# SALINITY TOLERANCE OF THE ENDANGERED ATLANTIC WHITEFISH *(COREGONUS HUNTSMANI)*: UNRAVELLING THE CONSERVATION PHYSIOLOGY OF AN ENIGMATIC FISH

by

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Dalhousie University is located in Mi'kma'ki, the
ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

## Dedication

To the Atlantic Whitefish and Dr Jeffrey Hutchings, without both of whom I would not be here today.

# Table of Contents

# List of Tables

# List of Figures

# Abstract

Amidst the ongoing global decline in biodiversity, the preservation of at risk, endemic species is increasingly important. The Atlantic Whitefish (*Coregonus huntsmani*) is an endangered, anadromous fish endemic to Nova Scotia, Canada. The only remaining population, located in the Petite Rivière watershed, has been effectively isolated in three lakes by dams since at least 1901; these dams are thought to have prevented anadromous migrations. Restoring anadromy is an important part of the recovery strategy for Atlantic Whitefish. However, the effects of salt water on the physiology and performance of the Petite Rivière population following a century without anadromy are not fully understood. I investigated the effect of salinity (0, 15, 30 ppt) at two different temperatures (~12 – 17ºC) on the growth, condition, baseline stress level (blood lactate and glucose, plasma cortisol) and osmoregulatory capacity (plasma ion content and osmolarity) of lab-bred, $F_1$ juvenile Atlantic Whitefish. I also conducted preliminary experiments of swimming performance and oxygen uptake during exercise but the sensitivity of Atlantic Whitefish to tail damage acquired in the respirometer, forced discontinuation of swim trials. I found that treatment salinity did not significantly affect growth, condition factor, or indicators of stress. There was a slight increase in total plasma osmolarity in response to full-strength seawater (30 ppt), but no significant differences among salinities in plasma $Na^+$ or $Cl^-$ concentrations. Fish sampled following an increase in ambient temperature from approximately 11.66°C to 16.55°C did exhibit significantly lower relative condition and higher blood lactate concentrations. Together, these results indicate that the Atlantic Whitefish in the Petite Rivière population grow and generally perform well in brackish (15 ppt) and full-strength seawater (30 ppt). Therefore, they are capable of tolerating the salinity changes associated with an anadromous migration, but may be sensitive to temperature increases in their natural environment.

# List of Abbreviations and Symbols Used

| | |
|---|---|
| ANOVA | Analysis of Variance |
| COSEWIC | Committee on the Status of Endangered Wildlife in Canada |
| DD | Degree day |
| DFO | Department of Fisheries and Oceans Canada |
| DPH | Days post-hatch |
| $F_0$ | Generation 0 (nought) |
| $F_1$ | Generation 1 |
| FL | Fork length |
| g | Grams |
| ga | Gauge |
| km | Kilometres |
| L | Litres |
| mL | Millilitres |
| mm | Millimetres |
| mmol | Millimole |
| mmol $L^{-}$ | Millimoles per litre |
| mOsm | Milliosmoles |
| ng | Nanograms |
| ng/mL | Nanograms per millilitre |
| ppt | Parts per thousand |
| RCF | Relative condition factor |
| SARA | Species At Risk Act |
| SD | Standard deviation |
| µL | Microlitre |
| $w\ W^{-}$ | Observed weight (g) per expected weight (g), unit of RCF |
| %Growth $DD^{-}$ | Percent growth per degree day |

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# Chapter 1 – Introduction

## 1.1 Biodiversity losses and conservation priorities for diadromous fishes

Global biodiversity loss is a pressing concern. Estimates place the proportion of species that have become threatened or extinct since the year 1500 at about 30%, but potentially up to 50% of all species (Isbell et al., 2023), and losses are expected to increase further with climate change (Urban, 2015). The expected reduction of ecosystem function and resilience (Srivastava et al., 2012) in response to biodiversity loss is of particular concern at higher latitudes, where species richness is lower to begin with (Gaston, 2000). In Canada, the rate of species endangerment is comparable with other countries with far more extensive habitat loss (Kerr & Deguise, 2004), suggesting a relatively high and increasingly severe rate of species loss (e.g. Kerr & Cihlar, 2004).

To effectively combat biodiversity loss with limited management resources, it has been suggested that ecologically distinct species which represent a disproportionate breadth of evolutionary history (e.g. last remaining species of a particular lineage) should be prioritized (Mazel et al., 2018; Enns et al., 2020; Kraus et al., 2023), to potentially maximize the conservation of functional diversity (Mazel et al., 2018). For the same reasons, endemic species are often targets for conservation efforts (Myers et al., 2000; Henson et al., 2005), as their occurrence often reflects unique ecological processes and conditions driving evolutionary diversity (Enns et al., 2020).

Freshwater systems have some of the highest estimates of species endangerment (Isbell et al., 2023). Canada contains 20% of the world's fresh water (Government of Canada, 2018), making this country responsible for an important component of global freshwater biodiversity.

Unfortunately, within Canada, 18.7% of freshwater-dependent species are considered “at risk” (Desforges et al., 2021). Diadromous fishes spend portions of their lifecycle in both marine and freshwater environments (Myers, 1949), and make important contributions to ecosystem function in their respective habitats by increasing connectivity between adjacent terrestrial, freshwater and marine ecosystems (Schindler et al., 2003; Walters et al., 2009; Hall et al., 2012). Their reliance on multiple ecosystems and the corridors that connect them make diadromous fishes particularly vulnerable to human impact (Waldman et al., 2016). As such, they have suffered some of the most marked declines of any vertebrate in North America, and these declines are closely associated with human activities (Waldman & Quinn, 2022). In particular, dams fragment freshwater environments by impeding movement between habitats crucial for feeding and reproduction (Waldman et al., 2016; Drouineau et al., 2018; Silva et al., 2018), and are the most common driver of diadromous fish species decline (Mattocks et al., 2017; Waldman & Quinn, 2022). Despite the presence of fish passage infrastructure on many dams, fishes traversing these barriers often perform poorly, which causes progressive reductions in fish numbers and delays to reach spawning grounds with each barrier passed (Waldman & Quinn, 2022). Fish access to more than 70,407 km of rivers (exclusive of habitat beyond the next upstream barrier) is impeded by terminal dams in the United States alone (Waldman & Quinn, 2022).

### 1.2 The Atlantic Whitefish

The Atlantic Whitefish (*Coregonus huntsmani*; Scott, 1987) is an endangered, anadromous, salmonid fish (in the Salmonidae family) endemic to Nova Scotia, Canada (Edge, 1984). It was the first fish to be assessed as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 1983 (Edge, 1984) and was among the first

group of species listed under Schedule 1 of the Species At Risk Act (SARA) in 2003 (Bradford et al., 2004). The Atlantic Whitefish is one of only three formally recognized species of freshwater and anadromous fishes endemic to Canada and the only vertebrate endemic to Nova Scotia (Enns et al., 2020). In addition to its endemicity, mitochondrial DNA (mtDNA) analysis confirmed that the Atlantic Whitefish represent an ancient lineage of coregonids unique to North America that diverged from the remainder of the genus during the Miocene Epoch, approximately 15 million years ago (Crête-Lafrenier et al., 2012; Figure 1). Extinction of the Atlantic Whitefish would thus represent an important loss to Canada's biodiversity.

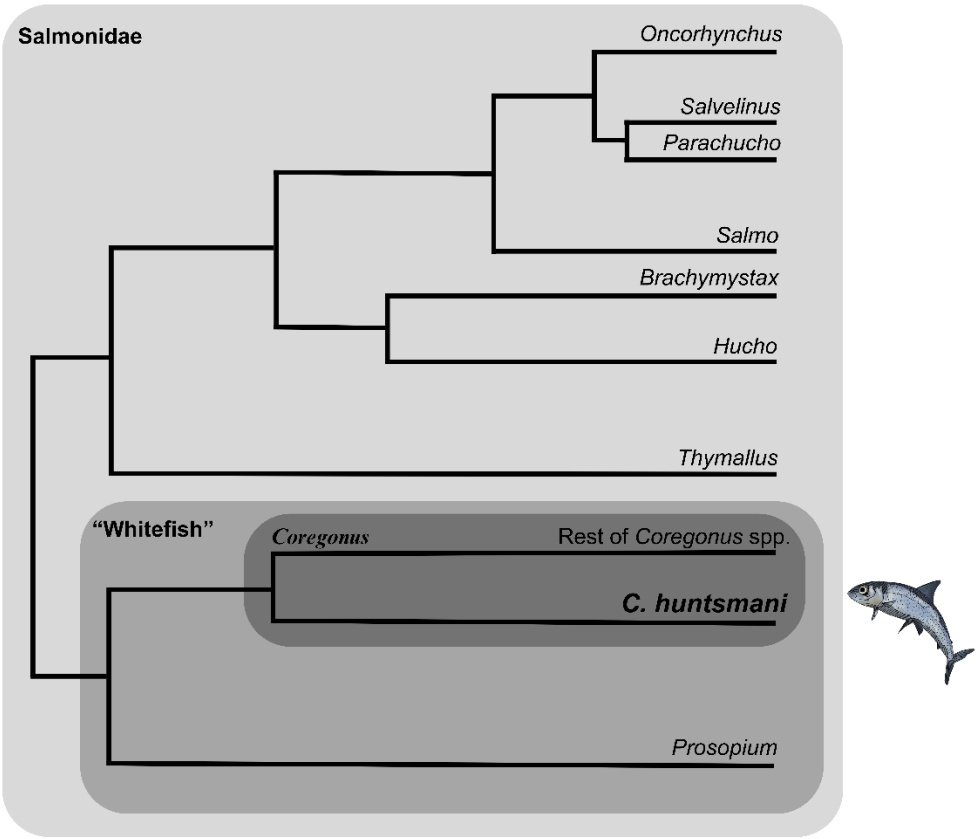


Figure 1. Cladogram of the relative position of Atlantic Whitefish (*C. huntsmani*) within the *Coregonus* genus and Salmonidae family. [Adapted from Crête-Lafrenier et al., 2012].

The Atlantic Whitefish has only been reported in the Tusket-Annis and Petite Rivière watersheds (Figure 2) in the southwestern region of Nova Scotia (Edge, 1984). The Tusket River population has since been assessed as extirpated, with the last whitefish sighted in the Annis River in October of 1982 (Edge & Gilhen, 2001; Bradford et al., 2004). The remaining range of the Atlantic Whitefish consists of three connected lakes in the Petite Rivière watershed totalling 16 $km^2$ in surface area: Hebb Lake, Milipsigate Lake and Minamkeak Lake (DFO, 2006; 2018a). Little is known about the historical population size and trends in the Petite Rivière, but enough fish were present to support a small recreational fishery prior to the 1900s (reviewed in Edge & Gilhen, 2001). However, catches during field surveys suggest a decline in the population from approximately 1925 onwards (Edge & Gilhen, 2001). Genetic estimates placed the effective population size ($N_e$) between 18 and 38 individuals (Cook, 2012), which is one of the smallest values for $N_e$ ever reported for a population of fish. Combined with reductions in the number of Atlantic Whitefish observed during field surveys and spring larval collections in 2018-2022 (Breen & Risto, 2019; Risto, 2020; Russell et al., 2022; Russell et al., 2023) these estimates suggest a critically low population size. Human alteration of the Petite Rivière and Tusket River watersheds, including acidification (Tusket only), changes in water quality due to agriculture and forestry, dam construction and the introduction of non-native fish species have been implicated in the extirpation of the Tusket River population and the declines of the Petite Rivière population of Atlantic Whitefish (Bradford, 2004; DFO, 2006; 2018a). In the Petite Rivière, pressing threats include competition and predation from invasive piscivores – Smallmouth Bass (*Micropterus dolomieu*), Chain Pickerel (*Esox niger*), were detected in the Petite Rivière in 2010 and 2013, respectively (LeBlanc, 2010; Themelis et al., 2014) – as well as a decreasing effective population size and associated reductions in genetic diversity and fitness (Cook, 2012). Future changes to

habitat suitability in the lakes available to Atlantic Whitefish resulting from climate change is also of increasing concern (Cook, 2012; DFO, 2006; 2018a).

Prior to the extirpation of the Tusket River population, gravid Atlantic Whitefish were observed migrating upstream in October and November in the Tusket River estuary (Gilhen, 1977; Scott & Scott, 1988). When paired with evidence that lab-bred and reared juvenile Atlantic Whitefish from the Petite Rivière prefer seawater (Cooke et al., 2010), these data suggest that the species is naturally anadromous. However, the construction of dams with inadequate fish passage pre-date the formal description of the species (Edge & Gilhen, 2001) and there is little evidence of returning migrants outside of a single group of 19 fish in 2012 (DFO, 2022). While released F1 captive-bred fish have been detected in the neighbouring LaHave and Medway River estuaries (DFO, 2018a), damming likely prevented most migratory individuals from returning to fresh water in the Petite Rivière system (Bradford et al., 2010). Currently, dams allow for very minimal upstream access to Minamkeak and Milipsigate Lake (DFO, 2006; 2018b). Hebb lake has only been accessible from the ocean since 2012, upon construction of a fish-way at Hebb Dam (DFO, 2012) and there has only been one recorded instance of use by 19 Atlantic Whitefish immediately after its construction (DFO, 2022). Existing passage at downstream dams (i.e., Crousetown, Conquerall) may also be ineffective for Atlantic Whitefish upstream movement (DFO, 2018a). Thus, the remaining whitefish in the Petite Rivière have been largely isolated from any returning migrants since the construction of Hebb Lake dam in 1901 (Edge & Gilhen, 2001).

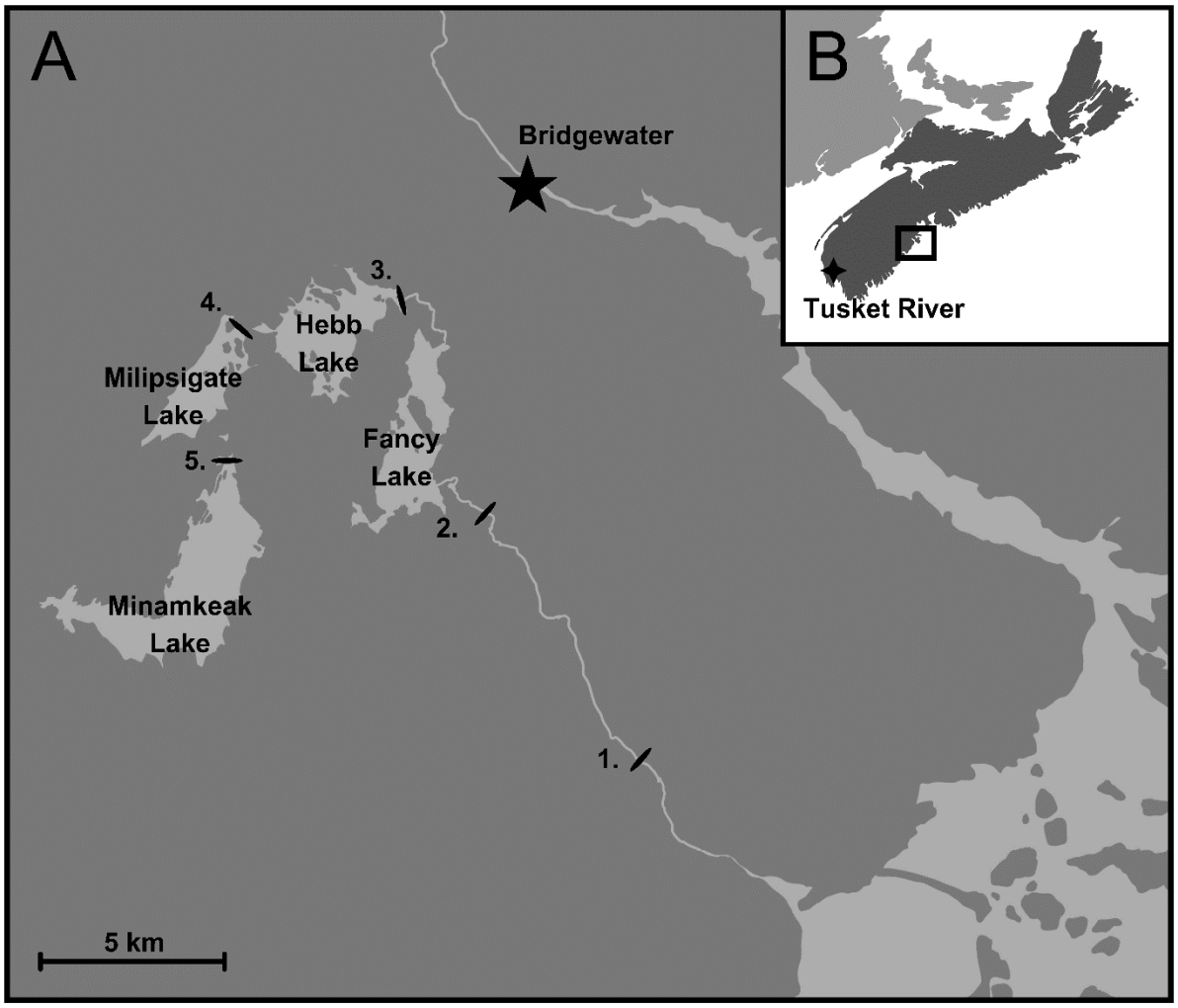


Figure 2. Map of the current range of the Atlantic Whitefish in the (A) Petite Rivière watershed in Lunenburg County, (B) Nova Scotia, Canada. Black bars indicate dams constructed along the waterway that connects Minamkeak, Milipsigate and Hebb Lakes to the Atlantic Ocean: 1. Crousetown dam; 2. Conquerall mills dam; 3. Hebbville dam; 4. Milipsigate outflow dam; 5. Minamkeak outflow dam. Dam characteristics and history can be found in the Atlantic Whitefish Recovery Strategy (DFO, 2006; 2018a)

### 1.3 Recovery plan for Atlantic Whitefish

A recovery strategy for the Atlantic Whitefish was proposed by the Department of Fisheries and Oceans Canada (DFO) in 2006 and finalized 2018 (DFO, 2006; 2018a). This strategy includes four broad objectives: (1) protect and conserve the species in its current habitat, (2)

increase the number and range of viable populations, (3) increase our understanding of the species and its habitat, and (4) increase public involvement and acceptance (DFO, 2006; 2018a). A main strategy of Objectives 1, 2 and 3 is to work towards re-establishing a self-sustaining anadromous population. To address Objectives (1) and (2) of the recovery strategy, the DFO has conducted releases of $F_1$-captive bred juveniles into the Petite Rivière system since the early 2000s (Whitelaw et al., 2015; Bradford et al., 2010).

Restoring anadromy may be beneficial to the remaining Atlantic Whitefish for several reasons. On northern coasts, migration across the freshwater-ocean boundary is widely considered to be beneficial, as marine and estuarine environments provide richer food sources than freshwater environments (McCormick & Saunders, 1987; Gross et al., 1988; McDowall, 2001a) at these latitudes. Indeed, anadromous individuals in the Salmonidae family, including coregonids, can experience substantial increases in their growth rate (Gross et al., 1988; McDowall, 1993, 2001b) and fecundity (Tallman et al., 1996; VanGerwen-Toyne et al., 2012) relative to their non-migratory conspecifics. Archived Atlantic Whitefish from the Tusket-Annis population similarly indicate that anadromous individuals were larger than those currently found in the Petite Rivière (DFO, 2006; 2018a). Estuarine and oceanic habitats may also provide a buffer to rising global temperatures, as they will remain cooler than inland lakes and can act as thermal refugia (Cook, 2012). Finally, despite the potential for novel challenges such as new predators, seaward migration may allow Atlantic Whitefish reprieve from introduced predators, including Smallmouth Bass and Chain Pickerel, which are restricted to fresh water (Edge & Gilhen, 2001; Themelis et al., 2014).

To successfully conduct an anadromous migration, the current Atlantic Whitefish population must be able to tolerate the changes in salinity they will encounter when moving from

fresh to saltwater. Studies on populations of other anadromous fishes suggest that reduced saltwater tolerance may evolve after selection for an anadromous lifestyle is relaxed. Stuarnes et al., (1992) found that Arctic Char (*Salvelinus alpinus*) from a landlocked population had a higher mortality rate (70%) following direct transfer to seawater than migratory conspecifics (20%). Velotta et al., (2016) found that genes regulating salt water ion secretion in landlocked forms of Alewife (*Alosa pseudoharengus*) had a blunted acclimation response to seawater relative to anadromous Alewife. Ouananiche, a form of non-migratory Atlantic Salmon (*Salmo salar*), exhibited a 100% mortality rate upon direct transfer to seawater in comparison to 80% in wild smolt of the same species (Burton & Idler, 1984). Similarly, a common garden experiment using three-spined stickleback (*Gasterosteus aculeatus*) suggested a local adaptation where populations habituated to low salinity environments (~5 ppt) had a reduced body size and survival probability when moved to high salinity (~20 ppt) compared to marine populations (Defaveri & Merilä, 2014).

Previous work shows that Atlantic Whitefish remain generally tolerant to high salinities (growth and high survival at ~ 30 ppt: Cook & Bentzen, 2009; Cook et al., 2010; Whitelaw et al., 2015), but less obvious reductions in tolerance may manifest as fitness consequences in long-term experiments and in the presence of other stressors. Among $F_1$ captive Atlantic Whitefish, both males and females produce gametes by age 2+ (Whitelaw et al., 2015), so it is possible that upwards of 50+ generations have since passed since the construction of Hebb dam in 1901 (Edge & Gilhen, 2001). Considering the low genetic diversity of this small population (Cook, 2012), it is possible reductions in upper salinity tolerance have evolved. Therefore, successful re-establishment of anadromous Atlantic Whitefish could be aided by better understanding how well Atlantic Whitefish from the Petite Rivière manage in higher salinities, particularly under

additional stressors that may be encountered during a migration, such as changes in water temperature and periods of strong flow.

In this thesis, I explored whether landlocked Atlantic Whitefish from the Petite Rivière have retained high tolerance to seawater to help assess the feasibility of restoring a self-sustaining anadromous Atlantic Whitefish population. To do so, I examined the physiology of juvenile Atlantic Whitefish acclimated to different salinities (0, 15, 30 ppt) and tested for differences in in growth, condition, stress response, aerobic scope, swimming performance and ion balance. I also took the opportunity to explore the effect of an unplanned, seasonal increase in temperature (~12 – 17ºC) in the fish housing facilities on body condition and stress indices.

# Chapter 2 – The effect of salinity and temperature on Atlantic Whitefish growth, metabolism and osmotic homeostasis

## 2.1 Introduction

### 2.1.1 *Atlantic Whitefish*

Diadromous fishes migrate between freshwater and marine habitats to complete their lifecycle (Myers, 1949). Among diadromous species are anadromous fishes, which spawn in freshwater habitats and migrate to the ocean to feed and mature (McDowall, 2001). Salmonids (fish in the family *Salmonidae*) are famous for this behaviour, as well as for variation in the degree to which anadromy is expressed within and between populations and species (Hutchings & Morris, 1985; McDowall, 2001a). Salmonid fishes in the *Coregonus* genus (coregonids) commonly exhibit anadromy in the northern parts of their range (reviewed in Howland et al., 2009), where the trait is considered beneficial to growth and fecundity due to increased productivity of marine and estuarine environments relative to freshwater (McCormick & Saunders, 1987; Gross et al., 1988).

Their reliance on both freshwater and marine environments, as well as the corridors that connect them, make diadromous fishes important components of ecosystems by increasing connectivity between these systems (Schindler et al., 2003; Walters et al., 2009; Hall et al., 2012). However, this reliance on two habitats also makes diadromous fish species additionally vulnerable to human impacts. Indeed, habitat fragmentation and loss of connectivity in between freshwater and marine environments is a common and widespread driver of declines in these migratory fishes (Waldman & Quinn, 2002).

The Atlantic Whitefish (*Coregonus huntsmani*) is an endangered salmonid fish endemic to Nova Scotia, Canada (Edge, 1984). The species is assumed to be ancestrally anadromous, as gravid whitefish were observed in the estuary of the Tusket-Annis River prior to the extirpation of this population of Atlantic Whitefish in the 1980s (Edge & Gilhen, 2001; Bradford et al., 2004). However, the last remaining population of Atlantic Whitefish is landlocked within the Petite Rivière watershed and has been largely isolated from any returning migrants for the last century due to the construction of dams. Natural anadromy has never been recorded in the Petite Rivière, either because dam construction preceded the observation of migration, or because the Petite Rivière whitefish are an ancestrally freshwater resident population (Bradford et al., 2010). Nonetheless, a major component of their recovery strategy (DFO, 2006; 2018a) is to restore a self-sustaining anadromous population. This raises the question, are the remaining Atlantic Whitefish capable of completing of an anadromous migration?

Cook et al. (2010), showed that $F_1$ juvenile and adult whitefish originating from the Petite Rivière were fully tolerant (100% survival) of fresh, brackish and salt water (15 and 30 ppt, respectively), and larval survival decreased only moderately from 100% in fresh water to 94% and 92% in brackish and salt water treatments, respectively. Similarly, housing data from the Mersey Tobeatic Biodiversity Institute reported that Atlantic Whitefish bred from the Petite Rivière were very tolerant of short-term and sudden changes in salinity (20 ppt) during routine prophylactic salt treatments (Whitelaw et al., 2015). However, maintaining homeostasis in salt water may impose some less obvious costs that may have fitness consequences in the context of a migration. Data from the Tusket River population indicates that adult anadromous whitefish move to fresh water in the autumn months (DFO, 2006; 2018a), but the age of migrant fish and the timing of out-migrations is not known. Given the large window of potential out-migration

times, temperature may also play an important role as a physiological stressor (Dean & Goodnight, 1964; Strange, 1980; Breau et al., 2011; Honsey et al., 2023), which in combination with osmotic stress, could influence the long-term persistence of an anadromous population.

### 2.1.2 *Physiological mechanisms facilitating osmo- and ionoregulatory homeostasis during anadromous migrations*

Salinity is an important factor that defines habitat for aquatic organisms, such as fishes (Kültz, 2015). Salinity affects fish survival through its influence on ion and water balance (Cook et al., 2010; Schreck & Tort, 2016), which in turn impacts biochemical and cellular processes, and the functioning of organs and organ systems (Kültz, 2015). As the tissue of teleost fishes is rarely iso-osmotic to the water in which they reside, fish are often exposed to osmotic stress due to passive movement of ions and water between bodily fluids and the hypo- (fresh water) or hyper- (seawater) saline environment (Takei & Hwang, 2018). For the purposes of this paper, I define *stress* as any deviation away from whole-organism homeostasis, and *stressor* as an external factor that challenges homeostasis.

Maintenance of body fluid and ion balance is a complex process that largely occurs through ion uptake and excretion across the membranes of major osmoregulatory organs, including the gills, kidneys, and digestive tract (Takei & Hwang, 2018). Ion movement is facilitated by a combination of co-transporters, pumps, and channels specific to the external environmental conditions (Evans et al., 2005; Hwang et al., 2011; Ern et al., 2014; Takei & Hwang, 2018). Teleost fish ionoregulate to maintain internal inorganic ion concentrations within a specific range (Fiol & Kültz, 2007), typically 10-12 ppt (Boeuf & Payan, 2001). Most fish species can tolerate only a limited range of salinity fluctuations (stenohaline fish), but there are

numerous species that have high tolerance for great fluctuations in salinity (euryhaline fishes) (Fiol & Kültz, 2007). A key characteristic of an anadromous life history is that fish must maintain homeostasis in both fresh and salt water.

To successfully transition between salinities, a euryhaline fish must be able to initiate acute and long-term physiological changes in ionoregulatory tissues needed to restore homeostasis in the new environment (Fiol & Kültz, 2007; Cook et al., 2010; Takei & Hwang, 2018). The acute phase occurs within minutes to hours following the introduction of osmotic stress, with the major outcomes being rapid changes in behaviour, altered blood flow to the osmoregulatory organs and changes to membrane ion transport protein composition and activity (reviewed in Fiol & Kültz, 2007; Kültz, 2015; Takei & Hwang, 2018). The long-term phase involves more enduring, hormonally-regulated changes (e.g., via changes in the concentrations of corticosteroids, growth hormone and corresponding receptors; Evans et al., 2005) that amount to organism-wide changes in ion transport capacity (reviewed in Foskett et al., 1983). For example, in response to hyperosmotic stress when moving from fresh water (0 ppt) to seawater (30 ppt), salinity detection by the osmosensors (cells that monitor body fluid osmolality; Kültz, 2012) immediately leads to changes in the ionocytes, specialized cells in osmoregulatory organs involved in ion homeostasis, to inhibit ion absorption and activate ion excretion (McCormick et al., 2012). Over the long-term, hormone expression induces changes to the composition of ionocyte types in osmoregulatory organs through the apoptosis of absorptive cells and differentiation of excretory cells (Takei & McCormick, 2012).

The processes required to osmoregulate in salt water and fresh water, as well as mechanisms of switching from ion-absorptive to ion-excretory pathways, are energetically costly (Ern et al., 2014; Sadoul & Vijayan, 2016). Costs may be particularly high in landlocked

populations of previously diadromous fishes in which relaxed selection has led to a reduction in osmoregulatory capabilities in higher salinities (e.g. Foote, 1989; Stuarnes et al., 1992; Velotta et al., 2014, 2015). In these populations, the cost-to-benefit ratio of diadromy is shifted due to the loss of access to productive estuarine and marine habitats (Kültz, 2015), and maintaining euryhalinity may no longer be beneficial (Gross, 1987). Alternatively, relaxed selection may simply increase the likelihood of saltwater tolerance loss (reviewed in Lahti et al., 2009). However, while coping with osmotic stress requires energy (Boeuf & Payan, 2001), the relative costs of osmoregulation in salt water and fresh water are unpredictable, and likely vary based on the species and circumstances (see Ern et al., 2014). For example, anadromous Sockeye Salmon (*Oncorhynchus nerka*) exhibit a better seawater tolerance than Kokanee, their non-anadromous counterparts (Foote, 1989) and previous work has suggested that there are likely trade-offs in osmoregulatory capacity and swimming performance that occur following freshwater isolation in Alewife (*Alosa pseudoharengus*) (Velotta et al., 2014, 2015). In contrast, some degree of persistent euryhalinity has been documented in landlocked Arctic Char in the British Isles (Roberts 1971; Finstad et al., 1989), and some landlocked Atlantic Salmon (*Salmo salar*) still retain remnants of a smolt phase (Burton & Idler, 1984). Therefore, the degree to which saltwater tolerance is reduced in landlocked populations of fishes likely depends on a combination of selective and random factors (genetic drift) that vary according to the species and circumstances (Delgado et al., 2020) such as time since landlocking, generation time, population size and diversity, and any fitness costs or benefits of maintaining the physiological capacity for osmoregulation (Lahti et al., 2009).

### 2.1.3 *Thesis goals*

In this thesis, I studied the physiology (growth, condition, metabolic and swimming capacity, and osmoregulatory capacity) of $F_1$ captive-bred offspring of wild-caught Atlantic Whitefish originating from the Petite Rivière in response to salinity and an increase in temperature that occurred during the course of the experiment. The goals of this study were to assess the ability of the remaining Atlantic Whitefish to effectively maintain iono- and osmoregulatory homeostasis at different salinities and the naturally occurring increases in water temperature allow for us to test these responses at two ecologically relevant temperatures.

The parameters measured in this study have been used previously to quantify the degree to which a fish is experiencing osmotic stress. Osmoregulation requires energy (Boeuf & Payan, 2001; Ern et al., 2014; Takei & Hwang, 2018), which, in theory, increases with the severity of deviation from homeostatic ion balance (osmotic stress) (Ern et al., 2014). The costs of maintaining osmotic homeostasis have been estimated to account for 10-50% of a fish's routine metabolic rate (RMR) (Boeuf & Payan, 2001). As such, maintaining osmotic homeostasis may require a shift in energy away from other processes such as growth and metabolism which may impede overall fish welfare and fecundity (Barton & Iwama, 1991). Condition is a measure of overall fish well-being (Froese, 2006) and available energy stores. It is considered a secondary response to stress, while growth represents a tertiary response (Barton & Iwama, 1991). Both traits are well-studied in response to stressors in aquaculture settings (e.g. McCormick et al., 1998; DiBattista et al., 2006; Sadoul & Vijayan, 2016) and many studies have reported reductions in growth and condition in response to osmotic stress (e.g. Mylonas et al., 2009; Liu et al., 2017; Monzanzadeh et al., 2021). Increased osmoregulatory costs could also be reflected by reductions in aerobic scope and swimming performance (Fang et al., 2019), which would

have considerable implications on migration success for anadromous fishes. Additionally, while the pattern and magnitude of the response tends to be specific to the species, most teleost fish respond to stress by increasing blood glucose, lactate and cortisol concentrations (Barton & Iwama, 1991; Wendelaar Bonga, 1997; Barton, 2002) and all have been reported to increase during the initial stages of salinity acclimation (e.g. Tsui et al., 2012; Monzanzadeh et al., 2021; McCormick et al., 2019). Cortisol is particularly important to osmoregulatory processes and has been shown to modulate hydromineral balance under osmotic stress (McCormick, 2001; Evans et al., 2005; Takei et al., 2014; Finstad, 2015) and promote long-term acclimation to hypo- and hypersaline environments through the reorganization of osmoregulatory organ epithelia (Wendelaar Bonga, 1997). Any changes to salinity that increase overall baseline stress levels are therefore expected to manifest as increases in blood cortisol, glucose and lactate. Finally, a change in plasma osmolarity is among the initial response to salinity, as movement of ions and water between internal and external milieu occurs before fish can compensate through changes to osmoregulatory mechanisms (Ordóñez-Grande et al., 2020), and this is reflected by changes in plasma ion concentrations. The degree of these changes is directly related to the intensity of osmotic shock (Varsamos, 2002).

In this thesis, I studied the extent to which Atlantic Whitefish are able to cope with varying degrees of ionic and osmotic stress by conducting a salinity acclimation experiment (0, 15, 30 ppt) with juvenile fish initially bred and reared in fresh water. Following acclimation, I attempted to measure aerobic scope and swimming performance, and measured fish growth, condition, stress and plasma ion content, an indicator of iono- and osmoregulatory homeostasis. Additionally, logistical difficulties during experimentation required that I increase water temperature from 12°C to 17°C across treatments to maintain uniformity. I took this opportunity

to explore whether the presence of an ecologically relevant temperature increase (A) induced a stress response and (B) influenced fish salinity tolerance.

## 2.2 Materials and Methods

### 2.2.1 *Atlantic Whitefish breeding, husbandry and salinity acclimation*

Early life fish husbandry (Day <1)

The 90 $F_1$, captive-bred fish used in this study were bred and reared at the Aquatron Laboratory, Dalhousie University (Halifax, NS). Parent fish ($F_0$) were captured as early juveniles from fyke nets and rotary screw traps at the inflow and outflow of Milipsigate Lake (Figure 2) in 2018 and 2019. They were then reared on-site in the Aquatron Laboratory in fresh water (0 ppt) at ambient tap water temperature (4.8°C – 17°C, kept under 17°C during summer months) and following a natural light cycle for Halifax, Nova Scotia. $F_1$ whitefish used in this experiment were hatched from artificially incubated eggs obtained through natural group spawning in $F_0$ housing tanks from likely around 3 pairs (personal communication, John Batt, Aquatron Laboratory). Thus, some of the individuals used in this experiment may be full or half-siblings; genetic parentage analysis would be needed to quantify the relatedness of individuals used in this experiment and it is not yet known. Once $F_1$ embryos hatched (~ March 25, 2022), they were reared under similar conditions as $F_0$ whitefish. $F_1$s were initially fed lab cultured *Artemia* (INVE Aquaculture), and weaned onto live *Daphnia* (Aquatron Laboratory) and salmon Nutra Sprint (Skretting, BC) at approximately 30 days post-hatch (DPH). They were then transitioned to Purina® Game Fish Chow (Land O' Lakes Inc, USA; minimum 32% crude protein, 3% crude fat, 0.8% phosphorus, maximum 6% crude fiber and 0.6% sodium1%, and 1-1.5% calcium), and

then to the pellet diet consisting of a 3:1 mixture of Corey Aquafeed 2.0 mm Optimum RAS (Corey Nutrition Company Inc, NB) and Skretting 1.2 mm Gemma Diamond (Skretting, BC) around 83 DPH and 114 DPH, respectively. It was not possible to determine sex via non-lethal methods during this experiment. Fish were not tagged.

Salinity acclimation and husbandry during salinity exposure (Day 1-252)

Whitefish were kept in nine (three per salinity treatment), 135 L fibreglass round tanks arranged on tiered racks. Each tank was initially stocked with 10 fish, and individually plumbed with a flow-through system operating at a rate of 3.6 L minute$^{-1}$ (Figure 3). Therefore, the three replicate tanks for each salinity shared flow through water. Target salinities [fresh water, 0 ppt (0.54 ppt ± SD 0.31); brackish, 15 ppt (15.03 ppt ± SD 0.06); seawater, 30 ppt (29.96 ppt ± SD 0.10)] were achieved through mixing of seawater from the Northwest Arm (Halifax, NS) and dechlorinated Halifax city tap water. $F_1$ Atlantic Whitefish had an average weight of 39.2 g ± SD 8.29 at the start of salinity acclimation. Fish were acclimated to treatment salinities between November 25, 2023, and December 9, 2023, at a rate of 2 ppt per day until desired salinity was achieved. Water temperature was maintained at 12°C (11.66°C ± SD 0.49) from November 25, 2022, to May 31, 2023 (days 1 to 187 in Figure 3) based on current housing temperatures for captive Atlantic Whitefish at the Aquatron Laboratory. However, seasonal increases in temperature resulted in tap water increasing the temperature of fresh water and brackish treatments relative to the seawater treatment from June 1, 2023, onwards. To maintain temperature uniformity, all tanks were slowly heated to and maintained at 17°C (16.55°C ± SD 0.83) over the course of 5 days starting on June 12, 2023 (day 199, Figure 3) until the salinity exposure experiment ended on August 4, 2023 (day 252, Figure 3). 17°C was chosen to avoid

making unnecessary increases to temperature while maintaining temperature uniformity long enough to finish experiments. Two mortalities occurred for unknown reasons in freshwater treatment tanks within the first 14 days of the study, after which no additional mortalities occurred, except for three fish used in preliminary respirometry trials (Table C.1).

During the salinity exposure experiment fish were fed twice daily by hand with a 3:1 mixture of Corey Aquafeed 2.0 mm Optimum RAS (Corey Nutrition Company Inc, NB) and Skretting 1.2 mm Gemma Diamond (Skretting, BC). Daily rations corresponded to 3% of the average body mass in each tank but was subsequently adjusted to 2% from experiment day 88 onwards to reduce fish growth. While consumption rates were not recorded, captive Atlantic Whitefish are generally willing to eat food off the tank bottom, and leftover food in each tank suggests that even a 2% body mass feed ration corresponded to *ad libitum* feeding. Ration masses were thus only updated with fish mass during initial growth measurements (days 1, 22, 50, 74, 116). At day 116, fish were split from the original nine tanks into 18 tanks to provide more space per individual (5 fish/135 L). Fish from each tank were split equally into two new tanks, such that a particular fish's original tank was known.

Throughout the salinity exposure (days 1-252), the photoperiod approximately followed a natural light:dark cycle (facility errors resulted in an approximately 1 month offset, with the shortest day falling mid-January). Water parameters (salinity, temperature, pH and dissolved oxygen (DO)) were measured daily with waterproof pocket pH and salinity testers (HANNA® Instruments, QC) and Polaris C hand-held DO meter (OxyGuard®, DK) (12.88°C ± SD 2.16, 8.08 ± SD 0.26, 95.44 % ± SD 7.16, see Table A.1 for salinity). Due to scheduled facility maintenance in the Aquatron, heat was temporarily shut off on days 161 and 162 of the

experiment, resulting in a decrease in temperature (average decrease 2.39°C) across all treatment groups (Fig A.1). During this time, fish were left undisturbed to minimize additional stress.

All procedures involving the handling and care of Atlantic Whitefish were approved by the Dalhousie University Committee on Laboratory Animals (UCLA) under protocols 21-127 and 23-019. Following the conclusion of experiments, Atlantic Whitefish from all treatments were returned to the care of the Aquatron Laboratory to act as backup broodstock for the laboratory breeding program.

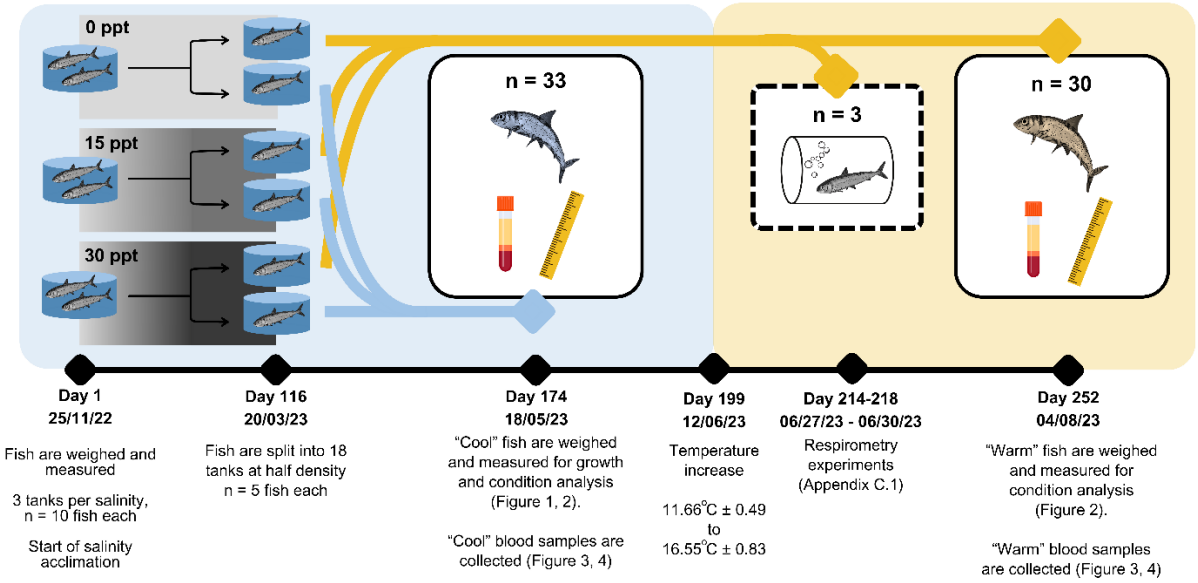


Figure 3. Experimental design and sampling of $F_1$ Atlantic Whitefish over the 252-day salinity acclimation. **Day 1:** all 90 juvenile (~265 DPH) Atlantic Whitefish were measured and separated into nine tanks, with three replicates per salinity treatment (0 ppt, 15 ppt, 30 ppt) Note that only one tank per treatment is shown for clarity. **Day 116:** fish were split from nine to 18 tanks to reduce density. **Day 174:** fish (~419 DPH) at "cool" temperatures (11.66°C ± SD 0.49) were measured, and blood samples collected. These fish were not handled again after this blood collection. **Day 199:** temperature increased approximately 5°C in all tanks. **Day 214-217:** three fish (~458 DPH) were used in maximum metabolic rate and swimming performance trials, after which respirometry experiments were discontinued (Section 2.2.7). **Day 252:** Measurements and blood samples collected for a different group of fish (~ 497 DPH) at approximately 16.55°C ± SD 0.83 ("warm" fish exposure).

### 2.2.2 *Measurement of fish mass and length to calculate growth and condition factor (Days 1-252*)

Fish were fasted for a minimum of 24 hours to reduce risk of regurgitation and fecal contamination and sedated with tricaine methanosulphonate (50 mg $L^{-1}$) buffered in a 1:2 ratio with sodium bicarbonate prior to every handling session, except for the purposes of respirometry. All fish from a given tank were sedated at once on days where only growth measurements occurred (days 1, 22, 50, 74, 116) and exposed to MS-222 one at a time on days where blood samples were taken (days 174, 252). Fish were considered sedated while they had opercular motion but did not respond to a pinch on the caudal fin. Fork length (mm) and mass (g, wet weight) were measured using a Biomark ® BioLogic Electronic Measuring Board and OHAUS™ Valor 7000 Compact Bench Scale approximately once per month for the first 116 days of growth (days 1, 22, 50, 74, 116, Figure B.1) and again during blood sampling (days 174, 252) (Figure 3). Growth data from days 1-74 were collected and used for the purposes of a Bachelor of Science Honours thesis (Lamontagne, 2023; unpublishd). All handling procedures were completed in less than approximately 2 minutes, and fish recovered from anaesthesia within approximately 5 minutes following return to oxygenated water.

Even after uniform heating of the water, some differences in temperature among salinity treatments remained (~1-2$^{o}$C). To correct for the potential effects of temperature variation and any differences in starting fish size on measurements of growth rate, I calculated the percentage of growth (% growth) per degree day (DD). The number of degree days experienced by each fish were calculated using the following equation from (Honsey et al., 2023):

$$\boldsymbol{DD = \sum_{t=1}^{N} T_1 - T_0, \qquad T_1 > T_0} \tag{1}$$

$T_1$ is the average temperature for day 1, and $T_0$ is a species-specific temperature below which thermal energy is assumed to have no effect on physiological processes (e.g. growth) (Honsey et al., 2023). I used a $T_0$ value of 0℃ which is the recommended temperature for coregonids and many other salmonids (Chezik et al., 2014, Honsey et al., 2023). So, Equation 1 was simplified to:

$$DD = \sum_{t=1}^{N} T_1 \quad \textbf{(1.1)}$$

Any days where I was missing temperature data, I filled by averaging the temperature of the preceding and following days. Prior to day 110, water temperature was not measured, but water temperature settings were identical to temperatures between day 110 and 199, so daily temperature in each tank prior to this date was calculated as the average daily temperature of that tank for days 110-140.

Fish were not individually tagged, so tracking individual growth from one point to another was not possible. Therefore, to calculate percent growth (% growth), I reported the size of an individual fish measured on day 174 (n = 33 per salinity) as a percentage of the average size of all (n = 10) fish in its original tank (all at the same salinity) on day 1. Percent growth per degree day (%Growth $DD^{-1}$) was then obtained by dividing % growth by the number of degree days experienced by that fish. %Growth $DD^{-1}$ was calculated separately using both length (FL) and weight data.

My inability to track individuals meant measuring changes in individual condition across measurement periods was not possible. To compare fish condition preceding and following a change in ambient water temperature and across salinities, I calculated relative condition factor

($w\ W^{-1}$) (Le Cren, 1951) for each individual measured on days 174 and 252. Relative condition factor is the ratio of the observed weight over the expected weight based on a linear regression of a control group. Our linear regression produced the following equation ($R^2 = 0.967$) to predict weight from fork length.

$$\mathbf{log}(\boldsymbol{W}) = \mathbf{3.5051} * \mathbf{log}(\boldsymbol{L}) - \mathbf{6.1059} \quad \textbf{(2)}$$

$\boldsymbol{W}$ is expected fish wet weight (g) and $\boldsymbol{L}$ is fish fork length (FL, mm).

2.2.3 *Blood sampling of "cool" water housed fish*

Baseline blood samples were taken on day 174 from 33 fish, with n = 10-13 per salinity, due to difficulty obtaining blood from three fish. A volume corresponding to 1% body mass (102.03 μL ± SD 22.27) was extracted from the caudal vein of anesthetized fish using 23 ga, 1.5" hypodermic needle fitted to a 1 mL syringe. Syringes and needles were pre-heparinized following the protocol outlined in Zang et al., (2015). Once fish were sampled and measured, they were returned to an oxygenated tank to recover and were not reused for future blood samples or size measurements in the "warm" period to reduce handling of individual fish. All fish were out of water for < 2 minutes and had fully recovered in approximately 4 minutes.

Blood was transferred to microcapillary tubes and centrifuged at 3,000 G for three minutes and photographed for haematocrit measurement. I later discovered that these settings were not sufficient to ensure full separation of plasma and erythrocytes, which rendered the haematocrit data unusable and reduced the amount of plasma available for laboratory tests. Future studies should centrifuge blood samples at 10,000 G for 5 minutes. Available plasma was separated and stored at -80℃.

2.2.4 *Blood sampling of "warm" water housed fish*

On day 252, blood samples were collected from an additional 10 fish per salinity (n = 30 fish across all salinities). It is important to note that these blood samples were taken from different individuals than the "cool" samples. While repeat sampling of the same individuals could account for individual differences in temperature responses, the precautions required for animal welfare made this impossible. At the time of sampling, "Warm" fish were housed at 16.55°C ± SD 0.83. This was a 5°C rise in ambient water temperature compared to the "cool" (11.66°C ± SD 0.49) blood collection on day 174. Fish growth allowed us to safely collect a 60% larger sample volume (164.58 µL ± SD 39.01) than day 174 on day 252. Blood samples were processed and stored as in section 2.3.

2.2.5 *Measurement blood glucose, blood lactate and plasma cortisol*

To determine if salinity treatments or the increase in housing temperature led to increased stress, I measured plasma cortisol, the primary glucocorticoid hormone released to initiate the stress responses in fishes (Barton and Iwama, 1991), and measured total blood glucose and lactate as proxies for metabolic stress (Barton, 1997; reviewed by Vaage et al. 2023). Blood glucose and lactate levels were measured during blood sampling using the ContourNext EZ© glucose meter (Ascensia Diabetes Care, Canada) and The Edge™ lactate meter (the Edge, USA). These meters were selected for their practicality and user-friendly interface. Numerous studies have demonstrated their accuracy in mammals. Haldorsdottir et al., (2013) reported that the EZ meter exhibited the lowest average deviation (4.7%) from laboratory reference glucose tests in the range 1.33-21.44 mmol $L^{-1}$, with over 99% of results falling within 10% of the reference

value according to previous studies (Warchal-Windham et al., 2012; Pflug et al., 2013). Similarly, Bonaventura et al., (2015) found that The Edge lactate meter consistently demonstrated a lower total error across a range of 1.0 – 23.0 mmol $L^{-1}$ than the Lactate Pro™ (Akray Inc, Japan), which has been validated for use with Atlantic Cod (*Gadus morhua*) (Brown et al., 2008) and Atlantic Salmon (Breau et al., 2011). However, it is important to note that neither meter used in this study has itself been validated for use in fish, so comparability of lactate and glucose values presented here to those outside of this study is limited.

Plasma cortisol was measured using an enzyme-linked immunosorbent assay (Neogen® corporation, USA) on a SpectraMax M3 spectrophotometer (Molecular Devices, USA), according to manufacturer's instructions with the following changes. During the extraction, ethyl ether was evaporated in-bulk using a vacuum desiccator outfitted with argon gas ($A_2$) instead of individually using a stream of nitrogen gas ($N_2$). Instead of 100µL of plasma, I extracted cortisol from only 10 µL plasma plus 90 uL urine diluent (Diamond Diagnostics, USA). Consequently, to ensure that samples would fall within the detection range of the assay, I reduced the dilution of sample to extraction buffer from 100-fold to 10-fold.

### 2.2.6 *Measurements of plasma osmolarity and $Na^+$ and $Cl^-$ concentrations*

Total plasma osmolarity was measured in 50 µL sample volumes using a µ·Osmette™ micro-osmometer (Precision Systems Inc., USA). Where necessary, plasma samples were diluted to 50 µL with urine diluent – resulting in a variety of plasma dilutions across samples. Sample osmolarity was calculated based upon the dilution with this diluent (average osmolarity of 317.66 mOsm and [Na+] = 117 mmol $L^{-1}$ and [Cl-] = 119.66 mmol $L^{-1}$).

As osmolarity measurement were non-destructive, the same sample was used to measure ion concentrations. An additional 35 µL of urine diluent was added to the 50 µL sample, to bring the sample volume up to the minimum volume of 85 µL required by the instrument (Smartlyte Electrolyte analyzer, Diamond Diagnostics, USA) to measure the concentration of $Na^+$, $Cl^-$, and $K^+$ ions in each diluted sample. Plasma osmolarity and ion concentrations were calculated using Equation (3) and (3.1), respectively, to correct for the osmolytes and ions added via dilution with the urine diluent.

$$\boldsymbol{plasma\ [Na^+]} = \frac{(\boldsymbol{Read[Na^+] * total\ vol(L)}) - (\boldsymbol{diluent\ vol\ (L) * diluent\ [Na^+]})}{\boldsymbol{plasma\ vol\ (L)}} \quad \textbf{(3)}$$

$$\boldsymbol{plasma\ mOsm} = \frac{(\boldsymbol{Read\ mOsm * total\ vol}) - (\boldsymbol{diluent\ vol * diluent\ mOsm})}{\boldsymbol{plasma\ vol}} \quad \textbf{(3.1)}$$

Where $[Na^+]$ is the concentration of $Na^+$ ions in mmol $L^{-1}$ and mOsm is osmolarity in milliosmoles.

### 2.2.7 *Respirometry trials*

I attempted to measure standard and maximal metabolic rates as well as swimming performance of Atlantic Whitefish using an intermittent flow respirometry protocol conducted in a 10 L swim tunnel respirometer (Loligo® Systems, Denmark). However, the fish responded poorly to containment within the swim tunnel respirometer; they were extremely sensitive to caudal fin damage, resulting in mortality. This prompted several modifications to the experimental protocol and respirometer design aimed at reducing stress. However, due to continued animal welfare concerns, respirometry experiments were discontinued entirely.

Instead, I shifted focus to assessing stress and the maintenance of osmoregulatory homeostasis through blood parameters. The increase in water temperature of the holding tanks following baseline blood sampling on day 174 allowed for the opportunity to investigate the effect of salinity at two different temperatures. For further details on respirometry, see Appendix C.1.

### 2.2.8 *Blood smears*

To capitalize on the opportunity to handle Atlantic Whitefish, blood smears (Fig C.1) were prepared from blood collected on day 252 (n = 30) for future analysis. Slides were crafted in duplicate and stained with VWR® Quick I™ Single Step Wright's stain (Avantor, USA) following the provided instructions. Blood smear imaging was conducted using oil immersion (100X objective) on a Zeiss Axiovert 200M inverted microscope (Zeiss, Germany). Images were captured using a Zeiss AxioCam ICc5 camera with AxioVision SE64 software.

### 2.2.9 *Statistical analysis*

All statistical analyses were conducted using R v4.3.2 (R Core Team, 2021) with RStudio (RStudio Team, 2020). Prior to analyses, all data were tested for outliers (z-score > 3), revealing outliers in both size (n = 1), condition (n = 2), cortisol (n = 1) and plasma ion data (n = 2). The outlier in size data (fish 8D) is a notably large fish acclimated to brackish water (15 ppt), and fish 3A and 16B exhibited lower relative conditions than other fish in this study. Cortisol outlier 12C had a considerably higher plasma cortisol concentration (6.69 ng $mL^{-1}$) than all other fish (1.807 ng $mL^{-1}$ ± SD 0.90). However, there was no biological justification for removal of any of these outliers, so they were retained in the study. Outliers for $Na^{+}$ and $Cl^{-}$ ion content were associated with the most diluted samples, those containing only 5 µL of plasma within the final 85 µL sample. To assess any further impact of plasma volume on the accuracy of the Smartlyte

Electrolyte analyzer, I plotted the ion concentration data against plasma volume (Figure D.3). I found for that samples containing > 10 μL of plasma, primarily from "cool" fish sampled on day 174, the Smartlyte meter appeared to overestimate the concentration of $Na^+$ and $Cl^-$. These samples were consequently removed from $Na^+$ and $Cl^-$ analysis, leading to insufficient sample sizes for 'cold' acclimated fish. Therefore, I could not test the effects of temperature increase on plasma ion concentration. Despite failing the homoscedasticity assumption, a one-way ANOVA indicated that differences in plasma dilution also significantly affected measures of osmolarity ($p = 2.06*10^{-5}$), so samples with less than 30 μL of plasma were removed from osmolarity data, after which plasma volume did not have a significant impact on plasma osmolarity measurement ($p = 0.065$). This resulted in a loss of enough data to make testing for differences in plasma osmolarity between "cool" and "warm" groups impossible. There were also insufficient data to test for the effect of temperature on plasma cortisol concentrations due to low sample volumes collected from these smaller fish. Therefore, cortisol, osmolarity and ion concentration data collected from only the "warm" fish that had experienced a temperature change (day 252) were used in analysis.

Impact of salinity on %Growth $DD^{-1}$, "warm" plasma cortisol (ng $mL^{-1}$), and "warm" plasma ion concentrations (mmol $L^{-1}$) was evaluated using a one-way ANOVA. For cortisol analysis and %Growth $DD^{-1}$ as measured by weight (g), a logarithmic transformation was applied to meet the normality of residuals assumption. Similarly, plasma $Na^+$ data was reciprocally transformed to meet this assumption. Two-way ANOVAs were used to examine the effects of both salinity (0, 15, or 30 ppt) and temperature (11.7°C or 16.6°C) on relative condition factor ($w\ W^{-1}$), blood lactate (mmol $L^{-1}$), and blood glucose (mmol $L^{-1}$). A Tukey's range test (Faria et al., 2023) was used to identify any sample groups with significantly different

means *post-h*oc. Tank was initially included as a random effect for all tests but did not improve model fit (Table D.1) and was therefore omitted. All plots were generated using the ggplot2 package (Wickham, 2016).

## 2.3 Results

### 2.3.1 *The effect of salinity and temperature on fish growth and condition*

Growth of juvenile Atlantic Whitefish was calculated as percent growth per degree day (%Growth $DD^{-1}$) at each salinity from experimental days 1-174 (0 ppt, 15 ppt, 30 ppt, n = 10-13 per salinity, with the same individuals measured repeatedly, but not paired) using both fork length (FL, mm) (Figure 1A) and weight (g) (Figure 1B). Fish acclimated to 15 ppt had a slightly higher average %Growth $DD^{-1}$ and variation compared to those at 0 ppt and 30 ppt, but there was no significant effect of salinity on %Growth $DD^{-1}$, measured by either length ($t_{1,30} = -1.302$, $p = 0.203$) or weight (*logarithmically transformed*; $t_{1,30} = -1.513$, $p = 0.141$). The relative condition factor (RCF, $w\ W^{-1}$) of Atlantic Whitefish before ("cool"; 11.66°C ± SD 0.44) and after ("warm"; 16.55°C ± SD 0.83) an increase in ambient water temperature, significantly differed (Figure 2; $F_{1,55} = 4.846$, $p = 0.032$); younger (~458 DPH), "cool" fish had a significantly higher RCF (0.99 ± SD 0.042) than older (~497 DPH) "warm" fish (0.96 ± SD 0.060). However, there was no difference in the RCF of fish from different salinity treatments at a given temperature ($F_{1,55} = 0.470$, $p = 0.496$). There was no significant difference in RCF between groups at the onset of salinity acclimation (day 1) ($F_{1,88} = 1.628$, $p = 0.205$, data not shown) or after a full week acclimating (day 22) to the treatment salinity ($F_{1, 87} = 0.972$, $p = 0.327$, data not shown).

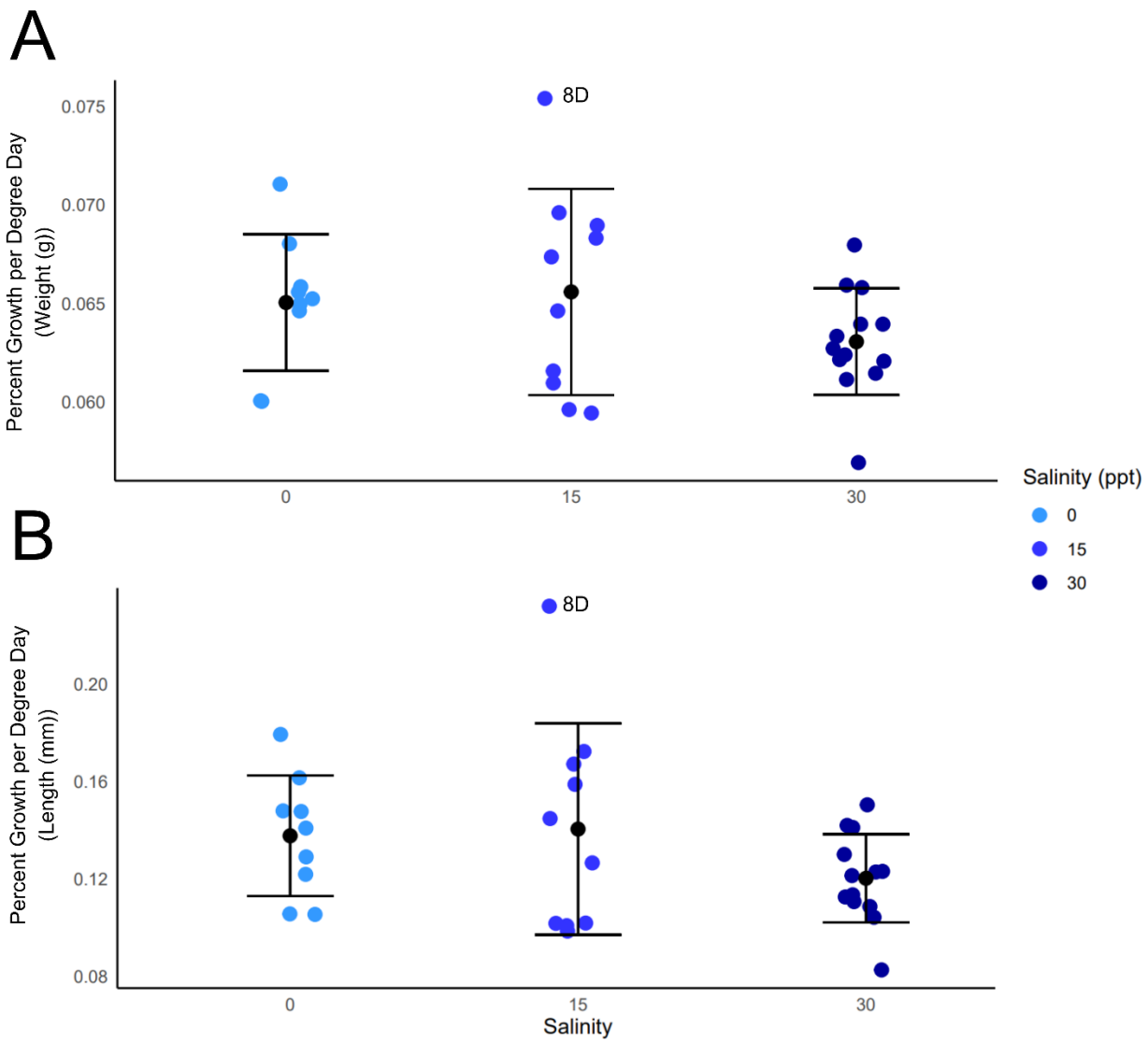


Figure 4. Percent growth per degree day (%Growth $DD^{-1}$) of $F_1$ captive-bred, Atlantic Whitefish at three different salinities (0 ppt, 15 ppt, 30 ppt), as measured by: A) weight (g) and B) fork length (FL, mm) from experimental days 1-174. Salinity did not have a significant effect on %Growth $DD^{-1}$ as measured by length or weight (test on logarithmically transformed data, with untransformed data shown for comparison to other studies). The black point and error bars represent the mean and standard deviation of each treatment with each dot representing individual fish. Fish 8D was identified as an outlier (z-score > |3|) but remains in the analysis.

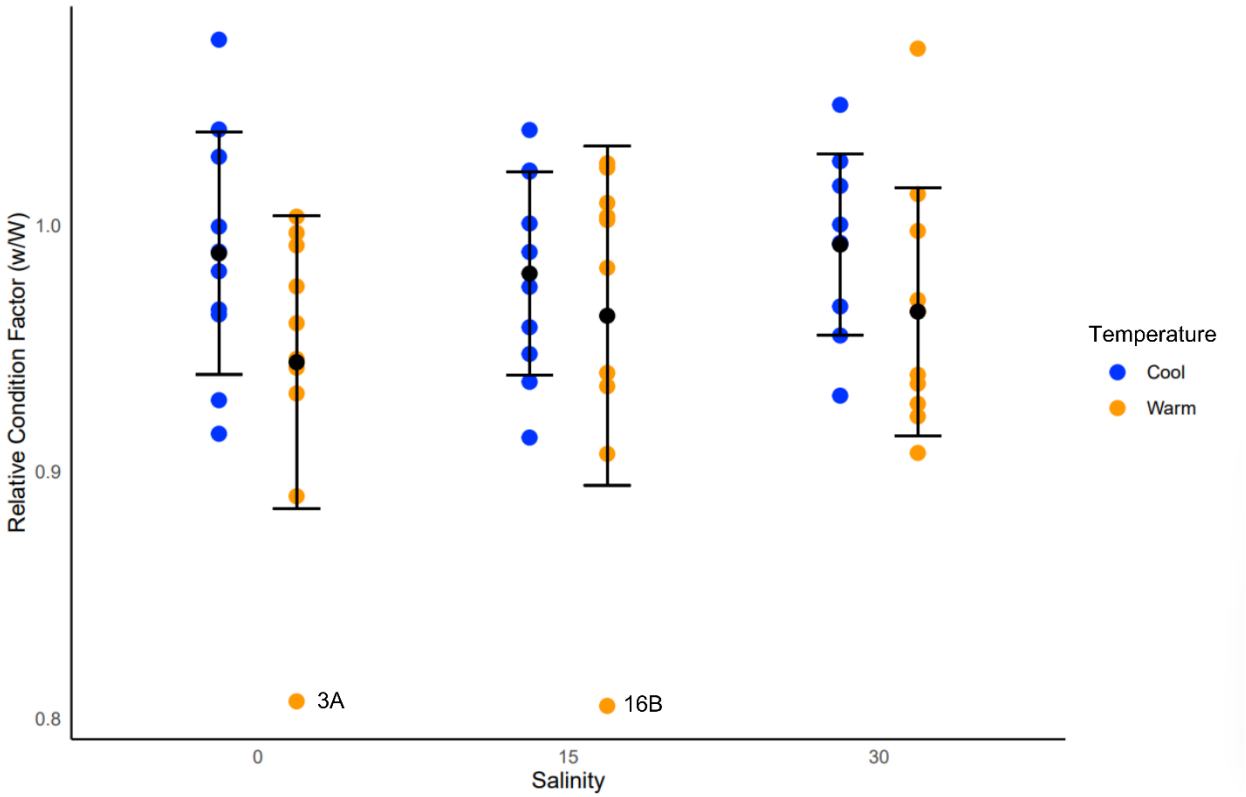


Figure 5. Relative condition factor (RCF, $w\ W^{-1}$) of $F_1$ captive-bred, Atlantic Whitefish in response to salinity (0 ppt, 15 ppt, 30 ppt) at two different temperatures. RCF is defined as the ratio of the observed weight (g) to expected weight based on length (mm). Water temperature of "cool" samples averaged 11.66°C ± SD 0.44 (day 1-199) and in "warm" samples averaged 16.55°C ± SD 0.83 (day 199 – day 252). Salinity did not have a significant effect on RCF but fish in "warm" conditions had a significantly lower relative condition factor than those measured before in "cool" conditions. The black point and error bars represent the mean and standard deviation of each treatment with each dot representing individual fish from three replicate tanks. Fish 16B and 3A were identified as outliers, (z-score > |3|) but remain in the analysis.

### 2.3.2 *The effect of salinity and temperature on indicators of stress*

There was no significant effect of salinity ($F_{2,54} = 0.882$, $p = 0.4197$), or significant interactions between salinity and temperature on blood lactate ($F_{2,54} = 0.256$, $p = 0.7753$), but the effect of temperature alone was significant (Figure 3A: $F_{1,54} = 6.610$, $p = 0.0129$). The older fish (~497 DPH) sampled in "warm" conditions on day 252 had a slightly higher blood lactate concentration than younger fish sampled in "warm" conditions on day 174 (~458 DPH) at all

salinities (Figure 3A). There was no effect of salinity ($F_{2,56} = 0.583$, $p = 0.562$), temperature, (Fig 3A: $F_{1,54} = 2.734$, $p = 0.104$) or their interaction ($F_{2,56} = 0.581$, $p = 0.563$) on blood glucose.

There was not a sufficient sample size to test for the effect of temperature on plasma cortisol, so only data from "warm" samples were analyzed. There was no significant effect of salinity on cortisol concentration in fish from the "warm" group (Figure 3C: tested on *logarithmically transformed data;* $F_{2,27} = 0.372$, $p = 0.693$). Average cortisol concentration for "cool" samples ($n = 4$) was 2.28 ng $mL^{-1}$ ± SD 1.74, which is within the range of "warm" plasma cortisol concentrations.

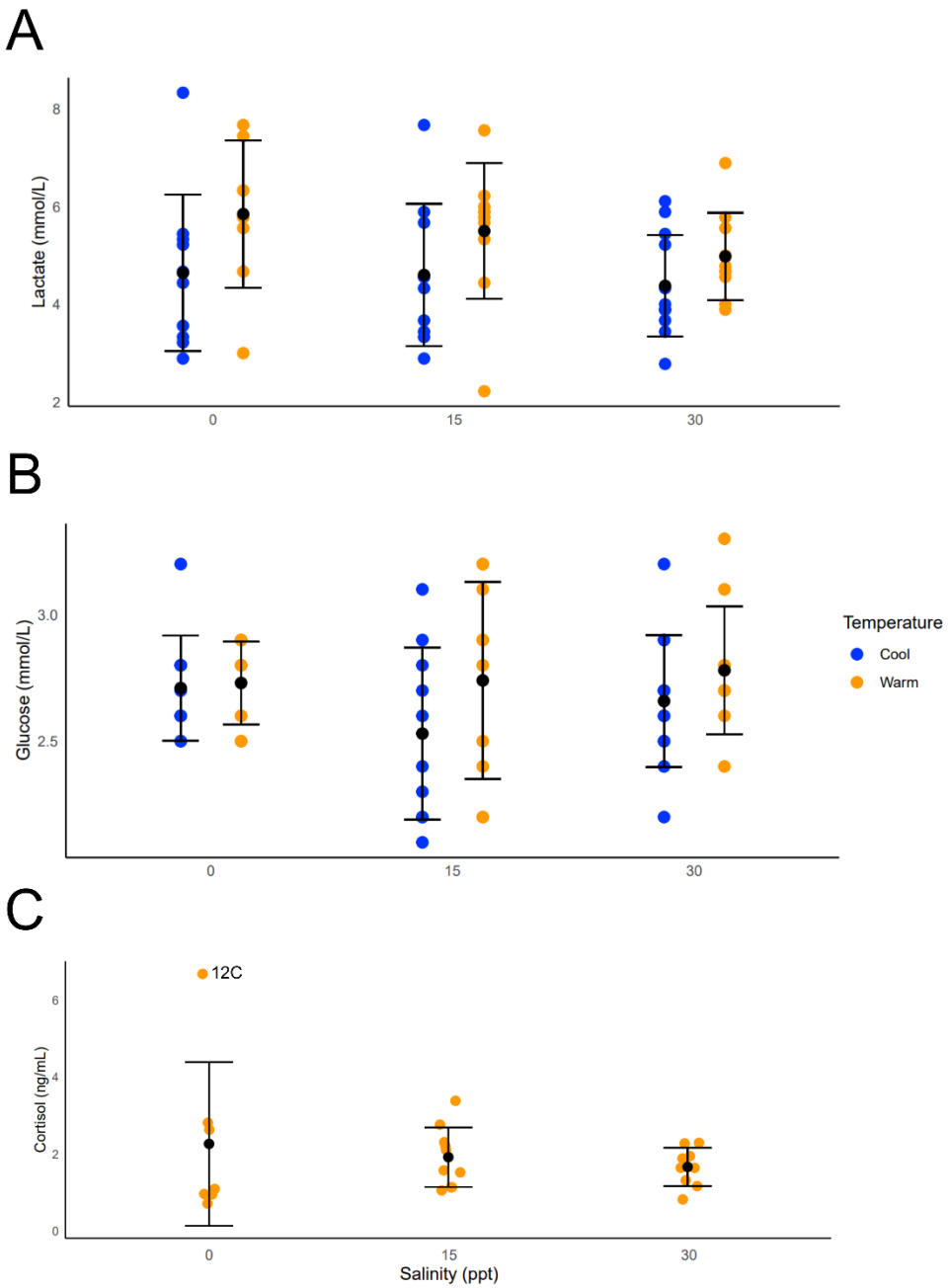


Figure 6. (A) whole-blood lactate (mmol $L^{-1}$), (B) whole-blood glucose (mmol $L^{-1}$) and (C) plasma cortisol (ng $mL^{-1}$) (C) of $F_1$ captive-bred, Atlantic Whitefish in response to salinity acclimation (0 ppt, 15 ppt, 30 ppt) at two different temperatures. "Cool" samples were obtained on day 174 (avg 11.66°C ± SD 0.44) and "warm" samples were obtained on day 252 (16.55°C ± SD 0.83). Salinity did not have a significant effect on lactate ($F_{1,54} = 0.882$, $p = 0.420$), glucose ($F_{1,56} = 0.583$, $p = 0.562$) or plasma cortisol ($F_{1,27} = 0.372$, $p = 0.693$), but fish measured in "warm" ambient water temperature had a significantly higher lactate concentrations ($F_{1,54} = 6.610$, $p = 0.0129$) than "cool" samples. Water temperature had no effect on plasma glucose levels ($F_{1,56} = 2.734$, $p = 0.104$) and effects of temperature cortisol could not be tested as only the "warm" group had a sufficient sample size. The black points and error bars represent the mean and standard deviation of each treatment. Fish 12C was identified as an outlier in cortisol data but remains in the analysis.

2.3.3 *The effect of salinity and temperature on osmoregulatory homeostasis*

Plasma osmolarity and ion concentrations could only be calculated for "warm" samples due to sample volume limitations (plasma ≤ 30 μL). There was a significant effect of salinity ($F_{2,19} = 5.605$, $p = 0.012$) on plasma osmolarity from these "warm" fish. While the tested sample size is small (n = 22) due to limitations from plasma volume, retaining all samples with plasma volume ≤ 30 μL (n = 44) produced the same result ($F_{2,38} = 3.314$, $p = 0.0472$). Fish held at 30 ppt had a higher mean plasma osmolarity than those kept in fresh (0 ppt) and brackish water (15 ppt), but the difference was only significant between brackish and saltwater treatments ($p = 0.010$).

As with plasma osmolarity, there were insufficient data to test for an effect of temperature on $Na^+$ and $Cl^-$ concentrations. Only two samples produced detectable concentrations of $K^+$ (fish 6A: 2.5 mmol $L^{-1}$, fish 11A: 1.9 mmol $L^{-1}$), so the effect of salinity on $K^+$ concentration could not be assessed. There was no effect of salinity on $Na^+$ (Figure 4B: reciprocally transformed; $F_{2,18} = 3.335$, $p = 0.0586$) or $Cl^-$ ion (Figure 4C: $F_{2,18} = 1.462$, $p = 0.258$) concentrations in the plasma of "warm" fish alone.

Note: plasma $Na^+$ and $Cl^-$ should add up to slightly less than total plasma osmolarity, which is not the case for one third (n = 6, total n = 18) of the samples for which we have both we have both ion and osmolarity data after removal of low volume samples (plasma volume ≥ 30 μL for $Na^+$ and $Cl^-$ data; ≥ 10 μL for osmolarity data). Values reported in this study are also frequently above those reported in the literature for euryhaline teleosts (Edwards & Marshall, 2013). This indicates that despite the removal of low volume samples, ion concentrations are likely being overestimated, plasma osmolarity is being underestimated, or both.

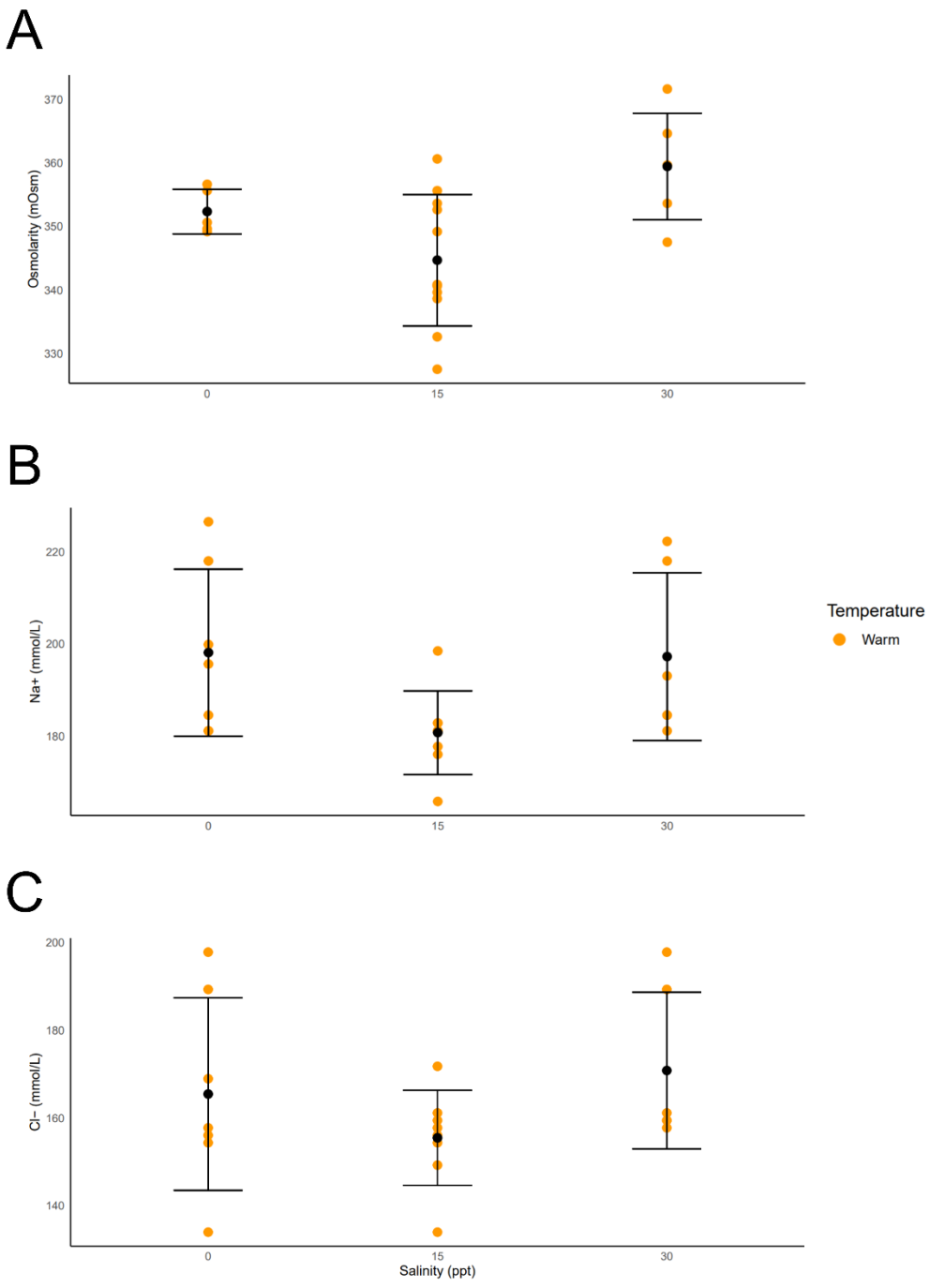


Figure 7: (A) plasma osmolarity (mOsm), (B) $Na^+$ (mmol $L^{-1}$) and (C) $Cl^-$ (mmol $L^{-1}$) plasma concentrations of $F_1$ captive-bred, Atlantic Whitefish in response to salinity acclimation (0 ppt, 15 ppt, 30 ppt) at the "warm" temperature. Only data from the "warm" group (16.55°C ± SD 0.83), collected on day 252, had sufficient samples to test for differences in all osmoregulatory variables. Salt water (30 ppt) fish had a significantly higher plasma osmolarity than brackish fish ($F_{2,19} = 5.605$, $p = 0.012$), but not freshwater fish. Salinity did not have any effect on plasma $Na^+$ (test on reciprocally transformed data, untransformed data shown, $F_{2,18} = 3.335$, $p = 0.0586$) or $Cl^-$ ($F2,18 = 1.462$, $p = 0.258$). The black point and error bars represent the mean and standard deviation of each treatment.

## 2.4 Discussion

The Atlantic Whitefish an endangered fish endemic to Nova Scotia, which is thought to be an ancestrally anadromous species. However, the last wild Atlantic Whitefish individuals have been essentially landlocked in three lakes in the Petite Rivière watershed, Nova Scotia, by dams for 120+ years. In this thesis, I explored the capacity of Atlantic Whitefish from the Petite Rivière to tolerate the increases in salinity associated with an anadromous life cycle. Specifically, I measured growth and condition factor of $F_1$ captive-bred juveniles during long-term exposure to the range of salinities they will experience during anadromous migrations (0, 15, 30 ppt). I also examined whether an increase in ambient water temperature (11.66°C ± SD 0.44 to 16.55°C ± SD 0.83) affected Atlantic Whitefish stress responsiveness and osmoregulation. I found Atlantic Whitefish exhibited little difficulty coping with changes in salinity, but observed a slight increase in blood lactate and decrease in condition factor at the higher temperature, which may be indicative of thermal sensitivity.

### 2.4.1 *The effect of salinity on Atlantic Whitefish growth, osmotic and ionoregulatory homeostasis*

The minimal effect of salinity on Atlantic Whitefish was not surprising as evidence from previous work (Cook & Bentzen, 2009; Cook et al., 2010; Whitelaw et al., 2015; personal communication, Jeremy Broome) have also found a high tolerance to salt water in Atlantic Whitefish as larvae, juveniles and adults.

Prior to maturity, resources are partitioned into somatic growth and maintenance costs. Coping with stress alters this balance as the animal activates energy-consuming pathways to restore and preserve homeostasis (Barton & Iwama, 1991; Barton, 2002). As such, stress has

been shown to reduce growth in several studies (Iwama et al., 1995; Pickering, 1990; McCormick et al., 1998; Barton, 2002). My results suggest that when fed to satiation, the cost of osmoregulation at higher salinities (15 ppt, 30 ppt) does not differ from that in fresh water (0 ppt) enough elicit a difference in growth or condition. Even among euryhaline species, Atlantic Whitefish appear to be among the very few species for which salinities between 0 and 30 ppt do not influence growth. For example, Shi Drum (*Umbrina cirrosa*) can maintain a constant plasma osmolality and electrolyte levels across salinities ranging from 4 – 40 ppt, but specific growth rates were significantly lower at 4 ppt than 10 ppt and 40 ppt (Mylonas et al., 2009). Similarly, Liu et al. (2017) observed that condition of euryhaline juvenile American Shad (*Alosa sapidissima*) was higher at 7, 14 and 21 ppt than at salinity extremes (28 ppt and the freshwater control).

However, the effect of salinity on the growth of euryhaline fish may change during ontogenetic development (Peterson et al., 1996; Da Silva Rocha et al., 2004; Liu et al., 2017), and may differ in a food-limited environment. While my results indicate that there is no difference in growth or condition for ~419-497 days post hatch (DPH) Atlantic Whitefish, other studies (Mylonas et al., 2009; Liu et al., 2017) demonstrate that salinity may influence growth of fish at different life stages despite the high survival reported by Cook et al. (2010). Without directly examining maintenance costs (standard metabolic rate/resting metabolic rate) it is also not possible to determine whether salinity has no effect on osmoregulatory costs from 0 – 30 ppt, as in Fang et al. (2019), or if the effect is present but not strong enough to manifest as differences in growth or condition in well-fed fish. It is important to note that I did not control for, or record, consumption rates, so it is possible that there were within-tank hierarchy effects on feeding and differences in growth efficiency (i.e. one group requires more food to grow the same amount) in

Atlantic Whitefish across salinities (0 – 30 ppt), as in Morinville & Rasmussen (2003), that were not captured by my analysis. Future work should examine the influence of diet on salinity tolerance and growth, especially during early development.

Similarly, I noted a lack of response in general proxies for stress (glucose, lactate, and cortisol) to salinity (0-30 ppt). These parameters are known to increase in response to osmotic stress and have been reported in most studies of euryhaline fish during salinity acclimation (e.g. Tsui et al., 2012; Monzanzadeh et al., 2021; McCormick et al., 2019). While some studies have reported an absence of a response in glucose, cortisol, or lactate to salinity (Laiz-Carrión et al., 2005; Mylonas et al., 2009) these tend to be measured over a period of weeks to months and stress metabolites have normally been reported to decrease in the hours, days or weeks following the onset of a stressor with the return to homeostasis (Pickering & Stewart, 1984; Laiz- Carrión et al., 2002; Mylonas et al., 2009; Tsui et al., 2012). Therefore, it is likely my results represent a return to baseline concentrations following an initial response to salinity acclimation, and suggest fish at all salinities are able to do this after an initial challenge. Indeed, the concentration of plasma cortisol in my samples generally fall well within the normal range reported for unstressed Brown Trout (*Salmo trutta*) and European Whitefish (*Coregonus lavaretus*) in fresh water (0-5 ng $mL^{-1}$, Pickering & Pottinger, 1989; Lappivaara, 2001). In addition, cortisol regulates hydromineral balance under both hyperosmotic and hypoosmotic stress (Wendelaar Bonga, 1997; Evans et al., 2005; McCormick, 2001; reviewed in Takei & Hwang, 2018), so there is likely an interplay between seawater acclimation and the stress response. Future work measuring the time course of the acute salinity acclimation response (e.g. Marshall et al., 1999) of Atlantic Whitefish after transfer to both fresh and saltwater are needed to fully assess how these fish will cope with salinity changes during anadromous migration. Despite these

unknowns, it is clear Atlantic Whitefish used in this study showed no signs of stress at salinities from 0 – 30 ppt after long-term acclimation.

A higher plasma osmolarity in fish at 30 ppt than at 15 ppt was the only significant response to salinity I found. adult euryhaline teleosts maintain plasma osmolality within a range from about 300 to 350 mOSm in tolerable salinities (Greenwell et al., 2003; Laiz-Carrión et al., 2005) and after successful acclimation to seawater from freshwater, plasma osmolarities are normally slightly higher (e.g. Brown et al., 2018). Given that the mean plasma osmolarity in my 30 ppt fish is 359.44 (± SD 8.38) mOsm, the significance of this relationship on animal physiology is likely marginal and represents a normal acclimation response.

Fish from all treatment groups (0, 15, 30 ppt) had similar plasma ion concentration ($Na^+$, $Cl^-$). Most studies report an acute response increase of inorganic of $Na^+$ and $Cl^-$ with increasing salinity (Stuarnes et al., 1992; Laiz-Carrión et al., 2005; Fang et al., 2019). However, given the long-term nature of my study (149 and 227 days) acclimation to salinity change, the relationship presented here likely represents reestablished ion concentrations (Tsui et al., 2012; Brijs et al., 2017; Ordóñez-Grande et al., 2020), suggesting that Atlantic Whitefish can successfully acclimate to salinities from 0-30 ppt. However, $Na^+$ and $Cl^-$ concentration values presented here are likely overestimates resulting from sample dilution (Figure D.1), as the sum of $Na^+$ and $Cl^-$ concentrations exceeding values for total plasma osmolarity in approximately a third of the fish in this experiment. The majority of individual data points also exceed those reported for most teleosts (Clarke & Blackburn 1978; Edwards & Marshall, 2013; Takei & Hwang, 2018). Considering this and the small sample size due to plasma shortage, more data and a refinement of laboratory protocols would be needed to make conclusions on plasma ion concentration and osmolarity data. However, though not directly comparable to other values in the literature due to

these limitations, plasma osmolarity and ion concentrations are still comparable across treatments in my study. With this caveat, and in the context of other results in this section, my ion concentration and osmolarity data still suggest that the $F_1$ captive-bred juvenile Atlantic Whitefish used in this study were able to appropriately adjust osmoregulatory mechanisms to cope with hyperosmotic salinities.

### 2.4.2 *The effect of temperature on Atlantic Whitefish growth, osmotic and ionoregulatory homeostasis*

I was only able to test for the effect of temperature on condition, blood lactate and blood glucose for juvenile Atlantic Whitefish. The remaining variables, (excluding growth) were tested across salinities using data from "warm" fish only. Fish that were sampled following a temperature increase exhibited slightly higher blood lactate concentrations and a decrease in condition factor but did not differ from "cool" fish in their ability to osmoregulate across salinities (0-30 ppt). This suggests that while temperatures around 17°C itself may induce a minor stress response in Atlantic Whitefish, it does not affect their osmoregulatory capacity at the salinities tested in this study.

Atlantic Whitefish from the "warm" group (11.44°C ± SD 0.44) had a significantly lower condition factor than fish in the "cool" group (16.55°C ± SD 0.83). As condition factor is a measure of fish well-being and energy stores (Froese, 2006), this reduction reflected a depletion in energy reserves in "warm" fish. This reduction may be the consequence of an increase in the costs of maintenance processes, or a decrease in food consumption and or food conversion efficiency of Atlantic Whitefish at a higher temperature (Dennis & Bulger, 1995; McCormick et al., 1998; Breau et al., 2011). While a lower condition factor in stressed whitefish relative to a

control group has been shown before (Lappivaara, 2001), whether either of these mechanisms was responsible for the decrease in condition factor observed in this study is not known due to an inability to track individual condition over time and lack of consumption rate data. This result is also confounded by fish age and size, as the "warm" group was sampled at a later date (day 252) than the "cool" group (day 174). I also cannot rule out a sex-based effect on condition, as I could not sex the fish in this study. Thus, it is possible that "cool" sample group may have included a higher number of males by chance, which tend to have a higher condition factor than females before maturity (Whitelaw et al., 2015).

I also found that Atlantic Whitefish from the "warm" group exhibited a higher blood lactate than "cool" fish. Lactic acid can build up in white muscle tissue when anaerobic glycolysis increases in response to the hypoxic conditions (Rees et al., 2009) that often accompany increases in water temperature or due to hypoxemia from exercise. However, oxygen in all treatment tanks was maintained at 95% (95.44% ± SD 7.159349) and the fish sampled were at rest, so this is likely not the case. In addition, the lactate values reported for warm fish are similar with some baseline values reported for other salmonids (Crespel et al., 2017; Hvas et al., 2018). The higher lactate values reported here are also lower than most reported in wild Vendace (*Coregonus albula*) after handling (Pasanen et al., 1979). While the lactate meter used in this experiment has not been cross validated with a traditional assay, this does indicate that the significant relationship between lactate and temperature reported here might not be biologically meaningful.

The slightly lower condition factor and higher blood lactate concentration, taken together, could represent the presence of a thermal stress response in "warm" fish upon handling, as Whitelaw et al., (2015) also reported an increased sensitivity to handling stress in Atlantic

Whitefish above 16°C. Thus, the increased blood lactate in "warm" fish may not be a meaningful indicator of stress in response to temperature alone. The nature of the relationship between blood lactate and condition to temperature should be confirmed with an experiment specifically designed to test the effect of temperature on animal physiology.

I further found no evidence of a relationship between blood glucose, suggests that by day 252 (53 days following the temperature increase), Atlantic Whitefish were not experiencing thermal stress. In teleosts, the presence and magnitude of a blood glucose response to temperature varies with species and ontogeny (Dean & Goodnight, 1964; Chavin & Young, 1970; Pérez-Casanova et al., 2008; Breau et al., 2011) as well as the presence of additional stressors such as confinement (Strange, 1980) and exercise (Dean & Goodnight, 1964). It is likely the glucose concentrations presented here represented a return to pre-stress levels following acclimation to temperature (Pérez-Casanova et al., 2008), or there was no change in blood glucose during temperature increases. These results are more difficult to interpret without cortisol data from "cool" fish, as the responses of blood lactate and blood glucose to temperature reported in other studies are not always in agreement (Dean & Goodnight, 1964; Breau et al., 2011). My results suggest that while the Atlantic Whitefish might be more sensitive to handling stress at warmer temperatures, they likely are not experiencing considerable stress as a result of the temperature increase alone (~11.4°C to 16.6°C). Indeed, the optimum growth temperature calculated for juvenile Atlantic Whitefish using a physiological model is 16.5°C (Cook et al., 2010). Overall, my results indicate that while Atlantic Whitefish are more sensitive to handling stress at warmer temperatures (~16.5°C), but an examination of stress metabolites and condition factor in a time-series accompanying temperature acclimation is necessary to test this hypothesis.

### 2.4.3 *Significance and future directions*

The recovery strategy for the Atlantic Whitefish highlights four goals (DFO, 2006; 2018a): (1) protect and conserve the species in its current habitat, (2) increase the number and range of viable populations, (3) increase our understanding of the species and its habitat, and (4) increase public involvement and acceptance. Performance indicators under Goal 1 include (a) establishment of anadromy in the Petite Rivière population, (b) establishment of a self-sustaining population in another freshwater waterbody, (c) establishment of an anadromous population in a second watershed in the Southern Uplands eco-region of Nova Scotia, and (d) re-assessment of the feasibility of repatriating an anadromous population to the Tusket River (DFO, 2022). The results of this study have narrowed the knowledge gaps regarding the biology and life history of the species (goal 3), related to their salinity tolerance. In this thesis, I have presented evidence that Atlantic Whitefish are able to successfully acclimate and grow in salinities between 0 and 30 ppt after being landlocked for ~120 years.

The DFO is currently conducting releases of Atlantic Whitefish into Milipsigate Lake and downstream of Hebb dam (personal communication, Jeremy Broome; DFO, 2022) to support the Petite Rivière population. Concurrently, the DFO has established an ongoing review of suitable lake habitats throughout the mainland portion of Nova Scotia for the establishment of anadromous and resident populations of Atlantic Whitefish outside of the Petite Rivière (DFO, 2018b; 2022). The results of my study support efforts to restore anadromy, and provide preliminary data suggesting that seasonal and climate-related increases in temperature should continue to be considered when selecting suitable habitats (i.e., lake depth and access to thermal refugia).

Importantly, salinity tolerance is not the only important component of a fish's ability to migrate. The Atlantic Whitefish in the Petite Rivière have had access to the ocean since the construction of a fishway in 2012, but there has been no evidence of use in the last decade. Therefore, an exploration of willingness to use fishways of this kind represents a natural next step.

## 2.5 Conclusion

In this thesis, I have demonstrated that Atlantic Whitefish from the Petite Rivière are still strongly euryhaline and can osmoregulate at salinities between 0 and 30 ppt for long periods of time (> 6 months) with no signs of stress or costs to condition or growth. Their high osmoregulatory capacity at salinities from 0-30 ppt confirms that the remaining Petite Rivière whitefish are likely descended from a previously anadromous population for which migration was impeded by the construction of dams in the Petite Rivière watershed. The preservation of osmoregulatory capacity at these salinities suggests that restoring anadromy remains a feasible life history strategy for the species, but it also highlights that the lack of migration since the opening of the Hebb dam fish way in 2012 is not due to physiological barriers to saline environments and warrants further investigation.

# Chapter 3 – Conclusion

## 3.1 Summary

The Atlantic Whitefish is an endangered (Edge, 1984), historically anadromous fish found only in three lakes in Nova Scotia, where it has been largely isolated from the ocean and any returning migrants for over 120 years (Edge & Gilhen, 2001; Bradford et al., 2004). As such, restoring anadromy to the species is a major component of the recovery strategy (DFO, 2006; 2018a). In Chapter 2, I compared the growth, condition, proxies for stress and osmotic balance of $F_1$ captive-bred, juvenile, Atlantic Whitefish originating from the Petite Rivière population in response to salinities that they will encounter during an anadromous migration (0 ppt, 15 ppt, 30 ppt). I found no evidence of osmotic stress or increased energetic costs of coping with osmotic stress at any of the salinities tested, except for a marginal effect of salinity on plasma osmolarity. I attempted to measure metabolic rates (resting metabolic rate, maximum metabolic rate) and swimming performance ($U_{crit}$), but Atlantic Whitefish were highly sensitive to containment stress and fin damage, so respirometry experiments were discontinued. I also noted a statistically significant effect of an increase in ambient water temperature on condition and stress (blood glucose, blood lactate), but this significance may not be biologically meaningful, rendering the effect of temperature increase on condition and stress inconclusive pending further investigation.

## 3.2 Applications for Conservation

A major driver of this decline in diadromous fish populations is the presence of dams and other water management systems with insufficient fish passage that fragment habitat and impede movement to critical habitats for feeding and reproduction (Waldman et al., 2016; Mattocks et al., 2017). The construction of dams, among other factors, have been implicated in the decline of

the Petite Rivière population and the extirpation of the Tusket River population of Atlantic Whitefish (Edge & Gilhen, 2001; Bradford et al., 2004; DFO, 2006; 2018a). Despite the presence of fish passage infrastructure at dams downstream of the remaining Atlantic Whitefish habitat there has only been one isolated observation of use by Atlantic Whitefish (DFO, 2022).

Releases of $F_1$ captive-bred Atlantic Whitefish into the Petite Rivière watershed (Milipsigate Lake and downstream of Hebb dam) are ongoing and soon to be accompanied by acoustic telemetry (personal communication, Jeremy Broome, DFO) to monitor habitat use and movement post-release. Additionally, a survey of lakes in the Southern Uplands region of Nova Scotia is being conducted for potential introduction sites to establish resident and anadromous populations of Atlantic Whitefish outside of the Petite Rivière (DFO, 2018b; 2022).

Here, I demonstrated that $F_1$ captive-bred juvenile Atlantic Whitefish are strongly euryhaline at salinities between 0 and 30 ppt and exhibits no signs of difficulty coping with osmotic stress, suggesting that Atlantic Whitefish from the Petite Rivière is still capable of re-adopting an anadromous life cycle after a century of relaxed selection pressure for salinity tolerance. These results are relevant to the current conservation efforts, begin to fill the knowledge gaps regarding the biology of the species, and point to specific future research priorities.

In the past, there was much uncertainty surrounding the anadromous nature of Atlantic Whitefish from the Petite Rivière, as to date there has been limited evidence of an anadromous migration in this population (Bradford et al., 2004; DFO, 2022). Previous work has shown that despite being isolated from the ocean for over a century, larval, juvenile and adult Atlantic Whitefish still retain a high tolerance for salt water (Cook et al., 2010; Whitelaw et al., 2015). However, until now, whether salinity exposure elicited long-term primary or secondary stress

responses in Petite Rivière whitefish was not known. Given that Atlantic Whitefish in this study showed signs of being more effective osmoregulators at salinities within 0-30 ppt than many other euryhaline and anadromous species (Mylonas et al., 2009; Liu et al., 2017; Fang et al., 2019; Mozanzadeh et al., 2021), this suggests the remaining Petite Rivière whitefish were almost certainly anadromous prior to landlocking.

However, this high tolerance means that the current barriers to fish migration (since the installation of the Hebb Dam fish passage) are not due to a deleterious response to saltwater. Given the strong response of Atlantic Whitefish to caudal fin damage and exercise stress exhibited during respirometry trials, it is possible wild Atlantic Whitefish are unwilling to use physically intensive fish passage. Similarly, fish ladders and small steep by-pass streams do not appear to be effective for North Sea Houting (*Coregonus oxyrinchus*), with no evidence of spawning upstream of even small obstacles (Jensen et al., 2003). Given this uncertainty, the use of fish passage structures by Atlantic Whitefish should continue be explored to determine if existing structures on the Petite Rivière can be successfully used by this species. Similarly, for the purposes of establishing anadromous populations outside of the Petite Rivière, candidate waterbodies with few hydrological features (i.e. rapids) likely to cause extensive fin damage during passage.

As salinity tolerance varies with ontogeny (Cook et al., 2010), future experiments should focus on testing tolerance at different ages and seasons. In addition, studying the time-course of acclimation and measuring acute responses to ecologically-relevant salinity changes, both from fresh to saltwater and from saltwater to freshwater, should help inform us of any potential 'costs' to salinity acclimation. My results also indicate that Atlantic Whitefish might be starting to display signs of handling stress at warmer temperatures (approximately 16.5℃). This is

consistent with observations from the past captive breeding program at the Mersey Biodiversity Facility, which found that Atlantic Whitefish were highly sensitive to handling stress above 16℃ (Whitelaw et al., 2015). However, Cook (2012) found the thermal growth optimum in juvenile Atlantic Whitefish (TL = 60 – 100 mm) is 16.5℃ in freshwater (0 ppt). While my findings are observational, they highlight the importance of considering seasonal and climate related increases in temperature both in the Petite Rivière and potential reintroduction sites, particularly for resident populations lacking access to the marine environment for thermal refuge. In particular, experimentally testing the effects of concurrent salinity and temperature changes on Atlantic Whitefish physiology at ecologically-appropriate life history stages should help to better describe the physiological limits of this species.

Anadromy is common for many coregonids in the northern parts of their ranges (e.g. Bernatchez & Dodson, 1985; Howland et al., 2009; VanGerwen-Toyne et al., 2012; Fagard, 2015; Jensen et al., 2015), as well as in the *Salmonidae* family as a whole (e.g. McCormick & Saunders, 1987; Stuarnes et al., 1992; McCormick et al., 2019; Nevoux et al., 2019). It is also quite common for populations of anadromous salmonid species to become landlocked (e.g. Burton & Idler, 1984; Stuarnes et al., 1992; Cowley et al., 1994; Godbout et al., 2011), and there have been some documented cases of successful reestablishment of anadromy (Godbout et al., 2011; Quinn et al., 2017). Notably, Godbout et al. (2011) report a reestablishment of anadromy by adult descendants of non-anadromous Sockeye Salmon, which were observed returning upstream for the first time after 20-25 generations of landlocking. Godbout et al. (2011) found that the number of returning fish from this volitional migration exceeded the number of returns of Kokanee of the same population that were forced to migrate during previous experiments (Foerster, 1947; Godbout et al., 2011). Previously landlocked Bull Trout (*Salvelinus confluentus*)

in the Elwha River, Washington, began to use marine food resources following dam removal, indicating the resumption of anadromy and a consequential expansion in foraging opportunities after 100 years of isolation (Quinn et al., 2017). These studies are on a similar time scale as the estimated landlocking of Atlantic Whitefish, lending support to the feasibility of restored anadromy for the Petite Rivière whitefish.

The areas of research that should be prioritized to effectively conserve the Atlantic Whitefish are well outlined (DFO, 2022). However, the results of this study point to specific and important directions for future work related to the restoration of anadromy. Specifically, my data suggests that with abundant food resources and sufficient acclimation time, Atlantic Whitefish can perform well from 0 to 30 ppt. However, measuring the acute metabolic costs of initial salinity exposure at different life stages may highlight ontogenetic differences in salinity tolerance that could provide information on the age at which Atlantic Whitefish may migrate. Research should also focus on metabolic rates and aerobic scope at relevant salinities (15 ppt, 30 ppt), to examine if there are any fine-scale costs of osmoregulation that are not captured by differences in growth or condition indices in well-fed, laboratory housed fish (e.g. Hvas et al., 2018).

### 3.3 Final Thoughts

I have provided additional evidence supporting the hypothesis that restoring anadromy to the Atlantic Whitefish is a feasible recovery goal. Working with species-at-risk comes with additional considerations that must occasionally be prioritized over experimental outcomes. Regardless of the hiccups in the research process, the findings here fill some key knowledge gaps related to ongoing recovery efforts. Future work should focus on learning more about how

salinity tolerance may fluctuate with life stage and other possible barriers to migration, such as willingness to navigate fish passage infrastructure and areas of turbulent current, as well as exploring interactions between salinity tolerance, thermal tolerance and exercise capacity of the Atlantic Whitefish.

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# Appendix

## Appendix A: Housing Data

Table A1. Summary of water parameters (salinity (ppt), temperature (°C), pH, dissolved oxygen (DO, %)) through the experimental period (days 1-252) by salinity (0 ppt, 15 ppt, 30 ppt) and temperature group ("cool", "warm"). All parameters were collected daily except for salinity, which was excluded from daily measurements following day 120 due to its stability and time constraints.

| **Parameter** | **(0 ppt)** | **(15 ppt)** | **(30 ppt)** |
|---|---|---|---|
| Salinity (ppt) | 0.54 ± SD 0.31 | 15.03 ± SD 0.06 | 29.96 ± SD 0.10 |
| Temperature (°C) | | | |
| Days 1- 252 | 12.88 ± SD 2.16 | 12.71 ± SD 2.10 | 12.55 ± SD 1.95 |
| Days 1-199: "cool" | 11.79 ± SD 0.46 | 11.64 ± SD 0.37 | 11.56 ± SD 0.45 |
| Days 200-252: "warm" | 16.85 ± SD 0.93 | 16.61 ± SD 0.82 | 16.18 ± SD 0.56 |
| | **Overall** | | |
| pH | 8.08 ± SD 0.26 | | |
| DO (%) | 95.44 ± SD 7.16 | | |

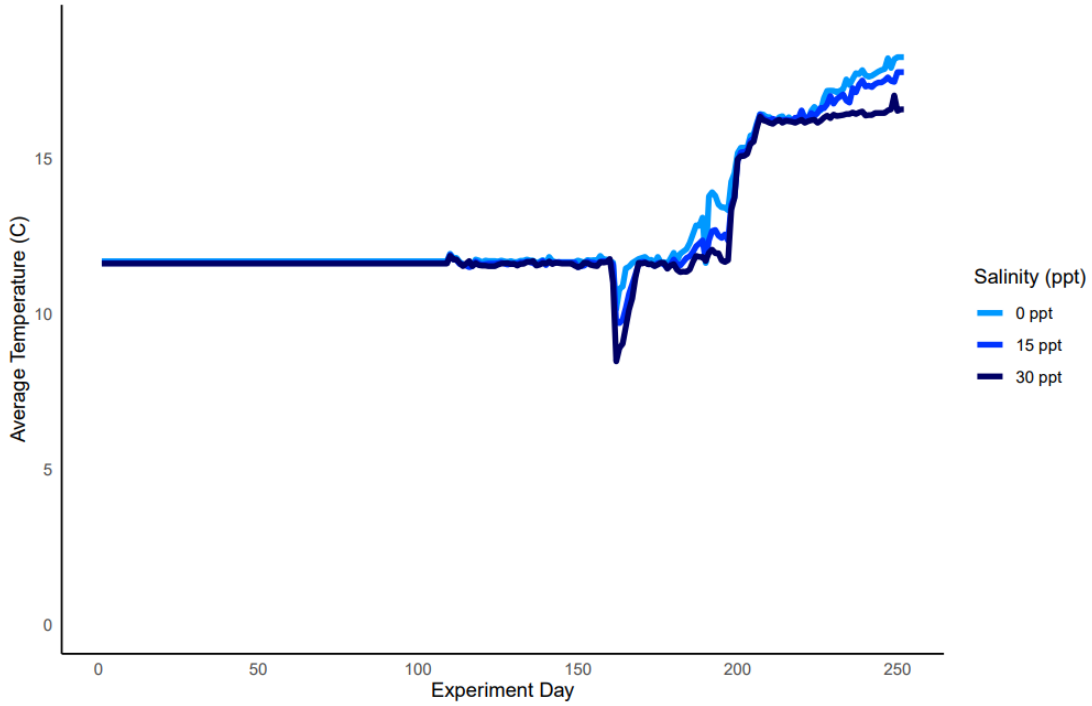


Figure A 1. Average water temperature (°C) by treatment salinities from days 1-252. Missing temperature data from before day 110 was averaged using daily temperature from the following 30 days (110 to 140) for each tank. Steam was turned off on days 161 and 162, resulting in a steep dip in average temperature for all tanks. Water temperature of all tanks was uniformly set to 16°C on day 199, but there was still some variation (~1-2°C).

**Appendix B: Size Data**

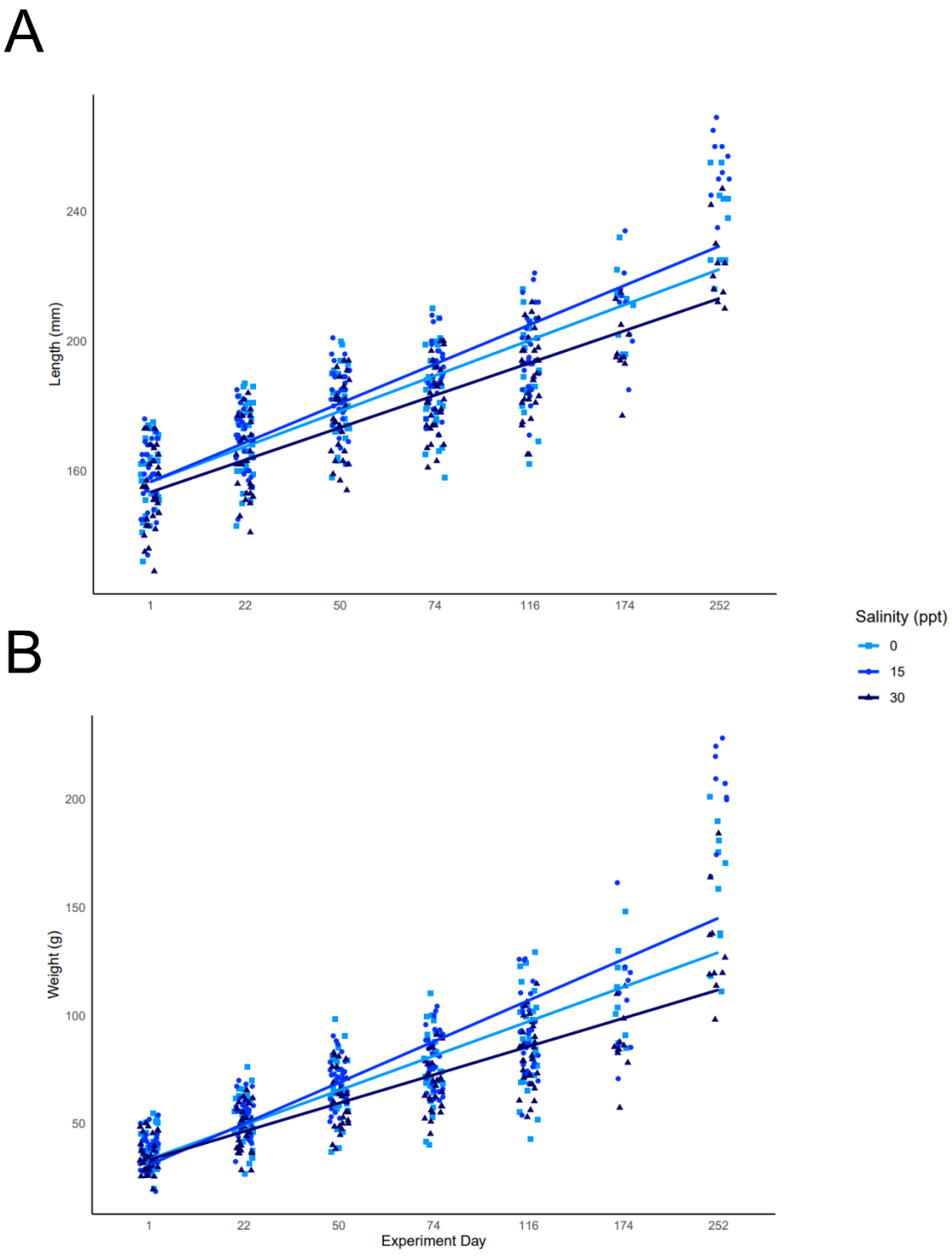


Figure B 1. Raw (A) fork length (FL, mm) and (B) weight (g) of $F_1$ captive-bred, juvenile Atlantic Whitefish from November 25, 2022, to August 4, 2023 (days 1-252) at three different salinities (0 ppt, 15 ppt, 30 ppt). Fish were measured experimental days (1, 22, 50, 74, 116, 174, 252). Data not corrected for initial size or temperature variation across treatment groups.

## Appendix C: Blood Smears and Respirometry

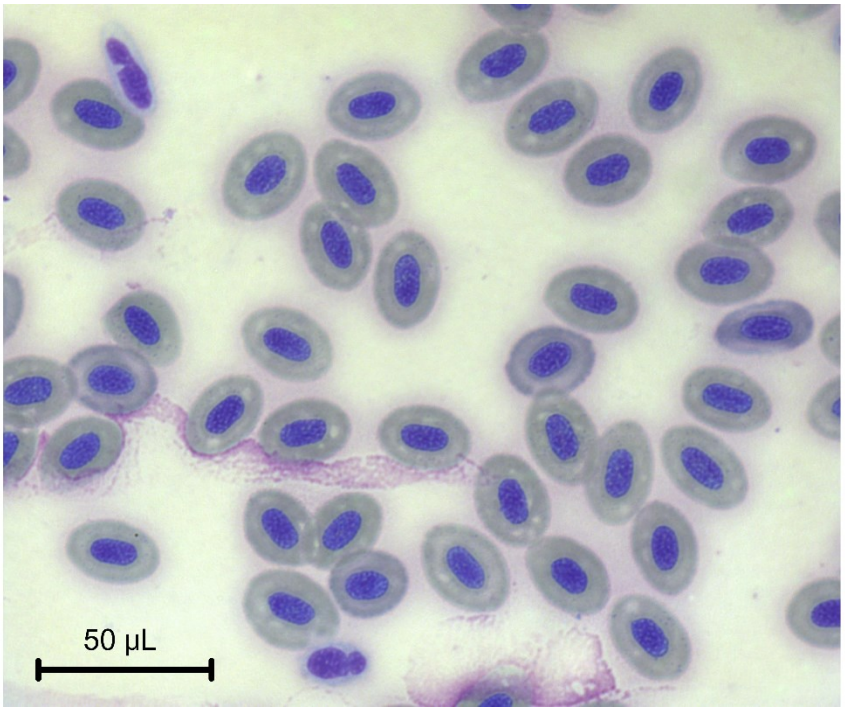


Figure C 1. A blood smear from fish 3A, an $F_1$, captive-bred, juvenile (~497 DPH) Atlantic Whitefish acclimated to 0 ppt and 16.55°C (± SD 0.83). Captured with oil immersion on a 100X objective. Stained with VWR® Quick 1™ single step Wright's stain according to manufacturer's instructions.

### Appendix C.1 *Respirometry*

Resting metabolic rate (RMR), maximum metabolic rate (MMR) and critical swimming speed ($U_{crit}$) were measured for three fish (17B, 13D and 13E) between June 26 and 28, 2023 (experiment days 213, 215). Respirometry experiments were conducted on individual fish using a Loligo® Systems 10 L swim tunnel respirometer (Loligo® Systems, n.d.) filled with water of the appropriate salinity and outfitted with a ClearPath® Servo motor to generate and control flow. I used a $U_{crit}$ ramp protocol adapted from Jain et al., (1997) outlined below.

C.1.1: Training protocol (~1 hour)

1. A fish was fasted 18 hours prior to being captured, weighed and measured in water on the day of the training protocol.
2. The fish was placed in the respirometer and allowed to acclimate for a minimum of 30 minutes in the swim tunnel in a light flow (0.5 body lengths per second (BL $s^{-1}$)).
    a. Water parameters (salinity, temperature, pH, DO) closely matched that of the holding tank, and was continuously flushed with oxygen-saturated water.
    b. During all respirometry periods, the front half of the swim tunnel was wrapped in black plastic, and the entire respirometer was surrounded by a black plastic screen to reduce visual disturbance.
3. If the fish appeared comfortable, it was run through a brief training protocol where water speed was increased by 0.25 BL $s^{-1}$ every 2 minutes until failure ($U_{crit\ initial}$)
4. The fish was promptly removed from the respirometer and moved to an empty recovery tank matching the water conditions of the respirometer and original housing tank and allowed to recover overnight.

C.1.2: RMR (~3 hours)

1. The following morning, the fish was returned to the swim tunnel section of the respirometer and allowed to acclimate for a minimum of 2 hours at a speed of 0.5 BL $s^{-1}$.
    a. Oxygenated water was continuously flushed into the respirometer during this period.
2. After acclimation, the respirometry chamber was sealed and oxygen consumption was measured over the course of 60 minutes. DO saturation did not fall below 80%.

C.1.3: Ramp MMR and $U_{crit}$ (~2 hours)

1. Immediately following RMR measurements, the tunnel was flushed with aerated water
2. During the ramp portion of the test, water speed will increase 0.25 BL $s^{-1}$ every 2 minutes until 50% of the failure speed from the previous day is reached.
3. After this period, water speed was increased every 20 minutes (+ 0.25 BL $s^{-1}$), during which the chamber was sealed and oxygen was measured for 15 minutes, and the respirometer was unsealed and flushed with oxygenated water for 5 minutes.
    a. DO saturation did not fall below 80%
4. This process continues until $U_{crit}$, defined as the speed when the fish cannot maintain position in the respirometer and falls against the back of the swim tunnel.
5. The fish was promptly removed from the chamber and immediately sedated for post-exercise blood sampling. It was then returned to the recovery chamber and monitored closely over the following days.

C.1.4: Respirometry outcomes

Of the three fish that underwent full respirometry protocols (training, RMR, MMR and $U_{crit}$), all three recovered well from sedation and were behaving normally for the first 8 hours following exercise. All three obtained some damage to the caudal fin from contact with the back of the swim tunnel, despite efforts to reduce this damage by covering the metal grate with finer window screen (only present during experiment with fish 17B).

Fish 13D and 13E did not in the one to two days following respirometry trials, and during this period, developed some difficulties swimming and regression of the caudal fin. Two to three days following trials, their caudal fin regressed entirely, and caudal peduncles had become infected and inflamed. At this point, all fish were approved to be treated with an antibiotic

(cefazolin, 30 mg $kg^{-1}$) via intramuscular injection into the dorsal muscle. However, in the following hours/day, condition declined to the point where the fish could no longer retain equilibrium, were euthanized with a lethal dose of TMS (400 mg $L^{-1}$) buffered in a 1:2 ratio with sodium hydroxide. Both 13D and 13E were euthanized within three days following respirometry trials.

The outcome was similar for fish 17B but was extended over a longer period, perhaps due to less severe damage from the window screen mesh. Buoyancy issues and tail regression did not become obvious until three days following the trial. The fish was treated with antibiotics on the eighth day, when caudal fin regression became more severe and there were early signs of infection (redness). Fish 17B was found deceased on the morning of the ninth day. It had no appetite during the entire recovery period.

During the recovery period for fish 13D, 13E and 17B, fish 5A and 18A were run through the training protocol only, after the window mesh was installed to reduce tail damage. Both fish received minimal fin damage and recovered their appetites within two days following the training session. These fish were moved back into a separate housing tank and were not used in the study. Following mortalities of fish 13D, 13E and 17B, adjustments were made to the experimental protocol to improve experimental outcomes and reduce tail damage. These included reducing the time spent in the respirometer, and elimination of MMR and $U_{crit}$ components, only including a 30 minute "RMR" measurement, followed by a small increase in speed (max 1.25 BL $s^{-1}$) to capture how oxygen consumption increases with an increase in swimming speed. I attempted to run this new protocol on one fish (12A). This fish did not respond well to confinement during the acclimation period, and I made the decision to discontinue respirometry experiments entirely. Further trial and error to refine the protocol was

not possible due to the species-specific nature of their response (Arctic char in preliminary experiment (training, RMR, MMR, $U_{crit}$) had a complete recovery), animal welfare concerns, and the conservation value of individual fish. It is important to note that these fish had not been exercised prior to this experiment, and were accustomed to life in relatively small tanks.

Table C1. Summary of outcomes from $U_{crit}$ experiments for fish 13D, 13E and 17B. Also included are recovery outcomes and dates of trials and euthanasia (date of death (DOD)).

| | **13D** | **13E** | **17B** |
|---|---|---|---|
| **Fish Information** | | | |
| Salinity (ppt) | 30 | 30 | 0 |
| Weight (g) | 89.1 | 87.5 | 128.1 |
| Length (mm) | 120 | 205 | 225 |
| ***$U_{crit}$* speed** | | | |
| BL $s^{-1}$ | 4.00 | 3.75 | 3.00 |
| cm $s^{-1}$ | 84 | 75 | 69 |
| **Blood parameters** | | | |
| Lactate (mmol $L^{-1}$) | 104 | 138 | 102 |
| Glucose (mmol $L^{-1}$) | 6.7 | 7.0 | 10.4 |
| **Date** | | | |
| Training protocol | 26/06/2023 | 28/06/23 | 26/06/23 |
| RMR and MMR | 27/06/2023 | 29/06/23 | 30/06/2023 |
| Euthanized/DOD | 30/06/2023 | 02/07/2023 | 09/07/2023 |

## Appendix D: Statistics

Table D1. AIC comparisons of models used to test for differences in across treatment groups. * denotes the model that was used for analyses.

| Model | AIC | BIC | Log likelihood |
|---|---|---|---|
| **Growth data** | | | |
| %Growth $DD^{-1}$ (length) | | | |
| Salinity * | -260.95 | -256.56 | -266.95 |
| Salinity + original tank | -259.12 | -253.26 | -267.12 |
| %Growth $DD^{-1}$ (weight) | | | |
| Salinity * | -130.01 | -125.62 | 68.007 |
| Salinity + original tank | -128.86 | -122.99 | 68.428 |
| **Stress data** | | | |
| Lactate | | | |
| Salinity * Temperature * | 211.1 | 226.17 | -98.753 |
| Salinity * Temperature + original tank | 213.51 | 230.26 | -98.753 |
| Glucose | | | |
| Salinity * Temperature * | 25.362 | 40.252 | -5.6808 |
| Salinity * Temperature + original tank | 27.362 | 44.379 | -5.6808 |
| Cortisol | | | |
| Salinity * | 89.19 | 94.23 | -40.60 |
| Salinity + original tank | 91.19 | 97.48 | -40.60 |
| **Ion Balance data** | | | |
| $Na^+$ | | | |
| Salinity * | 178.82 | 182.99 | -85.408 |
| Salinity + original tank | 178.42 | 183.64 | -84.210 |
| $Cl^-$ | | | |
| Salinity * | 183.79 | 187.97 | -87.894 |
| Salinity + original tank | 182.86 | 188.08 | -86.430 |
| Plasma Osmolarity | | | |
| Salinity * | 169.33 | 172.61 | -81.66 |
| Salinity + original tank | 165.72 | 170.08 | -78.86 |

Table D2: Outliers retained in analyses

| | Temp | Salinity (ppt) | z-score |
|---|---|---|---|
| **Weight (g) data** | | | |
| 8D | "cool" | 15 | 3.31 |
| **Condition data (RCF)** | | | |
| 3A | "warm" | 0 | -3.14 |
| 16B | "warm" | 15 | -3.17 |
| **Cortisol (ng $mL^{-1}$) data** | | | |
| 12C | "warm" | 0 | 3.97 |

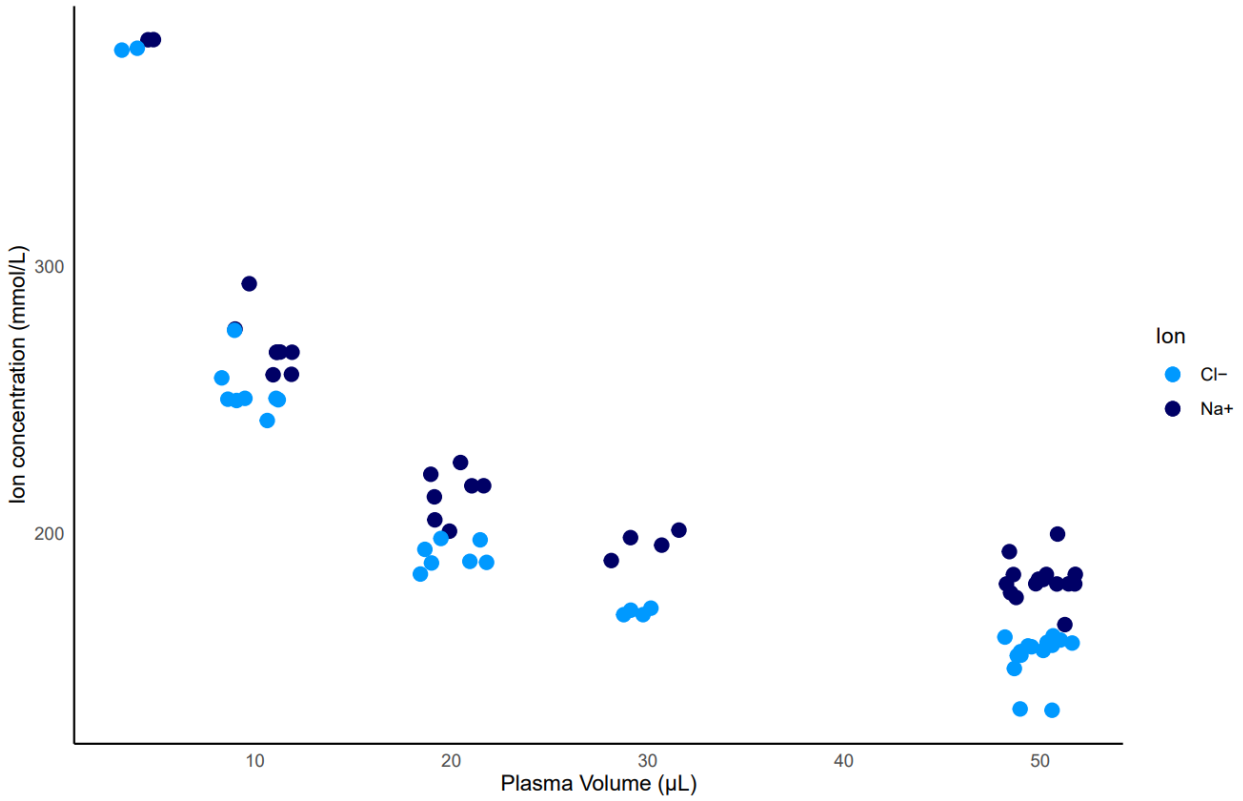


Figure D 1. Assay plasma $Na^+$ and $Cl^-$ concentration (mmol $L^{-1}$) by the volume of plasma (µL) within the total test sample volume (85 µL). Increasingly diluted samples (< 20 µL) overestimated the concentration of plasma $Na^+$ and $Cl^-$.