, 1984). Increases in gill permeability to both ions and water are also partially due to the presence of "leaky" tight junctions, which allow Na^{+} to be transported, but also increase the permeability of the gill epithelium (Chasiotis et al., 2012). Tight junctions are narrow seals between epithelial cells, which consist of various proteins, which limit the passage of ions in between epithelial cells (Chasiotis et al., 2012).

Based on the engineering plan, the discharge of brine into the Shubenacadie River estuary poses a very low direct threat to striped bass as the TOECs for five of the six early life stages were higher than the 28 ppt threshold at the normal summer water temperature

(18 - 20 ºC). At colder conditions (12 ºC), the TOECs were closer to the 28 ppt threshold, but the quick dilution in the estuary reduces the threat, as in the wild exposure will be short term (approximately 5 minutes to traverse the bypass channel), whereas, these values are based on a 1 h exposure. Temperature has an influence on the uptake of water via the gills; in both rainbow trout reared at 5 and 20 °C and tilapia (*Oreochromis niloticus*) reared at 21.5 and 33 °C there were increases in water weight gain of 1.5 to 3 fold, indicating that temperature may also play a role in osmoregulatory ability beyond the scope of this study (Robertson and Hazel, 1999). The interaction between temperature and salinity is important for the survival of striped bass early life stages, with a low of 12 ºC identified as a lethal factor among US stocks (Otwell and Merriner, 1975; Morgan et al., 1981; Rutherford and Houde, 1995). Poor egg survival at 12 ºC in Chesapeake Bay, estimated by Rutherford and Houde (1995), was in contrast to the similar acute salinity tolerance of eggs at 12 ° and 19 ºC (Figure 7). Striped bass in the Chesapeake Bay had better growth and recruitment when spawning occurred later in the year, where water temperature was warmer, and there was a decreased chance of temperatures dropping below 12 °C, which can lead to 100 % mortality of eggs and yolk sac larvae (Rutherford and Houde, 1995). In the Shubenacadie river, larval abundance was reduced from 340 larvae/m^3 to 29 larvae/m^3 following heavy rain and a reduction in water temperature from 19 to 11 °C in May 2015 (Duston et al., 2018). The vulnerability of yolk-sac larvae to low temperature, as evidenced by the 28% decrease in the median lethal brine concentration at 12 vs. 19 ºC (Figure 7), supports data on US stocks, where eggs and yolk-sac larvae were noted as vulnerable (Dey, 1981; Morgan et al., 1981; Rutherford and Houde, 1995). The cold tolerance of yolk-sac larvae of Shubenacadie stock, however, appears to be superior

to US stocks (Cook et al., 2010). Among feeding larvae, the independence in salinity tolerance between 12 and 19 ºC reported here, contrasts with reduced survival at 10 to 14 vs. 16 ºC in a six day trial in Instant Ocean, using 75 and 90 dph larvae 60 and 87 mm TL respectively (Cook et al., 2010). The longer exposure may have caused more disruption to osmoregulation than the 1 h tests, because of prolonged effects on Na^+/K^+/ATPase, an integral component of the salt pump (Donaldson et al., 2008). Larvae may not have fed in the lower temperatures, over the six day exposure, leading to mortality due to depletion of energy reserves, or potentially osmotic failure, as osmoregulation is energy expensive (Johnson and Evans, 1996; Cook et al., 2010).

The discharge of brine from the salt cavern in the mixing channel should not present any direct threat to the early life stages of striped bass, as it will be diluted to no more than 28 ppt, or 20 ppt when eggs or larvae are present at 5 m from the point of release due to the high mixing and dilution due to the macrotidal conditions in the estuary. Safeguards to prevent the loss of vulnerable striped bass life stages include the 24 day shutdown of brine release following detection of the first striped bass egg, ensuring that the vulnerable pre-gastrulation stage of egg development are protected from exposure. The early larvae are tolerant to salinities above 20 ppt, as they have developed chloride cells from hatch that allow them to adapt to changing salinities (Madsen et al., 1994; Varsamos et al., 2001).

In all treatment conditions there was an overall positive correlation between body length (mm) and salinity tolerance. This pattern of increased salinity tolerance with increases in body size is common for the ontogenetic development of salinity tolerance in larval and early life stage of many species of fish (Holliday and Blaxter, 1960; McCormick and Naiman, 1984). It is common for larval euryhaline or marine fish species to have high

LC_{50} values in relation to hypersaline solutions nearer 65 ppt (Varsamos et al., 2001). European sea bass larvae exhibited a similar pattern to striped bass in their development of salinity tolerance relative to age and body size. Maximum salinity tolerance for European sea bass was acquired at 17 mm total length and 63 dph, for striped bass tested in this study, maximum salinity tolerance was achieved in the 20 to 60 mm fork length, with an age range of 43 to 57 dph. This is due to the development of key osmoregulatory organs and behaviours, including early onset drinking behaviour, after mouth opening, where drinking rates and water absorption can be actively controlled during the yolk-sac stage for European sea bass (Varsamos et al., 2001). Later increases in osmoregulatory ability are tied to the morphological changes that characterize transformation to the juvenile stage, including full development of the gills, gut and the kidney, all key components of osmoregulation (Varsamos et al., 2001). The median lethal concentration was not statistically different from the LC_{50} determined for 120 mm juveniles, nor should it differ from adults; as in European sea bass there was no significant difference between the ability of 17 mm larvae and adults to osmoregulate and the osmoregulatory ability of fully grown adults (630 dph, 24 cm FL; Varsamos et al., 2001). There is evidence to suggest that through hatch to acquisition of maximum salinity tolerance there are minute increases in osmoregulatory capabilities, however there is no definitive study of these developments for striped bass or European sea bass (Varsamos et al., 2001). These changes could be indicative of ontogenetic development of salinity tolerance but are of little value to this project as they are small changes and would have little bearing on the overall trends in the data, which show striped bass have high salinity tolerance to both Instant Ocean synthetic sea salt and salt from underground formations.

Chapter 4: Conclusions and Future Studies

4.0 Limitations and Further Research

Despite the ability to determine the lethal effects of salt cavern brine on striped bass populations, the Shubenacadie plays host to many other estuarine species of teleosts and crustacea which play key roles in the trophic chain. Therefore, determining the impact of brine release on prey and predators of striped bass is also something worthwhile, as copepods are a key component of striped bass recruitment and first-feeding success.

Further studies on the mechanisms and ontogenetic development of salinity tolerance in striped bass could be useful to both the Alton gas project and the field of fish biology. These studies should include measures of blood plasma osmolality, osmoregulatory ability, and tissue moisture content. Determining the way chloride cells develop in key osmoregulatory organs is of equal use, and could be paired with the previously mentioned measures to better interpret the LC_{50} data, and explain how striped bass larvae from the Shubenacadie River cope with salinity, especially salinities that exceed that of the natural habitat. A final series of toxicology test on pre-blastulation striped bass eggs could be beneficial, as this would yield data showing how egg development impacts salinity tolerance.

Finally, complex hydrodynamic models of the river estuary need to be completed on a year-round basis. This will allow estimates of the transportation dynamics of the additional salt within the estuary, ensuring the salt from cavern development is dispersed in a safe manner. This work has been ongoing with the addition of overwinter CTD loggers, in November 2019 to aide in modeling efforts through the winter months.

4.1 Final Conclusions

The data clearly shows there are ontogenetic changes in the salinity tolerance of early life history stages of striped bass. The salinity tolerance of eggs and yolk sac larvae was high at 19 °C, which then declined for first feeding larvae and large larvae, then increased for early juveniles and 120 mm juveniles. Tests conducted in 12 °C suggest that temperature is not a lethal factor for striped bass eggs but has clear effects on yolk-sac larvae, first feeding larvae and large larvae. This is in contrast to some published literature and is noteworthy.

This thesis is a key component of regulatory compliance and regulation formation for the Alton Gas Storage Project's development of the Shubenacadie River Estuary. This data paired with three other theses and 10 years of baseline data serves as evidence that with proper regulation, monitoring and control, brine can be safely released into the Shubenacadie river with no direct impact on the striped bass population.

Additionally, this research aides in developing the state of knowledge for a few select fields. This includes fish physiology and development, estuarine sciences, commercial desalination (including coastal applications) in relation to the intrusion of salts, driven by anthropogenic means such as road salt, climate change or industrial usage.

References

Alton Gas. 2015. Alton Natural Gas Storage estuarial environmental monitoring and toxicity testing. Available: http://altonnaturalgasstorage.ca/doc/alton-river-site-monitoring-plan-dec-10-2015.pdf

Alton Gas. 2016. Alton Natural Gas Storage Project Newsletter, Issue 2. Available: http://altonnaturalgasstorage.ca/doc/ANGSP-News-Issue2-January2017.pdf.

Arnold, W.R., Cotsifas, J.S., Winter, A.R., Klinck, J.S., Smith, D.S., and Playle, R.C. 2007. Effects of using synthetic sea salts when measuring and modeling copper toxicity in saltwater toxicity tests. Environmental Toxicology and Chemistry. **26**(5):935-943.

Bailey, H.C., Alexander, C., Digiorgio, C., Miller, M., Doroshov, S.I., and Hinton, D.E. 1994. The effect of agricultural discharge on striped bass (*Morone saxatilis*) in California's Sacramento-San Joaquin drainage. Ecotoxicology. **3**:123-142.

Barbier, E.B, Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., and Silliman, B.R. 2011. The value of estuarine and coastal ecosystem services. Ecological Monographs: **81**(2): 169-193.

Becker, A.G., Goncalves, J.F., Toledo, J.D.A., Burns, M.D.M., Garcia, L.D., Vieira, J.P., and Baldisserotto, B. 2011. Plasma ion levels of freshwater and marine/estuarine teleosts from Southern Brazil. Neotropical Ichthyology. **9**(4): 895-900.

Berillis, P. 2015. Factors that can lead to the development of skeletal deformities in fish: a review. Journal of Fisheries Science. **9**(3):17-23.

Bradford, R.G., Halfyard, E.A., Hayman, T., and LeBlanc, P. 2015. Overview of 2013 Bay of Fundy striped bass biology and general status. DFO Canadian Science Advisory Secretariat. Research Document 2015/024. Iv + 36p.

Brown, G.S. 1984. The zooplankton of a turbid macrotidal estuary. MSc Thesis, Acadia University, Wolfville, Nova Scotia.

Burton, D.T., Hall., L.W., Klauda, R.J., and Margery, S.L. 1983. Effects of treated bleached kraft mill effluent on eggs and prolarvae of striped bass (*Morone saxatilis*). Water Resources Bulletin. **19**(6):869-879.

Carol, E. S., Alvarez, M. D. P. and Borzi, G. E. 2016. Assessment of factors enabling halite formation in a marsh in a humid temperate climate (Ajó Marsh, Argentina). Marine Pollution Bulletin. **106**: 323-328.

Cech, J.J., Bartholow, S.D., Young, P.S., and Hopkins, T.E. 1996. Striped bass exercise and handling stress in freshwater: physiological responses to recovery environment. Transactions of the American Fisheries Society. **125**:308-320.

Chasiotis, H., Kolosov, D., Bui, P., and Kelly, S.P. 2012. Tight junctions, tight junction proteins and paracellular permeability across the gill epithelium of fishes: A review. Respiratory Physiology and Neurobiology. **184**(3):269-281.

Clayton, R.D., and Summerfelt, R.C. 2010. Gas bladder inflation in walleye fry cultured in turbid water with and without a surface spray. North American Journal of Aquaculture. **72**(4): 338-342.

Cook, A. M., Duston, J., and Bradford, R. G. 2010. Temperature and salinity effects on survival and growth of early life stage Shubenacadie River striped bass. Transactions of the American Fisheries Society. **139**: 749-757.

Cook, A.M. 2003. Growth and survival of age 0+ Shubenacadie River striped bass (*Morone saxatilis*) in relation to temperature and salinity. MSc thesis. Nova Scotia Agricultural College, Truro.

COSEWIC. 2012. COSEWIC assessment and status report on the striped bass *Morone saxatilis* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. Available: (www.registrelep-sararegistry.gc.ca/default_e.cfm).

Cuthbert, A.W., and Maetz, J. 1972. The effects of calcium and magnesium on sodium fluxes through gills of *Carassius auratus* L. Journal of Physiology. **221**: 633-643.

Dalrymple, R.W., Knight, R.J., Zaitlin, B.A., and Middleton., G.V. 1990. Dynamics and facies model of a macrotidal sand-bar complex, Cobequid Bay-Salmon River Estuary (Bay of Fundy). Sedimentology. **37**:577-612.

Department of Fisheries and Oceans - Canada (DFO). 2016. Review of a method for identifying a window of principle striped bass *(Morone saxatilis*) spawning in the Shubenacadie River estuary. Canadian Science Advisory Secretariat. **026**:1-8.

Dey, W.P. 1981. Mortality and growth of young-of-the-year striped bass in the Hudson River estuary. Transactions of the American Fisheries Society. **110**:151-157.

Dolomatov, S.I., Zukow, W., Novikov, Y.N., Muszkieta, R., Bulatowicz, I., Dzierzanowski, M. Kazmierczak, U., and Strojek, K. 2012. The regulation of osmotic and ionic balance in fish reproduction and in the early stages of ontogeny. Russian Journal of Marine Biology. **38(5)**:365-374.

Donaldson, M.R., Cooke, S.J., Patterson, D.A., and Macdonald, J.S. 2008. Cold shock and fish. Journal of Fish Biology. **73**:1491-1530.

Douglas, S.G., R.G. Bradford, and G. Chaput. 2003. Assessment of striped bass (*Morone saxatilis*) in the Maritime Provinces in the context of species at risk. CSAS Res. Doc. 2003/008: iii + 49 p.

Duke, T.W. and Mount, D.I. 1991. Toxic effects on individuals, populations and aquatic ecosystems, and indicators of exposure to chemicals. Pages 393-404 *in* R.G Tardiff and B. Goldstein, eds. Methods for Assessing Exposure of Human and Non-Human Biota. John Wiley and Sons. New York City, New York, United States of America.

Duston, J., and Astatkie, T. 2012. Prey density and non-visual feeding by larval striped bass. Transactions of the American Fisheries Society. **141**:610-614.

Duston, J., Manríquez-Hernández, J., MacInnis, G.M., Reesor, C.M., and Astatkie, T. 2018. Striped bass early life history in the macrotidal Shubenacadie River. Transactions of the American Fisheries Society. **147**:919-938.

Edwards, S.L., and Marshall, W.S. 2013. Principles and Patterns of Osmoregulation and Euryhalinity in Fishes. *in* S.D McCormick., A.P Farrell., and C.J Brauner, eds. Euryhaline Fishes. Fish Physiology Series. Academic Press, Waltham, Massachusetts.

Eldridge, M.B., Joseph, J.D., Taberski, K.M., and Seaborn, G.T. 1983. Lipid and fatty acid composition of the endogenous energy sources of striped bass (*Morone saxatilis*) eggs. Lipids. **18**:510-513.

Environment Canada. 1990. Biological test method: Acute lethality test using threespine stickleback (*Gasterosteus aculeatus*). Report EPS 1/RM/10 July 1990 (including March 2000 amendments).

Environment Canada. 2013. Biological test method- acute lethality tests using rainbow trout. Environmental Protection Series. En49-24/1-9E.

EPA. 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Washington, USA. Available: https://www3.epa.gov/region6/water/npdes/wet/wet_methods_manuals/atx.pdf

EPA. 2018. Climate adaptation and estuaries. Available: https://www.epa.gov/arc-x/climate-adaptation-and-estuaries.

ETS. 2019. Reference toxicant testing. Available: http://www.etsnclab.com/quality-assurance-quality-control/reference-toxicant-testing

Findlay, K.R. 2019. The distribution of copepods in the Shubenacadie River Estuary relative to the diet of larval striped bass (*Morone saxatilis*). MSc Thesis. Dalhousie University.

Fisher, C., Bodinier, C., Kuhl, A., and Green, C. 2013. Effects of potassium ion supplementation on survival and ion regulation in Gulf killifish *Fundulus grandis* larvae reared in ion deficient saline waters. Comparative Biochemical Physiology: Part A Molecular Integrated Physiology. **164:**572-578.

Fisher, C., Bodinier, C., Kuhl, A., and Green, C. 2015. Application of physiological tests to determine specific monovalent and divalent ion supplementation for culture of marine species. Bulletin of Fisheries Research Agency. **40**:97-107.

Freadman, M.A. 1979. Swimming energetics of striped bass (*Morone saxatilis*) and bluefish (*Pomatomus saltatrix*): gill ventilation and swimming metabolism. Journal of Experimental Biology. **83**:217-230.

Giles, P.S., and Boehner, R.C. 2001. Windsor group stratigraphy and structure, drill core orientation field trip 2001. Targeted Geoscience Initiative 2000-2003. Open File Report Nova Scotia Natural Resources. Available: https://novascotia.ca/natr/meb /data/pubs/03ofr02/03ofr02.pdf.

Greenwall, M.G., Sherrill, J., and Clayton, L.A. 2003. Osmoregulation in fish mechanisms and clinical implications. Veterinary Clinics of North America: Exotic Animal Practice. **6**: 169-189.

Grizzle, J.M., and Cummins, K.A. 1996. Potassium flux in juvenile striped bass (*Morone saxatilis*): influence of external concentrations of sodium chloride and calcium. Fish Physiology and Biochemistry. **15**:181-186.

Grizzle, J.M., and Mauldin, A.C. 1995. Effect of calcium on acute toxicity of sodium chloride to larval and newly transformed juvenile striped bass. Journal of Aquatic Animal Health. **7**: 298-303.

Gros, N. 2013. Ion chromatographic analyses of sea waters, brines and related samples. Water. **5**:659-676.

Guggino, W.B. 1980. Salt balance in embryos of *Fundulus heteroclitus* and *F. bermudae* adapted to seawater. American Journal of Physiology. **238**(1): R42-R49.

Hall, L.W. 1987. Acidification effects on larval striped bass, *Morone saxatilis*, in Chesapeake Bay tributaries. Water, Air, and Soil Pollution. **35**:87–96.

Hamilton, M.A., Russo, R.C., and Thurston, R.V. 1978. Correction. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environmental Science and Technology. **12**(4):417.

Hardy, J.D. 1978. Development of fishes of the mid-Atlantic Bight. An atlas of egg, larval and juvenile stages. Vol. III. Aphredoderidae through Rachycentridae. Fishery Bulletin of the Fish and Wildlife Service. US Department of the Interior.

Hart, J.L. 1973. Pacific Fishes of Canada. Journal of the Fisheries Research Board of Canada Bulletin **180**:740.

Hickey, C.R., Young, B.H., and Bishop., D.R. 1977. Skeletal abnormalities in striped bass. New York Fish and Game Journal. **24**(1):68-85.

Hill, J., Evans, J.W., and Van Den Avyle, M.J. 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (South Atlantic): striped bass. U.S. Fish and Wildlife Service Biological Report. **82**.

Hirai, N., Tagawa, M., Kaneko, T., Secor, D.H., and Tanaka, M. 2002. Freshwater adaptation in Japanese sea bass and striped bass: A comparison of chloride cell distribution during their early life history. Fisheries Science. **68**(1):433-434.

Hirai, N., Tanaka, M., Tagawa, M., and Secor, D.H. 2000. Osmoregulatory responses in striped bass *Morone saxatilis* larvae: Survival, growth, yolk absorption, and development of chloride cells in body skin. International Congress on the Biology of Fish, Fish Migration and Passage, Symposium Proceedings. 83-95.

Holliday, F.G.T., and Blaxter, J.H.S. 1960. The effects of salinity on the developing eggs and larvae of the herring. Journal of the Marine Biological Association of the United Kingdom. **39**: 591-603.

Holt, N.M., García-Veigas, J., Lowenstein, T.K., Giles, P.S., and Williams-Stroud, S. 2014. The major-ion composition of carboniferous seawater. Geochimica et Cosmochimica Acta. 134:317-334.

Horita, J., Zimmermann, H., and Holland, H.D. 2002. Chemical evolution of seawater during the Phanerozoic: Implications from the record of marine evaporites. Geochimica et Cosmochimica Acta. **66**(21):3733-3756.

Hurst, T.P., and Conover, D.O. 1998. Effects of temperature and salinity on survival of young-of-the-year Hudson River striped bass (*Morone saxatilis*): implications for optimal overwintering habits. Canadian Journal of Fisheries and Aquatic Sciences. **59**: 787-795.

IEA. 2019. The role of gas in today's energy transitions. Available: https://www.iea.org/reports/the-role-of-gas-in-todays-energy-transitions.

Jensen, M. K., Madsen, S. S. and Kristiansen, K. 1998. Osmoregulation and salinity effects on the expression and activity of Na+, K+-ATPase in the gills of European sea bass*, Dicentrarchus labrax* (L.). The Journal of Experimental Zoology. **282(3)**: 290-300.

Jobling, M. 1995. Environmental Biology of Fishes. Chapman and Hall. T.J. Press, Cornwall, Great Britain.

Johnson, T.B. and Evans, D.O. 1996. Temperature constraints on overwinter survival of age-0 white perch. Transactions of the American Fisheries Society. **125**: 466-471.

Kaneko, T., Watanabe S., and Lee, K.M. 2008. Functional morphology of mitochondrion-rich cells in euryhaline and stenohaline teleosts. Aqua-Bioscience Monographs. **1**(1):1-62.

Katoh, F., Shimuzu, A., Uchida, K., and Kaneko, T. 2000. Shift of chloride cell distribution during early life stages in seawater-adapted killifish, *Fundulus heteroclitus.* Zoological Science. **17**: 11-18.

Kennish, M.J. 1994. Pollution in estuaries and coastal marine waters. Journal of Coastal Research. **12**: 27-49.

Keyser, F.M., Broome, J.E., Bradford, R.G., Sanderson, B., and Redden, A.M. 2016. Winter presence and temperature-related diel vertical migration of striped bass (*Morone saxatilis*) in an extreme high-flow passage in the inner Bay of Fundy. Canadian Journal of Fisheries and Aquatic Sciences. *73*:1777-1786.

Khaledi, K., Mahmoudi, E., Datcheva, M., and Schanz, T. 2016. Stability and serviceability of underground energy storage caverns in rock salt subjected to mechanical cyclic loading. International Journal of Rock Mechanics and Mining Sciences. **86**: 115-131.

Kidder, G.W., Petersen, C.W., and Preston, R.L. 2006. Energetics of osmoregulation: II. Water flux, and osmoregulatory work in the euryhaline fish, *Fundulus heteroclitus*. Journal of Experimental Zoology. **305A**:318-327.

King, J.A.C., and Hossler, F.E. 1991. The gill arch of the striped bass (*Morone saxatilis*). IV. Alterations in the ultrastructure of chloride cell apical crypts and chloride efflux following exposure to seawater. Journal of Morphology. **209**:165-176.

Krogh, A. and Ussing, H. H. 1937. A note on the permeability of trout eggs to D_2O and H_2O. Journal of Experimental Biology. **14**:35-37.

Kültz, D. 2015. Physiological mechanisms used by fish to cope with salinity stress. Journal of Experimental Biology. **218**:1907-1914.

Kupsco, A., Sikder, R., and Schlenk D. 2017. Comparative development toxicity of desalination brine and sulfate-dominated saltwater in euryhaline fish. Archives of Environmental Contamination and Toxicology.**72**:294-302.

Lal, K., Lasker, R., and Kuljis, A. 1977. Acclimation and rearing of striped bass larvae in sea water. California Fish and Wildlife Journal. **63**(4):210-218.

Larson, R.J., and Woltering, D.M. 1995. Linear Alkylbenzene Sulfonate (LAS). Pages 859-881 *in* G.M Rand ed. Fundamentals in Aquatic Toxicology. 2nd Edition. Taylor and Francis, Washington DC.

Lay, T. 1979. NS Department of Environment. Groundwater resources: Shubenacadie-Stewiacke River basin. Technical report. **26**.

Leblanc, N.M., Andrews, S.N., Avery, T.S., Puncher, G.N., Gahagan, B.I., Whiteley, A.R., Curry, R.A., Pavey, S.A. 2018. Evidence of a genetically distinct population of striped bass within the Saint John River, New Brunswick, Canada. North American Journal of Fisheries Management. **38**:1339-1349.

Lemm, C.A., Herman, R.L., Lemarie, D.P., and Arzapalo, A. 1993. Effects of diet and environmental salinity on the growth, mortality, and tissue structure of juvenile striped bass. Journal of Aquatic Animal Health. **5**:294-305.

Libralato, G., Avezz, L.F., and Ghirardini, A.V. 2009. Influence of the salinity adjustment methods, salts and brine, on the toxicity of wastewater samples to mussel embryos. Environmental Technology. **30**(1): 85-91.

Lowenstein, T.K., Hardie, L.A., Timofeeff, M.N., and Demicco, R.V. 2003. Secular variation in seawater chemistry and the origin of calcium chloride basinal brines. Geology. **31**(10):857-860.

MacInnis, G. M. 2012. Spatio-temporal distribution of eggs and age-0 striped bass (*Morone saxatilis*) in the Shubenacadie River estuary. Master's thesis, Dalhousie University, Truro, Nova Scotia.

MacNeil, L.A., Pufahl, P.K., and James, N.P. 2018. Deposition of a saline giant in the Mississippian Windsor Group, Nova Scotia, and the nascent Late Paleozoic Ice Age. Sedimentary Geology. **363**:118-135.

Madsen, S.S., Jensen, L.N., Tipsmark, C.K., Kiilerich, P., and Borski, R.J. 2007. Differential regulation of cystic fibrosis transmembrane conductance regulator and Na^+, K^+-ATPase in gills of striped bass, *Morone saxatilis*: Effect of salinity and hormones. Journal of Endocrinology. **192**:249-260.

Madsen, S.S., McCormick, S.D., Young, G., Endersen, J.S., Nishioka, R.S., and Bern, H.A. 1994. Physiology of seawater acclimation in striped bass, *Morone saxatilis* (Walbaum). Fish Physiology and Biochemistry. **13**:1-11.

Manríquez-Hernández, J., Breau, H.M., and Duston, J. 2017. Hyper-saline toxicity tests on striped bass (*Morone saxatilis*) 2016/2017. Interim Report. Alton Gas.

Manríquez-Hernández, J., Breau, H.M., and Duston, J. 2020. Acute toxicity of salt cavern brine on early life stages of striped bass (*Morone saxatilis*). Archives of Environmental Contamination and Toxicology. **78**(1): 124-136.

Mansueti, R.J. 1958. Eggs, larvae and young of the striped bass, *Roccus saxatilis*. Report of Chesapeake Biological Laboratory. **112**: 35.

Martec Limited. 2007. Numerical brine dispersion modeling in the Shubenacadie River. APPENDIX C: Dispersion modeling of discharged brine. Martec Technical Report no. TR-07-12.

Martino, E.J., and Houde E.D. 2010. Recruitment of striped bass in Chesapeake Bay: spatial and temporal environmental variability and availability of zooplankton prey. Marine Ecology Progress Series. **409**: 213-228.

McCormick, S.D., and Naiman, R.J. 1984. Osmoregulation in the brook trout, *Salvelinus fontinalis* -II. Effects of size, age and photoperiod on seawater survival and ionic regulation. Comparative Biochemistry and Physiology Part A: Physiology. **79**(1): 17-28.

Meng, L. 1993. Sustainable swimming speeds of striped bass larvae. Transactions of the American Fisheries Society. **122**: 702-708.

Middaugh, D.P., Couch., J.A., and Crane, A.M. 1977. Responses of early life stages of the striped bass, *Morone saxatilis* to chlorination. Chesapeake Science. **18**(1):141-153.

Morgan, R.P., and Prince, R.D. 1977. Chlorine toxicity of eggs and larvae of five Chesapeake Bay fishes. Transactions of the American Fisheries Society. **106**(4):380-386.

Morgan, R.P., Rasin, Jr V.J., and Copp, R.L. 1981. Temperature and salinity effects on development of striped bass eggs and larvae. Transactions of the American Fisheries Society. **110**:95-99.

National Aeronautics and Space Administration (NASA). 2019. The effects of climate change. Available: https://climate.nasa.gov/effects/

Nichols, K.M., Edo, A.C., Wheeler, P.A., and Thorgaard, G.H. 2008. The genetic basis of smoltification-related traits in *Oncorhynchus mykiss*. Genetics. **179**:1559-1575.

Nova Scotia Environmental Department (NSE). 2016. Approval to operate brine storage pond. Nº2008-061384-A03.Available: http://altonnaturalgasstorage.ca/doc/industrial- approval-esp-canada0001.pdf.

Nova Scotia Power. 2019. How we make power. Available: https://www.nspower .ca/about-us/electricity/producing.

Ogasawara, T., and Hirano, T. 1984. Changes in osmotic water permeability of the eel gills during seawater and freshwater adaptation. Journal of Comparative Physiology B. **154**:3-11.

Okamoto, T., Kurokawa, T., Gen, K., Murashita, K., Kazuharu, N., Kim, Sk., Matsubara, H., Ohta., H., and Tanaka, H. 2009. Influence of salinity on morphological deformities in cultured larvae of Japanese eel, *Anguilla japonica*, at completion of yolk resorption. Aquaculture. **293**(1-2):113-118.

Otwell, W.S., and Merriner, J.V. 1975. Survival and growth of juvenile striped bass, *Morone saxatilis*, in a factorial experiment with temperature, salinity and age. Transactions of the American Fisheries Society. **104:**560-566.

Palawaski, D., Hunn, J.B., and Dwyer, F.J. 1985. Sensitivity of young striped bass to organic and inorganic contaminants in fresh and saline waters. Transactions of the American Fisheries Society. **114**:748-753.

Paramore, L. 1998. Age, growth, and life history characteristics of striped bass, *Morone saxatilis*, from the Shubenacadie Stewiacke River, Nova Scotia. MSc Thesis. East Carolina University, Greenville, NC.

Reardon, I.S., and Harrell, R.M. 1990. Acute toxicity of formalin and copper sulfate to striped bass fingerlings held in varying salinities. Aquaculture. **87**(3-4): 255-270.

Reesor, C.M. 2012. Temporal distribution of *Morone saxatilis* eggs and larvae and *Neomysis americana* in the Shubenacadie Estuary. Master's thesis, Dalhousie University, Truro, Nova Scotia.

Reinert, T. R., and Peterson, J. T. 2008. Modeling the effects of potential salinity shifts on the recovery of striped bass in the Savannah River Estuary, Georgia–South Carolina, United States. Environmental Management. **41**(5): 753-765.

Robertson, J.C., and Hazel, J.R. 1999. Influence of temperature and membrane lipid composition on the osmotic water permeability of teleost gills. Physiological and Biochemical Zoology. **72**(5): 623-632.

Robichaud-LeBlanc, K.A., Courtenay, S.C., and Locke, A. 1996. Spawning and early life history of a northern population of striped bass (*Morone saxatilis*) in the Miramichi River estuary, Gulf of St. Lawrence. Canadian Journal of Zoology. **74**:1645-1655.

Rombough, P. 2007. The functional ontogeny of the teleost gill: Which comes first, gas or ion exchange? Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology. **148**:732-742.

Rulifson, R.A., and Dadswell, M.J. 1995. Life history and population characteristics of striped bass in Atlantic Canada. Transactions of the American Fisheries Society. **124**:477-507.

Rulifson, R.A., and Tull, K.A. 1999. Striped bass spawning in a tidal bore river: The Shubenacadie Estuary, Atlantic Canada. Transactions of the American Fisheries Society. **128**: 613-624.

Rutherford, E.S., and Houde, E.D. 1995. The influence of temperature on cohort-specific growth, survival, and recruitment of striped bass, *Morone saxatilis*, larvae in Chesapeake Bay. Fishery Bulletin. **93**:315-332.

Sanford, K.F. 1996. Solution salt mining in New York. Northeastern Geology and Environmental Sciences. **18**: 97-107.

Schmidt-Nielsen, K. 1983. Animal Physiology: Adaptation and Environment. Cambridge University Press, Cambridge, USA.

Schneider, S.H., and Chen, R.S. 1980. Carbon dioxide warming and coastline flooding: physical factors and climate impact. Annual Reviews of Energy and Environment. **5**:107-140.

Schoellhamer, D.H. 2001. Influence of salinity, bottom topography, and tides on locations of estuarine turbidity maxima in northern San Francisco Bay, *in* McAnally, W.H. and Mehta, A.J., ed., Coastal and Estuarine Fine Sediment Transport Processes: Elsevier Science B.V., p. 343-357.

Scott, W.B., and M.C. Scott. 1988. Atlantic Fishes of Canada. Canadian Bulletin of Fisheries and Aquatic Sciences 219. University of Toronto Press, Toronto, ON, Canada, in cooperation with the Minister of Fisheries and Oceans and the Canadian Government.

Secor, D.H., Houde, E.D., and Kellogg, L.L. 2017. Estuarine retention and production of striped bass larvae: a mark recapture experiment. **74**(6):1735-1748.

Setzler, E.M., Boynton, W.R., Wood, K.V., Zion, H.H., Lubbers, L., Mountford, N.K., Frere, P., Tucker, L., and Mihursky, J.A. 1980. Synopsis of biological data on striped bass, *Morone saxatilis* (Walbaum). NOAA Technical Report NMFS Circular. **433**.

Shelford., V.E. 1931. Some concepts of bioecology. Ecology. **12**(3):215-453.

Stone, B.R. 2015. Calculates the ED_{50} (effective dose) from a dose-response curve using the Trimmed Spearman-Karber method. https://rdrr.io/rforge/tsk/man/tsk.html.

Suga, N. 1963. Change of the toughness of the chorion of fish eggs. Embryologia. **8(1):** 63-74.

The Chronicle Herald. 2019. Alton Gas project should be stopped, report from opponents say. Available: https://www.thechronicleherald.ca/news/provincial/alton-gas-project-should-be-stopped-report-from-opponents-says-362935/

Tipsmark, C.K., Luckenbach, J.A., Madsen, S.S., and Borski, R.J. 2007. IGF-I and branchial IGF receptor expression and localization during salinity acclimation in striped bass. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. **292**: R535-R543.

Tipsmark, C.K., Madsen, S.S., and Borski, R.J. 2004. Effect of salinity on expression of branchial ion transporters in striped bass (*Morone saxatilis*). Journal of Experimental Zoology Part A: Ecological and Integrative Physiology. **301**:979-991.

Turner, J.L., and Farley, T.C. 1971. Effects of temperature, salinity, and dissolved oxygen on the survival of striped bass eggs and larvae. California Department of Fish and Wildlife. **57**(4):268-273.

United States Energy Information Administration (USEIA). 2019. Natural Gas and The Environment. Available: https://www.eia.gov/energyexplained/natural-gas/natural-gas-and-the-environment

Varsamos, S., Connes, R., Diaz, J. P., Barnabé, G., and Charmantier, G. 2001. Ontogeny of osmoregulation in the European sea bass *Dicentrarchus labrax* L. Marine Biology. **138(5)**: 909-915.

Varsamos, S., Nebel, C., and Charmantier, G. 2005. Ontogeny of osmoregulation in postembryonic fish: a review. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology. **141**:401-429.

Vinh, V.D., Ouillon, S., and Uu, D.V. 2017. Estuarine turbidity maxima and variations of aggregate parameters in the Cam-Nam Trieu Estuary, North Vietnam, in early wet season. Water. **10**(68):1-33.

Wellborn., T.L. 1969. The toxicity of nine therapeutic and herbicidal compounds to striped bass. The Progressive Fish-Culturist. **31**(1):27-32.

Williams, E.P., Peer, A.C., Miller, T.J., Secor, D.H., and Place, A.R. 2012. A phylogeny of the temperate seabasses (*Moronidae*) characterized by a translocation of the mt-*nd*6 gene. Journal of Fish Biology. **80:** 110-130.

Winger, P.V., and Lasier, P.J. 1994. Effects of salinity on striped bass eggs and larvae from the Savannah River, Georgia. Transactions of the American Fisheries Society.**123**:904-912.

Wirgin, I.I., Ong T.-L., Maceda, L., Waldman, R.J., Moore, D., and Courtenay, S. 1993. Mitochondrial DNA variation in striped bass (*Morone saxatilis*) from Canadian rivers. Canadian Journal of Fisheries and Aquatic Sciences. **50**: 80-87.

Wooster, W.S., Lee, A.J., and Dietrich, G. 1969. Redefinition of salinity. Journal of Geophysics. **35**:611-613.

Zar, J.H. 2014. Biostatistical analysis, 5th edn. Pearson New International

Appendix A: Copyright Permission Letters from Co-Authors

February 26th 2020

Juan Manriquez-
Hernandez

Dalhousie
University

I am preparing my Master's thesis for submission to the Faculty of Graduate Studies at Dalhousie University, Halifax, Nova Scotia, Canada. I am seeking your permission to include content from the manuscript version of the following paper(s) as a chapter in the thesis:

Manríquez-Hernández, J., Breau, H. M., & Duston, J. 2020. Acute toxicity of salt cavern brine on early life stages of striped bass (*Morone saxatilis*). Archives of Environmental Contamination and Toxicology. **78**(1): 124-136.

Canadian graduate theses are reproduced by the Library and Archives of Canada (formerly National Library of Canada) through a non-exclusive, world-wide license to reproduce, loan, distribute, or sell theses. I am also seeking your permission for the material described above to be reproduced and distributed by the LAC(NLC). Further details about the LAC(NLC) thesis program are available on the LAC(NLC) website (www.nlc-bnc.ca).

Full publication details and a copy of this permission letter will be included in the

thesis. Yours sincerely,

Hayden Matthew Breau

Permission is granted for:

a) the inclusion of the material described above in your thesis.

b) for the material described above to be included in the copy of your thesis that is sent to the Library and Archives of Canada (formerly National Library of Canada) for reproduction and distribution.

Name: JUAN MANRIQUEZ-HERNANDEZ Title: RESEARCH ASSISTANT II

Signature: Date: FEBRUARY 27, 2020

February 26th, 2020

James Duston

Dalhousie
University

I am preparing my Master's thesis for submission to the Faculty of Graduate Studies at Dalhousie University, Halifax, Nova Scotia, Canada. I am seeking your permission to include content from the manuscript version of the following paper(s) as a chapter in the thesis:

Manríquez-Hernández, J., Breau, H. M., & Duston, J. 2020. Acute toxicity of salt cavern brine on early life stages of striped bass (*Morone saxatilis*). Archives of Environmental Contamination and Toxicology. **78**(1): 124-136.

Canadian graduate theses are reproduced by the Library and Archives of Canada (formerly National Library of Canada) through a non-exclusive, world-wide license to reproduce, loan, distribute, or sell theses. I am also seeking your permission for the material described above to be reproduced and distributed by the LAC(NLC). Further details about the LAC(NLC) thesis program are available on the LAC(NLC) website (www.nlc-bnc.ca).

Full publication details and a copy of this permission letter will be included in the

thesis. Yours sincerely,

Hayden Matthew Breau

Permission is granted for:

a) the inclusion of the material described above in your thesis.

b) for the material described above to be included in the copy of your thesis that is sent to the Library and Archives of Canada (formerly National Library of Canada) for reproduction and distribution.

Name: JAMES DUSTON Title: Professor

Signature: Date: Feb 27/2020

Appendix B: Publication Agreement

Dear Author,

Please note: This e-mail is a confirmation of your copyright transfer and was sent to you only for your own records.

1.Publication

The copyright to this article, (including any supplementary information and graphic elements therein (e.g. illustrations, charts, moving images) (the ‘Article'), is hereby assigned for good and valuable consideration to Springer Science Business Media, LLC, part of Springer Nature (the 'Assignee'). Headings are for convenience only.

2. Grant of Rights

In consideration of the Assignee evaluating the Article for publication, the Author(s) grant the Assignee without limitation the exclusive (except as set out in clauses 3, 4 and 5 a) iv), assignable and sub-licensable right, unlimited in time and territory, to copy-edit, reproduce, publish, distribute, transmit, make available and store the Article, including abstracts thereof, in all forms of media of expression now known or developed in the future, including pre- and reprints, translations, photographic reproductions and extensions. Furthermore, to enable additional publishing services, such as promotion of the Article, the Author(s) grant the Assignee the right to use the Article (including the use of any graphic elements on a stand-alone basis) in whole or in part in electronic form, such as for display in databases or data networks (e.g. the Internet), or for print or download to stationary or portable devices. This includes interactive and multimedia use as well as posting the Article in full or in part or its abstract on social media, and the right to alter the Article to the extent necessary for such use. The Assignee may also let third parties share the Article in full or in part or its abstract on social media and may in this context sub-license the Article and its abstract to social media users. Author(s) grant to Assignee the right to re-license Article metadata without restriction (including but not limited to author name, title, abstract, citation, references, keywords and any additional information as determined by Assignee).

3. Self-Archiving

Author(s) are permitted to self-archive a pre-print and an author's accepted manuscript version of their Article.

a. A pre-print is the author’s version of the Article before peer-review has taken place ("Pre-Print”). Prior to acceptance for publication, Author(s) retain the right to make a Pre-Print of their Article available on any of the following: their own personal, self-maintained website; a legally compliant, non-commercial pre-print server such

as but not limited to arXiv and bioRxiv. Once the Article has been published, the Author(s) should update the acknowledgement and provide a link to the definitive version on the publisher's website: "This is a pre-print of an article published in [insert journal title]. The final authenticated version is available online at: https://doi.org/[insert DOI]".

b. An Author's Accepted Manuscript (AAM) is the version accepted for publication in a journal following peer review but prior to copyediting and typesetting that can be made available under the following conditions:

    a. Author(s) retain the right to make an AAM of their Article available on their own personal, self-maintained website immediately on acceptance,

    b. Author(s) retain the right to make an AAM of their Article available for public release on any of the following 12 months after first publication ("Embargo Period"): their employer's internal website; their institutional and/or funder repositories. AAMs may also be deposited in such repositories immediately on acceptance, provided that they are not made publicly available until after the Embargo Period.

An acknowledgement in the following form should be included, together with a link to the published version on the publisher's website: "This is a post-peer-review, pre-copyedit version of an article published in [insert journal title]. The final authenticated version is available online at: http://dx.doi.org/[insert DOI]".

4. Author's Retained Rights

Author(s) retain the following non-exclusive rights for the published version provided that, when reproducing the Article or extracts from it, the Author(s) acknowledge and reference first publication in the Journal:

a. to reuse graphic elements created by the Author(s) and contained in the Article, in presentations and other works created by them.

b. they and any academic institution where they work at the time may reproduce the Article for the purpose of course teaching (but not for inclusion in course pack material for onward sale by libraries and institutions); and

c. to reproduce, or to allow a third-party Assignee to reproduce the Article in whole or in part in any printed volume (book or thesis) written by the Author(s).

5. Warranties

The Author(s) warrant and represent that:

a. (i) the Author(s) are the sole copyright owners or have been authorised by any additional copyright owner(s) to assign the rights defined in clause 2, (ii) the Article does not infringe any intellectual property rights (including without limitation copyright, database rights or trade mark rights) or other third party rights and no licence from or payments to a third party are required to publish the Article, (iii) the Article has not been previously published or licensed, (iv) if the Article contains material from other sources (e.g. illustrations, tables, text quotations), Author(s) have obtained written permissions to the extent necessary from the copyright holder(s), to license to the Assignee the same rights as set out in Clause 2 but on a non-exclusive basis and without the right to use any graphic elements on a stand-alone basis and have cited any such material correctly;

b. all of the facts contained in the Article are according to the current body of science true and accurate.

c. nothing in the Article is obscene, defamatory, violates any right of privacy or publicity, infringes any other human, personal or other rights of any person or entity or is otherwise unlawful and that informed consent to publish has been obtained for all research participants.

d. nothing in the Article infringes any duty of confidentiality which any of the Author(s) might owe to anyone else or violates any contract, express or implied, of any of the Author(s). All of the institutions in which work recorded in the Article was created or carried out have authorised and approved such research and publication; and

e. the signatory (the Author or the employer) who has signed this agreement has full right, power and authority to enter into this agreement on behalf of all of the Author(s).

6. Cooperation

The Author(s) shall cooperate fully with the Assignee in relation to any legal action that might arise from the publication of the Article, and the Author(s) shall give the Assignee access at reasonable times to any relevant accounts, documents and records within the power or control of the Author(s). The Author(s) agree that the distributing entity is intended to have the benefit of and shall have the right to enforce the terms of this agreement.

7. Author List

After signing, changes of authorship or the order of the authors listed will not be accepted unless formally approved in writing by the Assignee.

8. Edits & Corrections

The Author(s) agree(s) that the Assignee may retract the Article or publish a correction or other notice in relation to the Article if the Assignee considers in its reasonable opinion that such actions are appropriate from a legal, editorial or research integrity perspective.

This is an automated e-mail; please do not reply to this account. If you have any questions, please go to our help pages.

Thank you very much.

Kind regards,

Springer Author Services