THE TRANSCRIPTOMIC PROFILES AND MARINE MIGRATION SUCCESS OF ATLANTIC SALMON SMOLTS (*SALMO SALAR*) FROM FIVE POPULATIONS IN CANADA

by

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The last word in ignorance is the man who says of any animal or plant: What good is it?

- Aldo Leopold, *A Sand County Almanac and Sketches Here and There*

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Abstract

Atlantic salmon smolts frequently experience high mortality during the marine phase of their migration, especially during the initial period after entering the sea. However, the mechanisms underlying marine mortality are difficult to determine due in part to the difficulty of observing freely migrating fish in the open ocean. I combined transcriptomic sampling with acoustic telemetry by non-lethally tissue sampling smolts (n = 262) from four stocks of wild Canadian Atlantic salmon as they emigrated down natal rivers, and followed their migration across the Gulf of St. Lawrence to the Labrador Sea ~800 km away. On average, it took fish ~40 days to emigrate to the Strait of Belle Isle. From the activity of 49 genes examined, there was no generalized transcriptomic profile that predicted mortality across populations over the time and distances covered in this study. However, I identified population-specific genes that contributed to mortality during migration. These genes played a role in immune system function and thermal stress, suggesting that smolts were under pressure from these stressors. Finally, I found that larger smolts were more likely to be successful migrants than smaller smolts.

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Chapter 1: Introduction

1.1 – Animal Migration

Migration is the predictable movement of an organism from one location to another for the purpose of exploiting spatiotemporally heterogenous resources and is a complex behavioural trait found in a huge variety of taxa. An often asked question is *why* animals undergo such lengthy journeys; the answer is ultimately to exploit a resource not found in their current environment. Different taxa migrate for different reasons, such as to take advantage of localized and periodic food blooms, sanctuary from predators, or to aggregate and reproduce *en masse* (Dingle, 2006). However, all movement incurs a cost. For any given individual, migration carries both risks and benefits, both of which are variable in time, space and severity (Alerstam *et al*., 2003). The severity of these costs, complicated by the organism's own contextual physical state, dictates whether or not a) that organism undergoes its migration and b) its probability of surviving the migration (Farmer and Wiens, 1998; Skov *et al*., 2011; Chaput *et al*., 2019). Hence, untangling the web of forces that affect an organism leading up to, during, and after migration is integral to understanding, and thus where possible managing, the flux in populations of migratory organisms, many of which are in decline globally (Saino *et al*., 2011; Flockhart *et al*., 2015). The onus is on researchers to try and predict how these same populations will respond to natural and anthropogenically-driven shifts in those factors in the future.

1.2 – Definition of Migration

When discussing a complex behaviour such as migration, it is important to have a clear working definition of what constitutes true migration as opposed to movement associated with typical ranging or foraging. Dingle (2006) proposed that migration has

the following six characteristics, distinguishing it from all other movement: 1) it is persistent and longer in both duration and range than normal ranging; 2) it is mostly linear, i.e., there is a predetermined "point a to point b" destination; 3) the animal exhibits different behaviours, ignoring resources or stimuli that would normally arrest its movement; 4) the animal exhibits different behaviour upon leaving and on arrival at the destination; 5) there is a secondary cue (such as photoperiod or temperature) associated with the instigation of the migration; 6) energy stores are re-allocated to fuel the migration. It is important to note that the distance travelled is not in itself a defining feature of migration, although many animal migrations can be vast relative to the animal's size. To concisely capture these six aforementioned characteristics in my own words: migration is a goal-oriented persistent movement of an organism brought about by changes in the organism's environment over time, requiring the atypical utilization of energy that is otherwise stored except for the specific purpose of migrating.

1.2.1 – Diadromy

Diadromy is a term used to describe a specific type of migration found in some fishes where individuals move between freshwater and saltwater (Bloom & Lovejoy, 2014). There are three types of diadromy: anadromy, where a species is born and reproduces in freshwater but migrates to saltwater in order to feed and grow; catadromy is opposite, catadromous fish are born and reproduce in saltwater but migrate to freshwater where they feed and grow; and amphidromy is a more complex combination of anadromy and catadromy where individuals move between freshwater and saltwater environments, often as juveniles, but not for the purpose of spawning (R. M. McDowall, 2007; Bloom & Lovejoy, 2014).

A diadromous life history necessitates the development of physiological adaptations, shaping the evolutionary history and population structures of such fish species (Bloom & Lovejoy, 2014; Delgado *et al*., 2019; Delgado & Ruzzante, 2020). For example, Closs *et al*. (2013) compared the egg sizes relative to body size of closely related amphidromous and non-migratory species of fish. The authors found that while amphidromous fish had typically higher body sizes, the egg:size ratio was smaller relative to freshwater resident species. This led the authors to propose that larger eggs in freshwater are advantageous due to low productivity (a sort of "jumpstart" at recruitment), while smaller larva released into the ocean are freed from this productivity limitation. Further, the authors concluded that the local proportion of fish exhibiting a migratory or non-migratory life history depends on the net return of each strategy. This study highlights how migration can shape not only the species, but also the local ecosystem.

Atlantic salmon are an iconic example of an anadromous fish, and exhibit remarkable plasticity in the degree of anadromy they express. Many populations conduct the "typical" anadromous migration whereby the juveniles (termed smolts) exit their home river, moving to their at-sea feeding grounds where adults subsequently spend one or more winters growing before returning to their home rivers to spawn (Thorstad *et al*., 2012). However, because Atlantic salmon are iteroparous and can spawn more than once in their lifetime, many salmon will make the anadromous migration multiple times. The proportion of repeat spawners is variable across space and time, and has indeed been shifting across a north-south latitudinal gradient where a decline in repeat spawners has

been observed in southern population contrast, while an increase in repeat spawners has been observed for more northern populations (Bordeleau *et al*., 2020).

1.3 – Factors Influencing Migration Success

The act of migration is the result of a complex web of variables. As such, it is beneficial to establish a foundational framework with which we can build upon as we discuss how, when, and why these variables affect migration in detail. Doing so allows us to more concisely describe the different ways that any one variable affects an organism's migration. Following the movement ecology framework put forth by Nathan *et al.* (2008) and expanded by Furey (2016), the mechanisms at play influencing animal movement can be grouped into one of four connected categories: internal state, movement capacity, navigation capacity, and external factors. For a summary of how these categories interact with each other and influence migration, see supplementary Figure B 1. Keeping this theoretical framework in mind, I examined the challenges that face Atlantic salmon (*Salmo salar*) during their migration from natal rivers to marine feeding grounds.

1.3.1 Internal State

Any research into the physiological incentives and consequences of migration fall under the framework category of internal state (Nathan *et al*., 2008). This refers to processes and mechanisms that occur within an animal's body. The internal state can flux wildly over an organism's lifetime; this is well-researched in species that migrate into environments with extremely disparate conditions such as salmonids which require a complex physiological overhaul to cope with their crossover from a hypotonic to hypertonic saline environment (McCormick *et al*, 1998). Complex physiological shifts within a migratory organism's body in preparation for migration are not uncommon,

especially in long-distance migrants, and thus require the facilitation of greater food intake (hyperphagy) and subsequent storage of large amounts of energy (McWilliams *et al.*, 1999; McCabe and Guglielmo, 2019). This makes body condition a topic of scientific interest when researching migratory animals. Metrics for body condition of organisms are generally a measure of absolute mass, or mass relative to body size (Fulton's Condition Factor) and can be used as a proxy for inferring an individual's accumulated energy stores. Body condition significantly affects behaviours such as departure timing, resting (or stop-over) and overall travel speed during migration (Anderson *et al*., 2019; Goosens *et al*., 2020). The body condition of an individual can even dictate whether or not an organism decides to undertake migration; a phenomenon known as partial migration where only a portion of a population migrates and the rest remain resident is common in salmonids (Thorstad *et al*., 2012; Crossin *et al*., 2016; Nilsson *et al*., 2016; Dujins *et al*., 2017).

Another important, but less studied, internal state factor to consider in a migration context is the immune system status of the organism. Migratory animals are exposed to a higher diversity of pathogens as they travel, and the pathogen load in an organism can directly affect survivorship and energy expenditure during the migration as the body both suffers from an illness and fights it (Alerstam *et al*., 2003; Jeffries *et al*., 2014; Koprivnikar & Leung, 2015). Parasite load can cause early migration in some species because of the energetic burden induced and some taxa may even migrate simply to escape seasonal parasite blooms. (Folstad *et al*., 1991; Birkeland & Jakobsen, 1997; Shaw *et al*., 2019).

Understanding the internal state within the context of migration is important because of how it intersects with the other two individual-specific categories within the movement ecology framework, motion capacity and navigation capacity, while being reflective of the external conditions the animal is facing.

1.3.2 Motion Capacity

The ability of an organism to physically travel its migration route is termed its motion capacity (Nathan *et al.*, 2008). Generally, this refers to the methods by which a given organism travels and its morphological adaptions towards that end, such as wing shape, size at migration, or adaptive camouflage (McCormick *et al*., 1998; Minias *et al*., 2015; Jonsson *et al*., 2016). Because migration can act as a force of natural selection, it favours animals that have adaptations to move efficiently and expend the least energy (Shepard *et al*., 2013). This is especially prominent in populations of animals that exhibit the phenomenon of partial migration, where different phenotypes specific to the migratory animals within the population can develop. For example, in sand crickets (*Gryllus firmus*) long-winged morphs are found to be capable of migratory flight, while short-winged morphs are not. Female crickets of the short-winged morphology produce more eggs and possess larger ovaries in an apparent energetic trade-off (Roff & Fairbairn, 2007). This is an example of how the internal state and motion capacity may overlap. In this case, the energetic cost of migration leads to an apparent reduced reproductive capacity, which also represents the idea of trade-offs being incurred at every stage of migration.

1.3.3 Navigation Capacity

The idea of navigation capacity refers to the ability of the animal to orient its movement to its migration destination (Nathan *et al.*, 2008). The hypothesized methods of navigation vary greatly among taxa; some animals use olfactory cues to imprint to their destinations, others may orient themselves by the earth's magnetic field (Bett & Hinch, 2015; Heyers *et al*., 2017). Successful navigation is imperative because deviating from the least-cost path of migration represents an energetic loss and increases the risk of mortality. Worse, if an animal gets lost on its migration it may be killed by hostile environmental conditions or another detrimental variable it had intended to escape by migrating (Truscott *et al.*, 2017; Ralph & Wolfe, 2018). Thus, any factor that has the potential to interfere with or damage an organism's navigation capacity can have serious consequences for the animal. An example of one such factor interfering with navigation capacity is the adverse effects of artificial light pollution on nocturnally migrating animals (Cabrera-Cruz *et al*., 2018; Truscott *et al*., 2017).

1.3.4 External Factors

Any outside influences that affect an organism's internal state, or motion and navigation capacity of this movement framework are termed external factors (Nathan *et al.*, 2008). This encompasses both abiotic influences such as climate and the geography of the migration route, but also influences with biotic origin such as predation pressure or food availability which may shape the movement behaviour of the animal in question. External factors are critical to the phenology of migrating organisms; it is common for migrating animals to begin preparing for migration at the same time that a shift in some seasonally changing variable such as photoperiod or temperature occurs because the

change in that variable instigates a change to that organism's internal state (McCormick, 1998; Goosens *et al*., 2020).

Many migrating animals have evolved to match their migrational phenology to that of their environment, using environmental cues to predict an optimal time to leave their current environment or enter a new one (McCormick, 1998; Durant *et al.,* 2007). This is formally recognized in the Match/Mismatch hypothesis postulated by Cushing (1969) which attempts to explain variation in population recruitment by the relationship between the phenology of important life history events (such as migration) in one species and the phenology of important seasonal behaviours in the species upon which it feeds (Durant *et al*., 2007). Particularly, an animal occupying a specific trophic level will have the highest population recruitment if the most energy-intensive part(s) of its life coincide in space and time with the period of highest abundance of the organism occupying the trophic level below it (the prey). Therefore, organisms that rely on these environmental cues to migrate at the most optimal time may be disproportionately affected by atypical environmental conditions that disrupt this critical timing.

In a similar vein, predation is one important biotic external factor that influences both the behaviour and survival of migrating organisms. In some populations of migrating animals, increasing predator presence may cause a given population to migrate sooner and seek refuge from their predators (Hulthèn *et al*., 2015). In other populations, migration coincides with the life stage where they are exposed to their highest levels of predation, a so-called mortality bottleneck, directly affecting recruitment for that season (Thorstad *et al*., 2012; Sergio *et al*., 2019).

Finally, the migration route itself can vary in space and time and plays an important role in year-to-year population recruitment and variability. Evolution favours those migrants that expend the least amount of energy while travelling, meaning that animals that take the least-cost path should be selected for (Farmer & Wiens, 1998; Shepard *et al*., 2013). Therefore, the animal must make use of both its navigational and movement capacities when migrating to avoid things such as predators or obstructions, all the while moving towards its goal. Physical obstructions caused by stochastic natural events such as windstorms or landslides can impede the movement of animals as they migrate, as can anthropogenically induced landscape fragmentation (Janin *et al.*, 2012; Thiem *et al*., 2016; Ralph & Wolfe, 2018). Unfavourable migratory conditions *en route* can have long-term impacts on the continued health and behaviour of the organism, even if it survives the initial migration (Strople *et al.*, 2018; Bordelea*u et al*., 2019). Such effects are called "carryover" effects of migration (O'Connor *et al*., 2014).

1.4 – Atlantic Salmon (*Salmo salar*) Migration

1.4.1 Status of Atlantic Salmon in Canada

Canada's Department of Fisheries and Oceans manages the population of Atlantic salmon in Canada by splitting the population into 16 distinct biogeographical regions termed "Designatable Units" (DUs, Supplementary Figure B 2). The last assessment made by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2010 assessed the population on a DU basis, and found that DUs ranged from being deemed Not At Risk to Extinct. Seven of the 16 DUs were deemed Threatened, Endangered or Extinct (Supplementary Table A 1). Only one DU was found to be extinct, which was the Lake Ontario (DU 11) population. The details of this assessment,

including the criteria used for status designation, can be found in COSEWIC's 2010 report.

Despite considerable government investment, including a full commercial fishing moratorium on the species, Canada's wild Atlantic salmon have been in decline since the 1930s where fisheries capture peaked at 6000t (Chaput 2012). More recent assessments show that small salmon or grilse (adult salmon that return to spawn after a single winter at sea) rebounded in 2018 with 581,000 individuals returning, exceeding the numbers predicted in 2017 by 29% (Atlantic Salmon Federation, 2019). However, large salmon (multiple sea-winter and repeat spawners) returns in 2018 fell under the predicted 2017 numbers by 24%, with 131,800 individuals returning (Atlantic Salmon Federation, 2019). Whether the apparent rebound of grilse will continue and translate into increased large salmon returns remains to be seen. However, it must also be noted that of the small salmon returns, 92% were concentrated in Newfoundland and Labrador and of the large salmon returns, 81% were in Quebec, Labrador, and the southern Gulf of St. Lawrence (Atlantic Salmon Federation, 2019).

Given the cultural, ecological, and economic (~$255 million CAD, Gardner Pinfold 2011) contributions Atlantic salmon make to Canada, it is alarming that their populations exhibit a declining trend despite intense management and multiple restoration projects. Equally alarming is that while at-sea mortality has been identified as the point in the life cycle that salmon are dying, there is not yet a consensus on why salmon are dying at sea, although several factors are hypothesized to contribute, including anthropogenic disturbances such as aquaculture and shifting climatic conditions (ICES, 2017). In particular, variation in sea-surface temperature (SST) has been shown to account for at

least some post-smolt mortality (Olmos *et al*., 2020) but climatic variation alone cannot explain the decline of Atlantic salmon across their native range (Soto *et al*., 2018).

1.4.2 The Smolt Migration

Atlantic salmon are well-known for their anadromous migrations, in addition to being iteroparous in contrast to their Pacific cousin's semelparity. After hatching from their freshwater-spawned eggs, the young salmon (termed alevin) will not feed until they have absorbed their egg yolks, after which they are termed fry. At the end of the summer, the fry develop into parr, which are characterized by cryptic black vertical bars on the dorso-lateral sections of the body. The next life stage, termed the smolt, refers to parr that are undergoing the so-called smoltification process whereby the individual undergoes an overhaul of its internal state in anticipation of migrating from a freshwater (hypotonic) environment into a saltwater (hypertonic) environment. This process is physiologically characterized by an increased ability to process lipids and an increased gill $Na^{\pm}$, $K^{\pm}$-ATPase activity consistent with higher salinity tolerance in addition to morphological changes where the fish becomes silvery so as to better camouflage itself in the pelagic environment (McCormick and Saunders, 1989; Sheridan, 1989; McCormick *et al,* 1998). This process demands high energy inputs, and is hypothesized to experience the highest mortality rate of any other life stage (Klemetsen *et al*, 2003). Once the fish reaches this critical stage, migration is triggered by environmental cues such as photoperiod and temperature. There is not a migration-inducing cue that is universal to all populations of Atlantic salmon, rather, one stock may have its migration induced by solely water temperature while in another stock the cue is a mixture of temperature and spring runoff (Thorstad *et al*., 2012).

Within Canada, populations of Atlantic salmon smolts exhibit remarkable phenotypic plasticity and variation in their migration strategies. Some populations can exhibit whole "true" migration, while others can exhibit partial migration. Partial migration refers to a fraction of the salmon within a population migrating out to sea while another fraction remains resident. The term "partial migration" also encompasses differential migration, where the fraction that migrates is predominately one sex, as is the case in rivers with a large population of precocious parr (Myers *et al*, 1986; Dingle & Drake, 2007; Strople *et al*, 2018). There is also variability in the age and size at which smolts migrate. Some populations of salmon will remain as parr for up to eight years, while others may only be parr for two years or even never smoltify. Males in some systems sexually mature and "sneak" spawn with returning females; a unique reproductive strategy that has led to such fish being termed "precocious" parr (See below for a description of precocious parr) (Legget & Power, 1969; Myers *et al*, 1986; Saunders & Schom, 1989). Klemetsen *et al*. (2003) illustrates that size and age at the time of smoltification typically increases with increasing latitude (see Bergmann's Rule), with large (>20cm) smolts being common in rivers that drain into cold oceans, while smaller (<13cm) smolts are found in rivers that drain into warmer oceans. Outmigrating smolts may then spend a single or several winters feeding at sea before returning to their natal rivers in the fall to spawn (Klemetsen *et al*. 2003; ICES, 2017).

While the migration strategies of Canadian Atlantic salmon are highly variable, the destination of said migration is generally the same. The majority of Atlantic salmon populations in Canada from rivers that drain into the Gulf of St. Lawrence migrate northwards through the Strait of Belle Isle towards the waters west of Greenland (ICES,

2017; Chaput, 2019). The destination of the North American Atlantic salmon migration has been known to researchers for years, but the at-sea period from when a smolt exits its natal river and actually migrates to Greenland is still largely a "black box" area in the field of salmon research, due to the difficulty of tracking such a small, mobile fish in a vast environment (Thorstad *et al*., 2012). With tagging technology in its current state, *S. salar* smolts are too small to attach satellite tags to; surgically or otherwise. Acoustic telemetry, therefore, is the best option to track these fish but is constrained by the need for a relatively fine network of receivers which is both expensive and hard to maintain in a pelagic environment. This thesis aims to describe part of this "black box" period of migration through the use of the natural geography of the Gulf of St. Lawrence, and large acoustic receiver arrays already in place and maintained by different authorities in Canada.

1.5 – Introduction to Transcriptomics in Fish Conservation

Transcriptomics is a term used to describe the assessment of processes happening at the cellular RNA level. Messenger RNA, or mRNA is the intermediate product between DNA translation and protein production. Therefore, measuring the abundance of known mRNA transcripts in a sample can be used as a proxy for gene expression in a sample (Jeffries *et al*, 2021). Transcriptomic technologies have benefited greatly from the advancement of quantitative polymerase-chain-reaction (qPCR) technologies which are necessary for amplifying the target mRNA sequences into the complementary DNA (cDNA) that is subsequently quantified. Modern micro- and nanofluidic arrays such as Fluidigm or OpenArray are both commercially available and able to process hundreds to thousands of qPCR cycles in parallel, while also requiring only a relatively small tissue

sample to begin with. Using these high throughput arrays, researchers can assess the expression of several genes of interest pertaining to multiple facets of an individual animal's physiological state within a single sample, creating a "snapshot" of the internal state in what is termed a transcriptomic profile. This makes transcriptomics well-suited to investigating whole body responses to some stressor of interest, rather than only considering a few molecular markers in the animal's system and then inferring biological significance (Wellband *et al*., 2018; Jeffrey *et al*., 2020; Jeffries *et al*., 2021).

Notably, animals can be sampled non-lethally (multiple times if the study requires it) and the resulting information can be linked to an ecological endpoint. For example, Ren *et al*. (2015) isolated target gene sequences from Channel catfish (*Ictalurus punctatus*) mucus and exposed groups of fish to *Flavobacterium columnare* bacteria. The authors then sampled mucus from three different surfaces: intestine, skin, and gill at different time periods after bacterial exposure. They found that expression patterns changed rapidly over the course of the catfish's response to pathogen exposure, and additionally found that the expression patterns were different depending on the tissue the mucus was sampled from. This study highlights that transcriptomics can be used to infer the progression of an animal's response to a stressor, and that the choice of sampled tissue is of consequence when planning transcriptomic studies.

In fish, several tissues have successfully been used in transcriptomic studies. For example, gill tissue was a popular target when studying pathogens and osmoregulation due to the gills being a significant interface between the external environment and the fish's internal state. Multiple studies (Martinelli-Lietdke *et al*., 1999; Zhao *et al*., 2014) have shown that the non-lethal biopsies of gill tissue do not cause a reduction in growth

or survival in fish when proper handling protocols are followed, but see Bass *et al*. (2020) who found evidence to show that gill biopsies may cause increased mortality in juvenile sockeye salmon (*Oncorhynchus nerka*) in the first ~14km following release. Other types of tissues that have been used in transcriptomic studies include skin (Arukwe & Røe, 2008), scales (de Vrieze *et al*., 2014), muscle (Vandhoen *et al*., 2009), fin (Andreasen *et al*., 2006), and blood (Lewis *et al*., 2010). The choice of which tissue to sample generally comes down to the study objectives – an ecological toxicology study interested in the biological effects and residency time of a pollutant in an organism would probably benefit from analyzing the tissue that the pollutant is suspected to be sequestered in, rather than a tissue it may not directly affect.

Handling procedures are another important variable to consider when planning a study using transcriptomics. Because fish, and animals in general, can begin reacting to stressors internally within minutes of exposure to that stressor, the sampled data acquired during handling can be confounded by the incidental stress of handling the animal (Barton, 2002; Jeffrey *et al*., 2020). Therefore, care must be taken to minimize the stress caused by any invasive procedures such as non-lethal sampling during the data collection process.

1.6 – Thesis Aims and Chapter Outline

This thesis was conducted in order to identify physiological mechanisms of mortality in seaward-migrating Atlantic salmon smolts. Specifically, I investigated whether different populations of smolts in Canada exhibit similar transcriptomic profiles to one another, and if the up- or down-regulation of certain genes can be used to predict migration success in an individual smolt.

In Chapter 1, I discussed a conceptual framework for understanding animal migration and introduced the key concepts and technologies used to accomplish this thesis' objectives. In Chapter 2, I present a study carried out where I combined acoustic telemetry and transcriptomics to investigate whether five populations of smolts in Canada exhibited similar transcriptomic profiles, and whether there were specific genes that the expression of which could predict a successful marine migration. In Chapter 3, I highlight why these data are important for policy-writers to consider when making decisions on how to best conserve Atlantic salmon populations in the context of a warming northern hemisphere and planning future studies.

Chapter 2 – Factors Influencing Smolt Migration Success Inferred from Acoustic Telemetry and Transcriptomic Profiling

2.1 – Introduction

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) outlines 16 Designatable Units (DUs) of Atlantic salmon within Canada, each composed of populations exhibiting different age structures, physical characteristics and migration strategies (COSEWIC 2010). For example, individuals of the Atlantic salmon population occupying Western Arm Brook in Newfoundland typically migrate out to sea at around ~4+ years of age with a mean fork length of 17.5 cm (Chadwick, 1981) and return to spawn after a single winter at sea (such fish are termed grilse) (DFO, 2020), while Atlantic salmon occupying the Margaree River typically begin their migration at 2-3 years of age with a mean fork length of 13.0 cm (Breau & Chaput, 2012) and return to spawn after multiple winters at sea. The populations of salmon in the Northwestern Atlantic (i.e., Canada) are genetically distinguishable from those in Europe, having diverged approximately 600,000 years ago (Lehnert *et al*., 2020). Lehnert *et al*. (2019) showed that populations of salmon in the North Atlantic which are in decline exhibited different genomic signatures from those that are not. There is also microsatellite allele evidence that shows Newfoundland populations are genetically distinct from other populations in North America and rivers which drain into the Gulf of St. Lawrence were found to cluster together (King *et al*., 2001; Spidle *et al*., 2003). Furthermore, Bradbury *et al*. (2014) showed that populations of Atlantic salmon in Newfoundland could exhibit high genetic divergence over a relatively small spatial scale connected to local environmental characteristics such as water chemistry and suggested that this was

evidence for local adaptation, an idea supported by the work of Pritchard *et al*. (2018). There is also variation in the timing of each population's migration due to latitudinal differences resulting in differential hours of sunlight and temperature (Otero *et al*., 2014). Consequently, smolts from different populations are thus exposed to different oceanographic conditions upon marine entry. The proportional contribution of such conditions to overall marine mortality remains unclear (Thorstad *et al*., 2012). The sizes of many of these populations are under historical levels and some continue to decline. This is despite considerable conservation investment and a commercial moratorium on Atlantic salmon introduced in 2000. The widespread and synchronous decline of populations suggests a common syndrome, yet no specific mortality mechanism has been identified (ICES 2020). Shifting temperature regimes, specifically persistently high sea-surface temperature anomalies in the first month at sea that restrict appropriate thermal habitat, have been proposed as a mechanism behind localized at-sea mortality in salmon (Friedland *et al*., 2003; Condron *et al*., 2005; Kilduff *et al*., 2015). However, recent evidence suggests that while SST accounts for a proportion of annual variation in mortality (Olmos *et al*., 2020), ocean warming cannot wholly explain this synchronous decline in Atlantic salmon across the species broad geographic range (Soto *et al*., 2018). Elevated river temperatures are known to be cellularly and metabolically taxing to Atlantic salmon smolts (Corey *et al*., 2017; Lennox *et al*., 2018). Higher temperatures are also known to compound other incidental stressors such as fisheries capture, leaving already physiologically stressed smolts vulnerable to pathogens and less able to adequately fight infection (Patterson *et al*., 2017; Chapman *et al.*, 2020). The net impact

of a warming northern hemisphere is predicted to ultimately be detrimental to populations of Atlantic salmon and other coldwater fish species (Alfonso *et al*., 2020).

For juvenile Atlantic salmon (*Salmo salar*), the transition from freshwater rearing areas to the sea and the early marine life-history is a period of high mortality (Thorstad *et al*., 2012; Chaput *et al*., 2019). At this life stage anadromous juvenile salmon must physiologically preadapt to oceanic conditions through the smoltification process. This process is characterized morphologically by the individual fish's body acquiring a silver colouration, and internally by increased gill K^+, Na^+-ATPase activity, increased gill ionocytes (also known as chloride cells, these cells pump Na^+ and K^+ ions against the concentration gradient to help maintain osmotic balance; Evans *et al*., 2005) and decreased water absorption through the gut in preparation for entry into the hyperosmotic environment (McCormick *et al*., 1998). This process is extremely energetically intensive, and individuals experience a decrease in total body lipids (Wedemeyer *et al*., 1980; Thorstad *et al*., 2012). All the while, smolts are struggling to cope with novel predators (Järvi, 1989; Halfyard *et al*., 2012; Strøm *et al*., 2019; Flavio *et al*., 2021), osmotic challenges (Halfyard *et al*., 2013), disease organisms (Miller *et al*., 2014 ; Vollset *et al*., 2021), and must learn how to capture new food resources (Rikardsen *et al.*, 2004; Haugland *et al*., 2006). These and other stressors may act alone or synergistically to cause mortality. For example, poor environmental conditions can lead to reduced food resources for young salmon, reducing their body condition (Tocher, 2010; Strople *et al*., 2018). As a result of poor body condition, fish can become more vulnerable to depredation via a reduced capacity to escape, or become favored targets to predators (Tucker *et al*., 2016; Hasegawa *et al*., 2021); or become vulnerable to infection from

pathogens that they would normally be capable of fighting off (Miller *et al*., 2014). For instance, the species of fungus belonging to genus *Saprolegnia* is a ubiquitous pathogen which causes saprolegniasis in many species of fish, but only in individuals who are immunologically compromised (Pickering, 1994; Mayer, 2000). In the case of *Saprolegnia* and other pathogens, juvenile salmon undergoing the smoltification process are known to be especially vulnerable due to stimulation of the hypothalamic-pituitary-axis releasing cortisol during the smoltification process which is a known immunosuppressant in salmonids (Pickering, 1994). For a review of pathogens that increase their pathogenicity in smolts, see Miller *et al*. (2014). The combination of undergoing an intense systemic physiological change, combined with a plethora of external threats and stressors makes the smolt life stage fraught with mortality. Chaput *et al*. (2019) found that the individual probability of survival for a smolt to enter the marine environment was highly variable both within and between populations, but could be less than 10%.

The majority of studies investigating smolt and post-smolt (the period immediately after a smolt enters the sea) movements have been limited to small areas such as fjords, estuaries, or coastal areas in the nearshore environment near the fish's home river. This has been enabled by the development of acoustic telemetry technology, where animals tagged with unique individual tags can be detected by strategically placed acoustic receivers that record the times and dates of fish passage. However, the technology has limited ranges and is costly, hence the typical focus on small or spatially explicit areas (Welch *et al*., 2010; Thorstad *et al*. 2012; Crossin *et al*., 2017; Birnie-Gauvin *et al*., 2019). Nevertheless, multiple studies indicate that predation pressure in

estuaries and nearshore waters is intense (Halfyard *et al*., 2012, 2013; Phillips *et al*., 2021), and smolts may have reduced foraging success in tidally mixed nearshore waters (James *et al*., 2020). Halfyard *et al*. (2012) acoustically tagged emigrating smolts from four rivers in Nova Scotia and found early migration cumulative survival rates through the river, estuary, and surrounding bay varied from 39.4% to 73.5%. Järvi (1989) proposed the concept of the synergistic stress effect; whereby stress induced by predator presence and osmoregulatory stress caused by the introduction to saltwater act synergistically to amplify mortality rates of emigrating smolts. The results from Halfyard *et al*. (2012) and Järvi (1989) suggest that smolts which quickly acclimatize to the osmotic change and move rapidly along the migration route subsequently benefit from reduced predation risk and an increased probability of survival. There is also evidence to suggest that food availability in the natal river prior to migration and subsequent body energetic condition of the migratory fish play an important role in the adult return rate, and thus migration success, of Atlantic salmon. Gregory *et al*. (2019) used state-space models to show that larger smolts had higher probabilities of returning to spawn, and Crossin *et al.* (2016) showed that smolts that enter the brackish Bras d'Or Lake in better nutritional condition were more likely to exhibit an alternative non-migratory life history and stay in the Bras d'Or as residents while poorer condition fish continued to the Atlantic Ocean. Few studies have been carried out in the open ocean to empirically elucidate levels and mechanisms of at-sea mortality in *S. salar* smolts during the marine migration. The work that has been done has documented instances of mortality but could only infer mortality causes (Lacroix, 2013; Crossin *et al*., 2016; Chaput et al., 2019). Lacroix (2013) investigated post-smolt movements through the Bay of Fundy (BoF),

Canada, and found that different migration strategies within the BoF led to differing survival rates among the sampled fish, but the sources of mortality were unidentified; a similar result was obtained by Kocik *et al*. (2009) who also found smolts making use of multiple migration strategies. Friedland *et al*. (2017) applied a Bayesian life-cycle framework to investigate the effects of various environmental parameters on Baltic post-smolts and postulated that changing environmental conditions favourable to post-smolt predators is increasing predation pressure on the post-smolts.

Transcriptomic profiles offer a novel, powerful and non-lethal methodology for revealing potential correlates of survival in migrating salmonids (Miller et al., 2011; Connon *et al*., 2018; Jeffries *et al*, 2021). Up- and down-regulation of sampled genes derived from non-destructive tissue samples in the field provide contextual data to researchers for addressing multiple hypotheses in a single sample by examining the levels of categorical gene expression as they pertain to different environmental forces acting positively or negatively upon the individual (Miller *et al*., 2011). Combining this transcriptomic technology with acoustic tracking technology allows researchers to easily match physiological shifts with fish behaviour and notably mortality in field studies (La & Cooke, 2011; Hussey *et al.*, 2015; Jeffries *et al*., 2021). Acoustic telemetry offers several benefits to physiological studies. It allows researchers to infer whether or not mortality has occurred and hypothesize whether it was a result of an external force such as depredation, and if the physiological variables of interest were at least partially responsible (Hussey *et al*., 2015; Lennox *et al*., 2017). For example, Evans *et al.* (2011) established transcriptomic profiles from genes affecting osmoregulation, temperature responses, and immune responses in wild female sockeye salmon (*Oncorhynchus nerka*)

by lethally and non-lethally sampling tissues as they returned to spawn. The authors found that the transcriptome was actively modulated in response to shifting environmental conditions reflecting the differences of a marine and a freshwater environment. They also identified differential profiles between successful and failed migrants through the use of acoustic telemetry. Gene expression involved in DNA repair suggested that migrating adults accumulated cellular damage in the Fraser River estuary that subsequently hampered their ability to successfully enter the river system. Similarly, Jeffries *et al*. (2014) found that differentially upregulated immune response genes were predictive of migratory failure in sockeye salmon smolts. Previous studies have also shown that gene expression can predict certain behaviours in salmonids months in advance, demonstrated by Amstutz *et al*. (2006). The study found that the expression of the transaldolase I gene predicted a sedentary versus migratory life history in brown trout up to three and a half months before migration occurred. Furthermore, the products of gene expression can remain within an individual's body for an extended period of time and can therefore influence behaviour and condition for days to weeks after sampling (Borbolis & Syntichaki, 2015; Jeffries *et al*., 2021).

Gene expression in salmonids can be used to infer causes of different aspects of behaviour and mortality, where acoustic telemetry can verify these behaviours and make them quantifiable (Lennox *et al*., 2017; Whoriskey *et al*., 2019). As both acoustic telemetry and transcriptomic technology advance, and through the use of large receiver arrays such as those maintained by the Ocean Tracking Network (Iverson *et al.*, 2019; Bangley *et al*., 2020), researchers will be capable of studying the interaction of physiological state and behaviour on a much vaster geographical scale. Pairing

transcriptomic profiles with acoustic telemetry of smolts provides the most comprehensive methodology available to investigate the mechanisms of mortality of this species at risk (Goetz & MacKenzie, 2008; Crossin *et al*., 2014; Connon *et al*., 2018; Jeffries *et al*., 2021) during this particularly sensitive life stage (Klemetsen *et al*., 2003; Thorstad *et al*., 2012).

Here, I present the first comparative multi-population study of Atlantic salmon to combine transcriptomic work with acoustic tracking technology. This study tests hypotheses about putative mechanisms underlying variation in the levels of mortality for migrating Canadian smolts/post-smolts during the first ~60 days at sea and 800 km of their movement from home rivers towards oceanic feeding areas in the Labrador Sea. I worked on populations from four rivers across a north-south latitudinal gradient and having salmon populations with different age structures and anticipated migration patterns and distances. Given the biological and behavioural differences among the populations, I expected that these populations should differ in their gene expression patterns. I then evaluated two hypotheses: that different populations of Atlantic salmon are exposed to different environmental stressors in space and time such as varied water chemistry or temperature, and should thus exhibit different transcriptomic profiles. Second, I hypothesized that successful migrants should exhibit different transcriptomic profiles from failed migrants, and if this is true, that I could then identify possible mechanisms of mortality by identifying groups of genes that are differentially expressed between the successful and failed migrants. My study objectives are: i) investigate if the gene expression of different *S. salar* populations is similar to one another, and ii) investigate which genes, if any, are predictive of marine migration success or failure.

Understanding the early marine migration of Atlantic salmon smolts is a key step towards identifying the spatially explicit stressors that this species encounters at sea and unlocking the details of individual and population-scale variance in fate.

2.2 – Methods

The Gulf of St. Lawrence provides a unique field laboratory for studying Atlantic salmon post-smolt migration movements due to the presence of key acoustic telemetry arrays spanning the breadth of the Cabot Strait and Strait of Belle Isle across the migration routes to the Labrador Sea of Atlantic salmon leaving rivers draining to the Gulf of St. Lawrence. These arrays are several hundred kilometers from the tagging sites, except for Western Arm Brook, providing a unique infrastructure to assess smolt/post-smolt mortality that occurred over the first few weeks fish were at sea (Figure 1). Smolts from rivers draining into the Gulf of St. Lawrence typically migrate north towards feeding grounds west of Greenland via the Strait of Belle Isle (Chaput, 2019).

To identify drivers behind marine mortality, I coupled acoustic tracking with qPCR of tagged smolt gill tissue samples. Using qPCR, tissues may be screened for biomarkers indicative of known pathogens, as well as tRNA that allows for the measurement of specific gene expression in what is known as a transcriptomic profile describing the up- and down-regulation of individual genes (Miller, 2016). Fish were sampled in May-June of 2019 from four rivers and implanted with small acoustic transmitters in the context of the Ocean Tracking Network acoustic telemetry array with key lines at marine straits providing important coverage of the migration pathway from the river. At the time of tagging, myself or the individual tagging also non-lethally sampled the gill tissue of each individual in order to create transcriptomic profiles for

each fish. Gill tissue was chosen because we predicted that it would exhibit the most fluctuation in the expression of our targeted genes (Table 1) as it is the largest interface between the external environment and the fish's internal environment. Gill tissue can also be biopsied with little subsequent harm to the animal (Zhao *et al*., 2014). This interface is extremely important for osmoregulation and often an entryway for pathogens (Evans *et al*., 2005). Previous studies on sockeye salmon where expression profiling was used on gill tissue has revealed profiles that predict some freshwater mortality in smolts (Jeffries *et al*., 2014) and the impacts of bycatch or environmental stressors in adults (Teffer *et al*., 2017). Many of our targeted genes related to immune system function or smoltification, and so we concluded that gill tissue was the most practical tissue to sample for our purposes.

2.2.1 – Study Areas

Margaree River

The Margaree river is located on the western shore of Cape Breton, Nova Scotia (46.2ºN, 61.5ºW) flowing about 120km from its headwaters and emptying into the Gulf of St. Lawrence at Margaree Harbour. This river has two main branches, the Northeast and Southwest branches, which are fed from the Cape Breton Highlands and Lake Ainslie, respectively. The two branches join at Margaree Forks. This population falls under the Gaspé-Southern Gulf of St. Lawrence Designatable Unit (DU 12), and its conservation status is noted as Special Concern by the Committee on the status of Endangered Wildlife in Canada (COSEWIC) (COSEWIC 2010). Smolts in this population are typically 2-3 years of age upon migration, and usually spend two winters at sea (Breau & Chaput, 2010). The Margaree Salmon Association operates habitat

restoration and hatchery activities in this river, and releases up to 50,000 hatchery reared salmon each year (http://www.margareens.com/hatchery.html). The smolt wheel used to capture smolts for sampling and tagging in this river was located at Tompkins' Pool, which is ~1km downstream from Margaree Forks and ~1km from the ocean (Figure 2). Tagging occurred on this river from May 24th 2019 to June 12th 2019, due to a delay in the smolt run caused by an unusually cold spring. The average river temperature during tagging was 10.76ºC.

Trinité River

The Trinité River is located in Quebec, on the North Shore of the Gulf of St. Lawrence (49.4ºN, 67.3ºW) and flows for 75km from its origin to enter the Gulf next to the village of Baie-Trinité. The salmon population in the Trinité River is part of the Quebec Western North Shore Designatable Unit (DU 8), and is judged to be of Special Concern (COSEWIC 2010). This river's salmon population has experienced a 24% decline in returning fish from 1997 to 2006 (COSEWIC, 2010). The smolts in this river typically migrate at around 3-4 years of age, and spend on average two winters at sea (Caron & Raymond, 2000). This is the only river in the study series to have a dam (low head) present, which is located at its mouth. There are no hatchery operations on this river. The smolt wheel used to capture fish on this river is located just off Highway 138, about 4km upstream from Baie-Trinité and the ocean (Figure 2). Tagging occurred on this river from June 16th 2019 to June 24th 2019. The average river temperature during tagging was 14.13ºC.

Western Arm Brook

Western Arm Brook is located on the Northeastern coast of Newfoundland (51.11ºN, 56.45ºW), and flows 27km from its origin to enter the Gulf of St. Lawrence in St. Barbe's Bay. The smolts in this river typically migrate at approximately four years of age, and generally only spend a single winter at sea before returning to spawn (Chadwick, 1981, DFO, 2020). This population is designated as DU 3 (COSEWIC 2010), and has been assessed as Not at Risk, however, recent reports suggest as much as a 57% decline in overall smolt survival (DFO, 2020). A smolt weir was used to capture fish for tagging and sampling on this system just at the estuary entrance, and it is located just off Highway 430, where the highway crosses the river (Figure 2). Tagging occurred on this river from June 15th 2019 to June 16th 2019. The average river temperature during tagging was 12.73ºC.

Miramichi River

The Miramichi River is located in New Brunswick, flows for approximately 250km and drains into the southern Gulf of St. Lawrence at the town of Miramichi, N.B (45.1ºN, 65.3ºW). The river has two major branches, the Northwest and the Southwest. We worked with fish from both branches in this study. The Miramichi system is part of the COSEWIC Gaspé-Southern Gulf of St. Lawrence Designatable Unit (DU 12) and is categorized as being of Special Concern (COSEWIC 2010). Salmon abundance in DU 12 has undergone an estimated 28% decline from 1997 to 2007, which is now estimated to have ~100,000 individuals, however, most of these salmon are present in the Miramichi. The smolt run consists of mostly 2-3 year old smolts (Strothotte *et al*., 2005) which then spend either one or two winters at sea (Chaput *et al*., 2016). In recent years, this river has seen an increase in striped bass populations in its estuary (local fishermen and hatchery

employees, personal communication). Smolt tagging and sampling occurred on the Northwest branch of the Miramichi from May 30th 2019 to May 31st 2019, and on the Southwest branch on May 27th 2019. The average temperature during tagging was 14ºC on the Northwest branch and 11ºC on the Southwest branch. The smolt wheel in the Northwest branch was located ~15km from the ocean between Johnson's Island and Metepenagiag Lodge, just upstream from Sunny Corner and the wheel on the Southwest branch is located ~60km from the ocean at Sisters Rapids, Stanley Parish (Figure 2). We considered the two branches as separate populations because previous studies have found evidence to suggest genetically distinct populations between the Northwest and Southwest tributaries (Møller, 1970; Chaput *et al*., 2016).

2.2.2 – Acoustic Tagging and Gill Sampling

All fish handling was performed in accordance with protocols approved by the Dalhousie University Committee on Lab Animals, permit number 18-126. Once captured, fish from each river were first measured, and only fish of 14 cm fork length or larger had tags implanted. By using fish in this size range, we kept the tag weight to body weight ratio less than or equal to 8% of the fish's mass in air, which previous studies have reported to minimize adverse handling effects (Lacroix *et al.,* 2004; Chittenden *et al*., 2009; Smircich & Kelly, 2014). The tagging methods used here are described in Cooke *et al*. (2011a).

Prior to surgery, fish were kept in groups of five in 10 gallon buckets full of fresh river water with airstones. Fish were placed in a clove oil solution (0.5 ml of oil per 10,000 ml of water) and considered anaesthetized when they were unable to right themselves and did not react to a gentle tail pinch. At this point, the fish were removed

from the bucket, weighed, measured for fork length once more, and a small (~2mm) biopsy of gill filaments was taken using small bone cutting forceps. Gill filaments were selected because it is the largest interface between the external environment and the fish's body, and it is known that fish sampled for gill tissue do not experience differential growth or survival after sampling (Jeffries *et al*., 2021). The gill tissue biopsy was placed in a small vial of RNAlater (Life Technologies, Grand Island, New York) where it was refrigerated at 4°C for 24 hours, after which it was placed into a freezer at -2°C until it could be placed into a -80°C freezer for long-term storage. Upon completion of the gill biopsy each fish was transferred, ventral side up, to a surgery table where its gills were constantly flushed with water from a maintenance bath. A small incision (8-10mm) was made in the ventral surface adjacent to the midline, between the pelvic and pectoral girdles. The tagger then inserted a VEMCO V8-4x acoustic tags (20.5mm length × 8mm diameter, 2g air/0.9g water; 69kHz, VEMCO Ltd., Bedford, Nova Scotia; www.vemco.com) into the incision which was then closed with 2-3 monofilament absorbable sutures (Ethicon monocryl 5-0 monofilament; www.ethicon.com). The fish was then placed into a new bucket of fresh river water aerated by an airstone where it was monitored until it had righted itself and was swimming strongly. At this point, the fish was deemed to be sufficiently recovered and was then released.

2.2.3 – Telemetry Infrastructure and Analyses

At each study site, acoustic receivers were deployed by the study's partners to create a gate near the ocean entry to detect emigrating smolts (Figure 2). Seven receivers were originally placed by the Department of Fisheries and Oceans (DFO) in St. Barbe's Bay at the mouth of Western Arm Brook, but heavy ice conditions caused the loss of five,

leaving the two marked in Figure 2. The Atlantic Salmon Federation maintains receiver arrays within the Miramichi River and estuary, as well as two lines of receivers across the Strait of Belle Isle (https://members.oceantrack.org/OTN/project ?ccode=ASF). The Ocean Tracking Network (OTN) also maintains receivers and hydrophone-equipped marine autonomous vehicles (Slocum electric gliders; Liquid Robotics Wave Gliders) throughout the Gulf of St. Lawrence. OTN also maintains lines of receivers across the Cabot Strait (https://members.oceantrack.org/OTN/project?ccode=CBS). These were used to detect the survival of this study's tagged smolts moving up to 800 km across the Gulf of St. Lawrence. Chaput (2019) reported that the vast majority of smolts from rivers draining to the Gulf of St. Lawrence exit the Gulf through the Strait of Belle Isle, but a small proportion may exit the Gulf through the Cabot Strait. Detection data were analyzed using the package *actel* for R (R Core Team, 2020; Flavio 2020). A detection was deemed to be "real" if the tagged fish was detected at least twice at two separate times by the same receiver or detected by more than one receiver in the array. A fish was deemed to have survived migration across the Gulf of St. Lawrence if its tag was detected at either of the Strait of Belle Isle or Cabot Strait arrays.

2.2.4 – Genetic Assays

Tissue samples were transported frozen with dry ice to the Molecular Genetics lab at the Pacific Biological Station in Nanaimo, British Columbia to be assayed. Here, each sample was thawed, the RNAlater decanted, and the samples subjected to organic phase extraction using Trizol and BCP (3-bromochloropentane). The tubules were then homogenized at 30 Hz for three minutes, shaken by hand for one minute, then incubated at room temperature for five minutes. This procedure was repeated a second time,

following which the samples were centrifuged at 3000 RPMs for five minutes. 100µl of this aqueous, RNA-containing solution was siphoned off, and the RNA extracted through an automated liquid handling instrument, and eluted by hand. The RNA was then normalized to 50mg/L and reverse transcribed to cDNA. Specific Target Amplification (STA) was used to identify specific markers for qualities relating to physiological states such as osmotic stress or immune system readiness. Finally, these amplified fragments were analysed using Fluidigm qPCR software (Bass *et al*, 2017) and delta-delta normalized (Pfaffl, 2001) using customized functions in R. Further details of the methods for the preparation of RNA for this analysis can be found in Houde *et al* (2019).

2.2.5 – Statistical Analyses

All analyses were performed using R software (version 4.0.3; R Core Team, 2013).

Principal Components Analysis

In total, I investigated the expression of 52 genes pertaining to ten different functional families; their sequences and additional information can be found in Table 1. To analyze how these genes influenced migratory survival, we first performed a principal component analysis (PCA), an unconstrained data reduction tool used to develop aggregate variables (PC axes) for subsequent analyses. PCA constructs these synthetic variables by evaluating linear relationships (eigenvectors) between columns in a matrix of data and selecting those relationships that maximize variance (i.e., the highest eigenvalues) in the dataset (Joliffe & Cadima, 2016). *Post hoc*, we can investigate these variables to examine their loadings; that is, the columns that had the highest weighting when constructing the variables (Table 2). This allows us to investigate which specific genes exhibited the most variance within the dataset. PC1 accounted for 23.66% of the

variance in the genetic dataset and was highly loaded with genes from the Viral Disease Development family, and thus I interpret this axis to be "Disease Effects". PC2 accounted for 18.59% of the variance and was highly loaded with a gene from the Osmotic Stress family, so I interpreted this axis to be "Osmotic Stress". PC3 accounted for 10.27% of the variance and was highly loaded with genes from the Immune Response family and one gene from the Imminent Mortality signature family, and so I interpreted this axis to mean "Disease-related Mortality". Finally, PC4 accounted for 7.86% of the variance and was highly loaded with one gene each from the Osmotic Stress family (the same one as PC2) and from the General Stress family; the specific gene that loaded codes for heat shock proteins. The same Imminent Mortality gene from PC3 loaded highly negatively, which indicates that variation within the expression of Osmotic Stress and General Stress genes was negatively associated with variation within the expression of the Imminent Mortality gene. Thus, I interpret this axis to be "Environmental Adaptation".

K-means Clustering

K-means clustering is an unsupervised machine learning method that uses a distance calculation to group observations. Specifically, K-means seeks to maximize the distances between or among clusters while also minimizing the distances within the clusters themselves. I used Euclidean distance, given by:

$$distance(a, b) = \sqrt{\sum n\ i=1\ (a_i - b_i)^2}$$

I plotted K-means clusters on our first four PC axes, which explain the highest proportion of variance, to investigate if different rivers had different transcriptomic profiles, as well as to determine if successful migrants had different transcriptomic profiles from the unsuccessful migrants.

Models

To investigate the effect transcriptomes had on migration success, I incorporated the first four PC axes that accounted for the most variance as explanatory variables in my models. I ran generalized linear models (GLMs) with whether or not an individual fish was detected at the Strait of Belle Isle as a binomial response variable for each stock individually, and one model for all of the sampled fish considered together, written as: Migration success ~ PC1 + PC2 + PC3 + PC4. I also ran GLMs on genes that loaded heavily (>0.5) on one or more of the first four PC axes, written as: Migration success ~ Gene A + Gene B + Other. Finally, I also looked for condition-dependent and environmental effects on migration success, running similar GLMs written as Migration success ~ Julian day (release date) + Fork length + River + Tag-to-mass-ratio (TMR, for those salmon where mass data was available.). While I also screened for three common pathogens of Atlantic salmon and incorporated them into different models, pathogen data never showed a statistical effect. However, a table showing information on fish that were infected with a given pathogen can be found in Table 3. Finally, I also included the chip number, which is an identifier used to keep track of which samples were put through qPCR in parallel, as a covariate in order to identify any group biases that occurred during analysis.

2.3 – Results

The biological characteristics of each population, along with survival data, can be found in Table 4 and Figure 3. A total of 38/262 (14.5%) of the tagged fish were detected at either the Strait of Belle Isle or Cabot Strait. A river by river breakdown can be found in Table 4. The Trinité river population had a low amount of successful migrants (3/50,

6.0%). The Western Arm Brook population had the highest proportion of successful migrants (14/50, 28.0%). A high proportion of fish from the Margaree population were detected completing their migration (16/62, 25.8%). The Miramichi stock fared poorly; just 1/50 (2.0%) and 4/50 (8.0%) fish from the Northwest and Southwest tributaries respectively were detected exiting the Gulf of St. Lawrence at the Strait of Belle Isle. Cumulatively, the Miramichi only had 5/100 (5%) successful migrants. While these success rates fall within the expected ranges proposed by Chaput *et al*. (2019), we did not apply the same modelled uncertainties and corrections, and thus the rates shown here may be underestimates, as I calculated the detection efficiency of the Strait of Belle Isle array to be approximately 66.7%. Smolts almost exclusively used the Strait of Belle Isle to exit the Gulf of St. Lawrence, 13 of the 16 fish that succeeded in their migration from the Margaree were first detected at the Cabot Strait line before later detection at the Strait of Belle Isle, suggesting that smolts from populations in this area stay close to shore for as long as possible before crossing the deeper waters of Cabot Strait. These smolts from the Margaree were the only fish detected at the Cabot Strait.

Of the 262 fish that were tagged and biopsied, 237 gill biopsies were successfully analyzed with qPCR. The raw gene expression data for each population, separated by migration success, can be seen in Figures 4-9. There were no significant differences in expression among populations, nor among successful versus unsuccessful migrants. PCA and K-Means clustering showed no differences among populations along any of the constructed PC axes (Figures 10-12). Genes which loaded heavily onto the constructed PC axis can be found on Table 2. Models with gene expression data were not significant when using any of the PC axes as coefficients, however, I did find significant

relationships when investigating singular genes that loaded heavily upon the axes. We found that two genes, “hepcidin” (IMMU3) and “heat shock protein 90” (GENST2) had a significant effect on migration success ($p = 0.0395$ & 0.0247, odds ratios = 1.35 & 0.708 respectively) when pooling all populations; where higher expression of IMMU3 increased the odds of a successful migration and higher expression of GENST2 decreased the odds of a successful migration. Broken down into population specific models, increased expression of GENST2 was negatively associated with migration success only in the Margaree population ($p = 0.049$, odds ratio = 0.528). IMMU3 was only significant when the populations were pooled and was not found to be significant in any single population. In Western Arm Brook, we additionally found a significant negative relationship between increased expression in the “C-type lectin domain family 4, member E” (IMORT2) gene and migration success ($p = 0.0429$, odds ratio = 0.739). I was unable to run a population-specific model for Northwest Miramichi as there was an insufficient sample size (n = 1 successful migrant). I did not detect a chip effect in any of the models.

Fork-length had a significant positive effect ($p = 0.0479$, odds ratio = 1.707) on migration success, but when this analysed further by population-specific models, fork length showed no significant effect on survival despite a consistent trend of larger smolts having higher odds of a successful migration (Figure 13).

2.4 – Discussion

In this study, I established transcriptomic profiles for five populations of wild Atlantic salmon smolts and then tracked them as they migrated from natal rivers to their marine feeding grounds, which depending on the population ranged from as little as 10 km to as much as 800km. Despite wide variation in life-history characteristic and age

structures, all of the salmon populations we examined exhibited very similar profiles of gene transcription at the time of sampling during down-river migration, and these profiles did not predict variation in survival or migration rate during seaward migration. Additionally, there was no common gene or gene profile across populations that predicted migration success. However, within populations the expression of specific genes could predict an individual's probability of successfully surviving the migration to the Strait of Belle Isle, and these genes varied from population to population (Table 5). My results suggest that transcriptomics can be used to diagnose the key physiological processes in wild populations of Atlantic salmon that occur in response to environmental stressors and contribute to annual migration-related mortality. These results give decision makers another tool with which to identify otherwise-invisible drivers of population decline.

Given that the Atlantic salmon populations that I considered are known to be genetically distinct from one another, especially those from Western Arm Brook (King *et al*., 2001; Spidle *et al*., 2003), and that smolts from each population experience very different sets of environmental conditions during riverine and marine migration (Chadwick, 1981; McCormick *et al*., 1998; Caron & Raymond, 2000; Strothotte *et al*., 2005; Breau *et al*., 2010), it is surprising that there were no distinct population-specific transcriptomic profiles. Recent work on sockeye salmon (*O. nerka*) has shown that smolts of different age classes exhibit different transcriptomic profiles and specifically differed in the expression of sampled immune response genes (Stevenson, 2018). Yet this trend does not appear to be the same for Atlantic salmon, as we studied five different populations with different age structures and found little difference among them. The

overall similarity among populations in their gene expression may indicate that the intense selective pressure experienced by Atlantic salmon favours rapid acclimatization to environmental stressors and, barring stochastic variation in such stressors, populations adapt to and modulate their gene expression to a similar baseline regardless of age or other population-specific characteristics. I suggest future studies investigate this local adaptation hypothesis through mesocosm experiments, comparing the transcriptomic profiles of *S. salar* smolts from different populations before and after manipulating some physical variable such as temperature or salinity.

While populations did not differ overall in their transcriptomic profiles, certain genes were more important in certain populations than others for predicting migration success. When the populations were considered collectively, "IMMU3" (Hepcidin), a gene that codes for hepcidin production, and "GENST2", a gene that codes for heat shock protein 90 (HSP90) production, were found to be significant in predicting whether an individual would succeed in its migration to the Strait of Belle Isle. Hepcidin is an important protein that regulates iron entry into the circulatory system, and is associated with inflammation (Ganz, 2003). Increased expression of IMMU3 was associated with higher odds of a successful migration. I interpreted this to mean that, because it is connected to inflammation, that higher expression of this gene is indicative of a healthy, functioning immune system that is combatting pathogens. HSP90 is a chaperone protein that assists in the stabilization of other proteins against thermal denaturing, along with assisting other proteins' appropriate folding (Pearl & Prodromou, 2001). Increased expression of GENST2 was associated with lower odds of a successful migration. I believe that fish with a high expression of this gene were under stress from temperature.

GENST2 was found to be a predictor of migration success specific to the Margaree River population, which suggests that this river may have been under stress from fluctuating river temperatures. In Western Arm Brook, high expression of the gene "C-type lectin domain family 4, member E" (CLCE4E, IMORT2) was found to be negatively associated with a successful migration. CLCE4E is a protein that recognizes patterns of cell death, precipitating immune system response, specifically antigen production (https://www.uniprot.org/uniprot/Q9ULY5), and has been shown to predict mortality in salmonids (Miller *et al*., 2011). High expression of this gene in Western Arm Brook, without seeing associated immune response genes also predicting migration success, may be indicative that smolts in this system are encountering pathogens that their bodies are inept to recognize.

Although it did not show significance in any models, the gene "hyperosmotic protein 21" (OSMST2) loaded heavily on the second PC axis in my PCA (Table 2), which accounted for 18.59% of the variance in the genetic dataset. This gene appeared to be highly expressed in fish from the Northwest Miramichi population (Figure 3). I was unable to run a model investigating this gene's effect due to an insufficient sample size as only a single fish from this population was detected to be a successful migrant (Table 4). It is likely though, that if I had a greater sample size this gene would have presented as a significant predictor of migration success for this population. SHOP21 is related to osmotic stress (Pan *et al*., 2002), perhaps indicating that the smolts of the Northwest Miramichi are having trouble adapting to the hyperosmotic conditions in the marine environment. However, this is somewhat confounded by my sampling occurring while these fish are still well above the head of tide, in a freshwater environment. Evans *et al*.

(2011) found that adult sockeye salmon upregulated osmotic genes in preparation for river entry, and I suggest that these smolts may be enacting a similar process, albeit in preparation for marine entry. Why this gene is so clearly upregulated compared to the other populations, and how it links to survivorship is unclear and merits further investigation. It may be possible that fish from this tributary are not exiting the river when they are supposed to, and remaining in freshwater for longer than they are physiologically prepared to. Similarly, given the body of evidence indicating that quickly acclimatizing to the osmotic conditions in the ocean increases the probability of an individual's survival (Halfyard *et al*. 2012, 2013; Philips *et al* 2021), I would expect to see upregulated osmotic stress genes in at least some of the mortalities. As fish were sampled prior to their saltwater entry, I was not able to investigate the transcriptomic response to osmotic stress. The absence of such an osmotic signature in all but one population, given that the smoltification process preadapts fish to the ocean, is interesting and warrants future studies – ideally where smolts can be recaptured and sampled more than once to elucidate further temporal effects on transcriptome-related osmotic preparedness.

Overall, the results from the telemetry data in this study reinforces the notion that at-sea mortality is high and of great consequence to the continued existence of these populations. Compounded with further mortality during the growth period, it is understandable why so few emigrants return to spawn successfully. Notably, I believe my data from the Trinité River is some of the first estimates of migration success for this system and the low proportion of successful ocean migrants is alarming. While survival estimates here do fall within those put forth by Chaput *et al*., 2019, I did not apply the

same rigorous corrections as those authors and may be over or underestimating survival. Here, I also provide more supporting evidence that most populations of Atlantic salmon from rivers draining into the Gulf of St. Lawrence almost exclusively migrate out of the Gulf via the Strait of Belle Isle as opposed to the Cabot Strait. This is evidenced by 13 fish from the Margaree river population that were detected first at Cabot Strait, and then again some days later at the Strait of Belle Isle, showing a clear preference to stay within the Gulf of St. Lawrence.

I suggest that studies seeking to identify mechanisms of marine mortality continue to include transcriptomics if possible because salmon rivers can differ radically in their environmental conditions. As the use of transcriptomics in conservation becomes more common and refined, newly identified genes can be included in future studies to investigate their predictive power on migration. Ideally, I would have identified an expression pattern that predicted migration success across all populations in this study. The lack of such a pattern in this study does not necessarily mean it does not exist; rather, the genes that make up such a pattern may simply not have been identified and their primers not yet available for analysis. If possible, sampling fish more than once would be ideal, perhaps after smolts encounter the head-of-tide. Because depredation is such an overwhelming force at this life-history stage, it also follows that evaluating how the transcriptome affects the probability of depredation should be investigated, possibly using predation tags. Future experimental studies establishing baseline responses to manipulated variables such as the work of Teffer *et al*. (2018), should investigate if real-world conditions are enough to elicit transcriptome level responses in Atlantic salmon and related species.

Overall, my results here suggest that the relationship between a warming northern hemisphere and pathogens will be of consequence to the continued existence of Atlantic salmon populations. The genes I found to be predictive of migration success related to immune system function or heat stress, and my most explanatory PC axis was comprised largely of viral disease development genes. Given what we already know about temperature stress leaving fish physiologically vulnerable to infection (Patterson *et al*., 2017; Chapman *et al.*, 2019) and the hypothesized spread of pathogens as temperate latitudes warm and become more favourable to their spread (Altizer *et al*., 2013; Semenza & Suk, 2018; Vargas-Chacoff *et al*., 2018), it is reasonable to conclude that without adaptation, already-imperiled populations will only face greater challenges.

Table 1: Table with information pertaining to the targeted genes. VDD = Viral Disease Development, MRS = Mortality Related Signature, MGL = Molecular Genetics Lab, Nanaimo, B.C.

Assay name	Gene name	Panel	F primer	R primer	Probe	Citation
CCL4_v1	CC chemokine 4	AA-smoltification	TCTCTTCATTGCAACAATCTGCTT	ACAGCAGTCCACGGGTACCT	CTACGCAGCAGCATT	Houde, et. Al. 2020
IL12B_v1	Interleukin-12 beta	AA-smoltification	GGAGCCTCCCATGCTCTTACT	TGGCGTGGACCACTTTGAC	CCCCTCACATTCCA	Houde, et. Al. 2020.
CA4_v1	Carbonic anhydrase 4	AA-smoltification	GGTCATTTTGGTTTTGTACACAGTCT	CCTAGATATAGCTATCCACGTACTCACCTA	TGATACGTGGTATAGAAAAG	Houde, et. Al. 2020.
NKAa1-b	Na/K ATPase α-1b (saltwater)	AA-smoltification	TGAAGAAGTGGTGGTTGGAGATC	GGCAGAGACAATACGCAAATCA	TGAAAGGAGGAGATAGAAT	Houde, et. Al. 2020.
ALD1_chr3	Fructose-bisphosphate aldolase A1	AA-thermal stress	CGTGATTCAGTGTTGTCATCTTGA	TTCCTCCAGTGTTTTTTTCAGTCA	AAGTACATGTGCCTTCTT	MGL
HSC70	Heat shock cognate 70kDa protein	General Stress	GGGTCACACAGAAGCCAAAAG	GCGCTCTATAGCGTTGATTGGT	AGACCAAGCCTAAACTA	MGL
78d	S100 calcium binding protein	Housekeeping	GTCAAGACTGGAGGCTCAGAG	GATCAAGCCCCAGAAGTGTTTG	AAGGTGATTCCCTCGCCGTCCGA	MGL
BSG_1 (not Tuba1C)	Basigin	Imminent mortality	CGTGGCCGAGGTCATCAT	TCAGGCTTTCTCCTCTTCTCGTA	TGGTCAGCATCATCTT	MGL
CLEC4E_2	C-type lectin domain family 4, member E	Imminent mortality	CCTGAGGGCTGGATTCATGT	TCGGCCAGTCCATCTTGTC	TGAGAAATGTTACTCCTTCAGT	MGL
GLUL_1	Glutamate-ammonia ligase (glutamine synthetase)	Imminent mortality	GTTCCAGGTTGGCCCTTGT	CCTAGCTGCCCAAAGGTGATC	AAGGCATCAGCATGGG	MGL

Assay name	Gene name	Panel	F primer	R primer	Probe	Citation
H1F0_1	H1 histone family, member 0	Imminent mortality	CCAATGGAC GTCAGCAAG ATT	AGCATAGAGT CCGCATTTGGA	TCATGTGATG CGTAATGG	MGL
IQGAP1_2	IQ motif containing GTPase activating protein 1	Imminent mortality	GAGGGTGTG GCTGTGATG AA	CAGGAAGATG AGCAGGTTGA CA	CTCTTCGACA GGGCC	MGL
ODC1_2	Ornithine decarboxylase 1	Imminent mortality	CCAGAAGGC TCCCTGTTTC A	GCAGCCATTTC CTGGAGAAG	ACAACCCAAT CTCA	MGL
TAGLN3_2	Transgelin 3	Imminent mortality	TGGCTCAAG GACGGATGT G	GGATCTTCCTG ATGGGCTTGT	TGTGTGAACT GATCAACAG	MGL
HEP	hepcidin	Immune Stimulation	GAGGAGGTT GGAAGCATT GA	TGACGCTTGA ACCTGAAATG	AGTCCAGTTG GGGAACATC AACAG	Raida et al. 2009
SAA	Serum amyloid protein a (SAA)	Immune Stimulation	GGGAGATGA TTCAGGGTTC CA	TTACGTCCCCA GTGGTTAGC	TCGAGGACAC GAGGACTCA GCA	Raida et al. 2009
IL15	IL-15	Immune Stimulation	TTGGATTTTG CCCTAACTGC	CTGCGCTCCAA TAAACGAAT	CGAACAACGC TGATGACAG GTTTTT	Raida et al. 2009
B2M	B2M	Immunity	TTTACAGCGC GGTGGAGTC	TGCCAGGGTT ACGGCTGTAC	AAAGAATCTC CCCCCAAGGT GCAGG	Hauglan d et al. 2005.
C5aR	C5a receptor	Immunity	ACGCACCTT GAGGGTCAT T	CAGTGGAAAC CAGCACAGG	TTGCCGTGTC GCTGAGCTTC TT	Raida et al. 2009
ILIR	IL-1 receptor I	Immunity	ATCATCCTGT CAGCCCAGA G	TCTGGTGCAG TGGTAACTGG	TGCATCCCCT CTACACCCCA AA	Raida et al. 2009
MMP13	Matrix metalloproteinase-13	Inflammation	GCCAGCGGA GCAGGAA	AGTCACCTGG AGGCCAAAGA	TCAGCGAGAT GCAAAG	Tadiso, et al. 2011

Assay name	Gene name	Panel	F primer	R primer	Probe	Citation
EPD_2	ependymin	Inflammation	ACAAGACATTCGGCCTGGAT	CGGTTCTTGTGGTTAATCGTATACA	CCCTTCTGCTCTTCA	MGL
ES1_1	ES1 protein homolog	Inflammation	CGGCAACTTCCATGAAGGA	GGACCTCCCCCACTTTCTTATT	TGGGCTGTAAACACG	MGL
GILT_1	gamma-interferon-inducible lysosomal thiol reductase (GILT)	Inflammation	CTGGTGCCCTATGGAAATGC	CCGTGCTGGCAGGTGAAC	ATCTTTTGATGGGAAGAAG	MGL
tgfb_2	transforming growth factor β	Inflammation	TGAGCTCCGTCTCCTCATCA	GCGATTGGCCCATTCCTT	AGAGGCTGGAACTCTACAG	MGL
IFNa	Interferon alpha	IPN Response	CGTCATCTGCAAAGATTGGA	GGGCGTAGCTTCTGAAATGA	TGCAGCACAGATGTACTGATCATCCA	Ingersle v et al. 2009
IL1B	Interleukin 1B	IPN Response	AGGACAAGGACCTGCTCAACT	CCGACTCCAACTCCAACACTA	TTGCTGGAGAGTGCTGTGGAAGAA	Ingersle v et al. 2009
FYB	FYN-T-binding protein	MRS	TGCAGATGAGCTTGTTGTCTACAG	GCAGTAAAGATCTGCCGTTGAGA	CTCAACGATGACATCCACAGTCTCCCC	Miller et al. 2011
HTA	HIV-1 Tat interactive protein	MRS	CTTGTAACAGTTCGACATGGCTTATT	TGGTGAAGCATTTCTGTATGTCAA	TCTGTACTGAGCATCCCCGCACATTACA	Miller et al. 2011
HSP90	Heat shock protein 90	MRS	TGGGCTACATGGCTGCCAAG	TCCAAGGTGAACCCAGAGGAC	AGCACCTGGAGATCAA	Miller et al. 2011
Coil-P84	Coiled-coil domain-containing protein 84	MRS	GCTCATTTGAGGAGAAGGAGGATG	CTGGCGATGCTGTTCCTGAG	TTATCAAGCAGCAAGCC	Miller et al. 2011
MrpL40	39S ribosomal protein L40, mitochondrial precursor	MRS	CCCAGTATGAGGCACCTGAAGG	GTTAATGCTGCCACCCTCTCAC	ACAACAACATCACCA	Miller et al. 2011

Assay name	Gene name	Panel	F primer	R primer	Probe	Citation
ATP5G3-C	ATP synthase lipid-binding protein, mitochondrial precursor	MRS	GGAACGCCACCATGAGACA	CGCCATCCTGGGCTTTG	AGCCCCATTGCCTC	Miller et al. 2011
C7	Complement component C7 precursor	MRS	GATGCTGACCACATCAAACTGC	ACCTCTGTCCAGCTCTGTGTC	AACTACCAGACAGTGCTG	Miller et al. 2011
IRF1	Oncorhynchus mykiss interferon regulatory factor 1 (IRF-1) gene, promoter region and partial sequence	MRS	CAAACCGCAAGAGTTCCTCATT	AGTTTGGTTGTGTTTTTGCATGTAG	CTGGCGCAGCAGATA	Miller et al. 2011
JUNB	Transcription factor AP-1	MRS	TTGTTGCTGGTGAGAAAACTCAGT	CCTGTTGCCCTATGAATTGTCTAGT	AGACTTGGGCTATTTAC	Miller et al. 2011
MMP25	Matrix metalloproteinase-25 precursor	MRS	TGCAGTCTTTTCCCCTTGGAT	TCCACATGTACCCACACCTACAC	AGGATTGGCTGGAAGGT	Miller et al. 2011
FKBP5_v1	FK506-binding protein 5	Osmotic stress	GGGCGTTCCTCTGGGTGTA	GCATGCAGCATTCTCCTTTCT	ACAGGGCCATGGAGA	Houde, et. Al. 2020
RGS21	Regulation of G protein signalling 21	Osmotic stress	TCCCGACTACAGCGCAGAT	TCCTCAGGGCTAAGTCGTTCA	TTCCCAATCCCCC	Houde, et. Al. 2020
SHOP21	Hyperosmotic protein 21 (Shop21)	Osmotic stress	GCGGTAGTGGAGTCAGTTGGA	GCTGCTGACGTCTCACATCAC	CCTGTTGATGCTCAAGG	Houde, et. Al. 2020
RIG1_MGLSYBR_1	RIG1 NM_001163699	Post Vaccine Immune Stimulation	ACAGCTGTTACACAGACGACATCA	TTTAGGGTGAGGTTCTGTCCGA	TCGTGTTGGACCCCACTCTGTTCTCTC	Larsen et al. 2012
CCL19_v1	C-C motif chemokine 19	Smoltification	ACCTGGGTTACAGACCTGATGAA	TGGTTTCGTGGCATTTCTTG	CTCATGGACCGCCTCA	Houde et Al. 2020
NKAA1C	NKA α-1c	Smoltification	AGGGAGACGTACTACTAGAAAGCAT	CAGAACTTAAAATTCCGAGCAGCAA	ACAACCATGCAAGAACT	Stefansson et al. 2007

Assay name	Gene name	Panel	F primer	R primer	Probe	Citation
PXMP2[UBL 1]	Ubiqitin-like protein-1, Peroxisomal membrane protein 2	VDD	GGCCTGCAT TCAGGATCT AA	TACAGTCTCAC CAGGCACCA	AGTGATGGT GCTGATTACG GAGCC	Miller et al. 2017
CA054694_ MGL_1	45217 pfam05316, VAR1, Mitochondrial ribosomal protein (VAR1).	VDD	CCACCTGAG GTACTGAAG ATAAGACA	TTAAGTCCTCC TTCCTCATCTG GTA	TCTACCAGGC CTTAAAG	Miller et al. 2017
HERC6_1	Probable E3 ubiquitin-protein ligase HERC6	VDD	AGGGACAAC TTGGTAGAC AGAAGAA	TGACGCACAC ACAGCTACAG AGT	CAGTGGTCTC TGTGGCT	Miller et al. 2017
IFIT5_MGL _2	Interferon-induced protein with tetratricopeptide repeats 5	VDD	CCGTCAATG AGTCCCTACA CATT	CACAGGCCAA TTTGGTGATG	CTGTCTCCAA ACTCCCA	Miller et al. 2017
GAL3_MGL _2	Galectin-3-binding protein precursor	VDD	TTGTAGCGC CTGTTGTAAT CATATC	TACACTGCTGA GGCCATGGA	CTTGGCGTGG TGGC	Miller et al. 2017
MX_ONTS	Mx	VDD	AGATGATGC TGCACCTCAA GTC	CTGCAGCTGG GAAGCAAAC	ATTCCCATGG TGATCCGCTA CCTGG	Eder et al. 2008
IFI44A_MG L_2	IFN-induced protein 44-1	VDD	CGGAGTCCA GAGCAGCCT ACT	TCCAGTGGTCT CCCCATCTC	CGCTGGTCCT GTGTGA	Miller et al. 2017
VHSVIP4_ MGL_3	VHSV-inducible protein-4	VDD	GCTCTCGTAA AGCCCCACA TC	GGGCGACTGC TCTCTGATCT	AAACTGCACG TCGCGC	Miller et al. 2017
RSAD_MGB 2	Radical S-adenosyl methionine domaine-containing 2	VDD	GGGAAATTA GTCCAATACT GCAAAC	GCCATTGCTGA CAATACTGACA CT	CGACCTCCAG CTCC	Miller et al. 2017

Table 2: Table depicting significant loadings on the first four PC axes. Yellow highlights are loadings greater than |0.2| and green highlights are loadings greater than |0.5|.

Assay Name	Gene Name	Gene Abb.	PC1	PC2	PC3	PC4
CCL4_v1	CC chemokine 4	"SMT3"	**-0.3168**	0.08929	0.05481	-0.08087
C5aR	C5a receptor	"IMMU2"	-0.0386	-0.1793	**0.26845**	-0.02668
HEP	hepcidin	"IMMU3"	-0.1125	**0.23541**	**0.54139**	0.10765
SAA	Serum amyloid protein a (SAA)	"IMMU10"	-0.1217	-0.0122	**0.21239**	-0.09170
SHOP21	Hyperosmotic protein 21 (Shop21)	"OSMST2"	-0.0471	**-0.67627**	**0.26899**	**0.40384**
CLEC4E_2	C-type lectin domain family 4, member E	"IMORT2"	0.03492	-0.03069	**0.50011**	**-0.43027**
ODC1_2	Ornithine decarboxylase 1	"IMORT6"	-0.0333	0.18138	-0.00693	**0.27125**
CA054694_MGL_1	45217 pfam05316, VAR1, Mitochondrial ribosomal protein (VAR1)	"VDD1"	**-0.3361**	-0.04046	-0.07077	-0.12444
HERC6_1	Probable E3 ubiquitin-protein ligase HERC6	"VDD3"	**-0.3264**	0.08606	-0.07055	-0.03790
IFI44A_MGL_2	IFN-induced protein 44-1	"VDD4"	**-0.2843**	**-0.32507**	-0.09204	**0.30301**
IFIT5_MGL_2	Interferon-induced protein with tetratricopeptide repeats 5	"VDD5"	**-0.2714**	0.02169	-0.07129	0.00041
PXMP2[UBL1]	Ubiqitin-like protein-1, Peroxisomal membrane protein 2	"VDD7"	**-0.4701**	0.05097	-0.11698	-0.17240
RSAD_MGB2	Radical S-adenosyl methionine domaine-containing 2	"VDD8"	**-0.2581**	0.02331	-0.07274	0.03283
VHSVIP4_MGL_3	VHSV-inducible protein-4	"VDD9"	**-0.2241**	-0.07153	0.09383	-0.0123
HSP90	Heat shock protein 90	"GENST2"	-0.0126	**0.4193**	0.18483	**0.40431**

Table 3: Proportion of pathogen infected smolts from each river.

River	*Candidatus Branchiomonas cysticola*	*Piscichlamydia salmonis*	*Flavobacterium psychrophilum*
Margaree	**5/60**	**18/60**	**4/60**
Trinité	**3/40**	**5/40**	**0/40**
Western Arm Brook	**4/42**	**6/42**	**6/42**
Miramichi Northwest	**4/47**	**2/47**	**6/47**
Miramichi Southwest	**1/48**	**2/48**	**3/48**

Table 4: Biological data and total successful migrants by section of the different populations in this study.

Population	Sample Size	Mean Length (cm) (SD)	Mean Mass (g) (SD)	Mean Tag to Mass Ratio	N Detected Leaving River	N Completed Migration
Margaree	62	15.35 (0.795)	33.75 (5.89)	0.0607	31	16
Trinité	50	14.76 (0.723)	29.48 (4.68)	0.0694	42	3
Miramichi NW	50	14.01 (0.880)	-	-	7	1
Miramichi SW	50	14.02 (0.653)	-	-	11	4
WAB	50	17.39 (1.45)	44.49 (12.54)	0.0478	21	14
All	262	15.12 (1.558)	35.91 (7.71)	0.0593	112	38

Table 5: Summary of significant generalized linear models on migration success. Note that a model could not be run on Miramichi Northwest due to the lack of a sufficient sample size (n=1).

Population Data	Model	Coefficients where Pr(0.05 > |z|)	Slope	Odds Ratio
All	Migration Success ~ PC1 + PC2 + PC3 + PC4 + Fork Length + Date	Fork Length – **$4.33 * 10^{-5}$**	0.621	1.86
All	Migration Success ~ IMMU3 + GENST2 + OSMT2 + IMORT2 + VDD7 + Fork Length	IMMU3 – **0.0395** GENST2 – **0.0247** Fork Length – **$1.86 * 10^{-4}$**	IMMU3: 0.303 GENST2: -0.344 Fork Length: 0.534	IMMU3: 1.35 GENST2: 0.708 Fork Length: 1.707
Margaree	Migration Success ~ IMMU3 + GENST2 + OSMT2 + IMORT2 + VDD7	GENST2 – **0.0493**	-0.637	0.528
Western Arm Brook	Migration Success ~ IMMU3 + GENST2 + OSMT2 + IMORT2 + VDD7	IMORT2 – **0.0429**	-1.56	0.739
Trinité	Migration Success ~ IMMU3 + GENST2 + OSMT2 + IMORT2 + VDD7	-	-	-
Miramichi Southwest	Migration Success ~ IMMU3 + GENST2 + OSMT2 + IMORT2 + VDD7	-	-	-

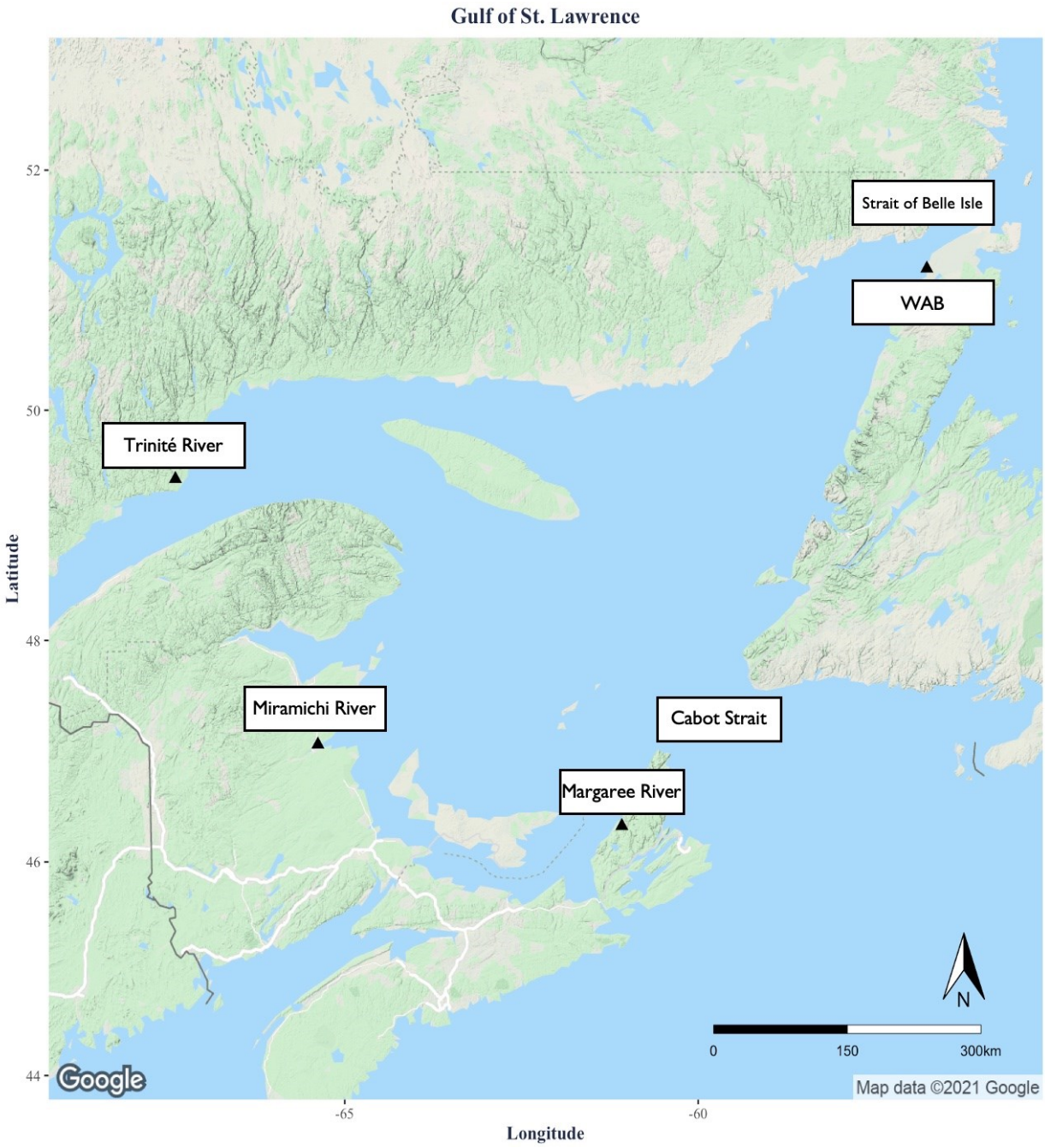

Figure 1: Map depicting the study area and tagging locations (Triangles) within the Gulf of St. Lawrence.

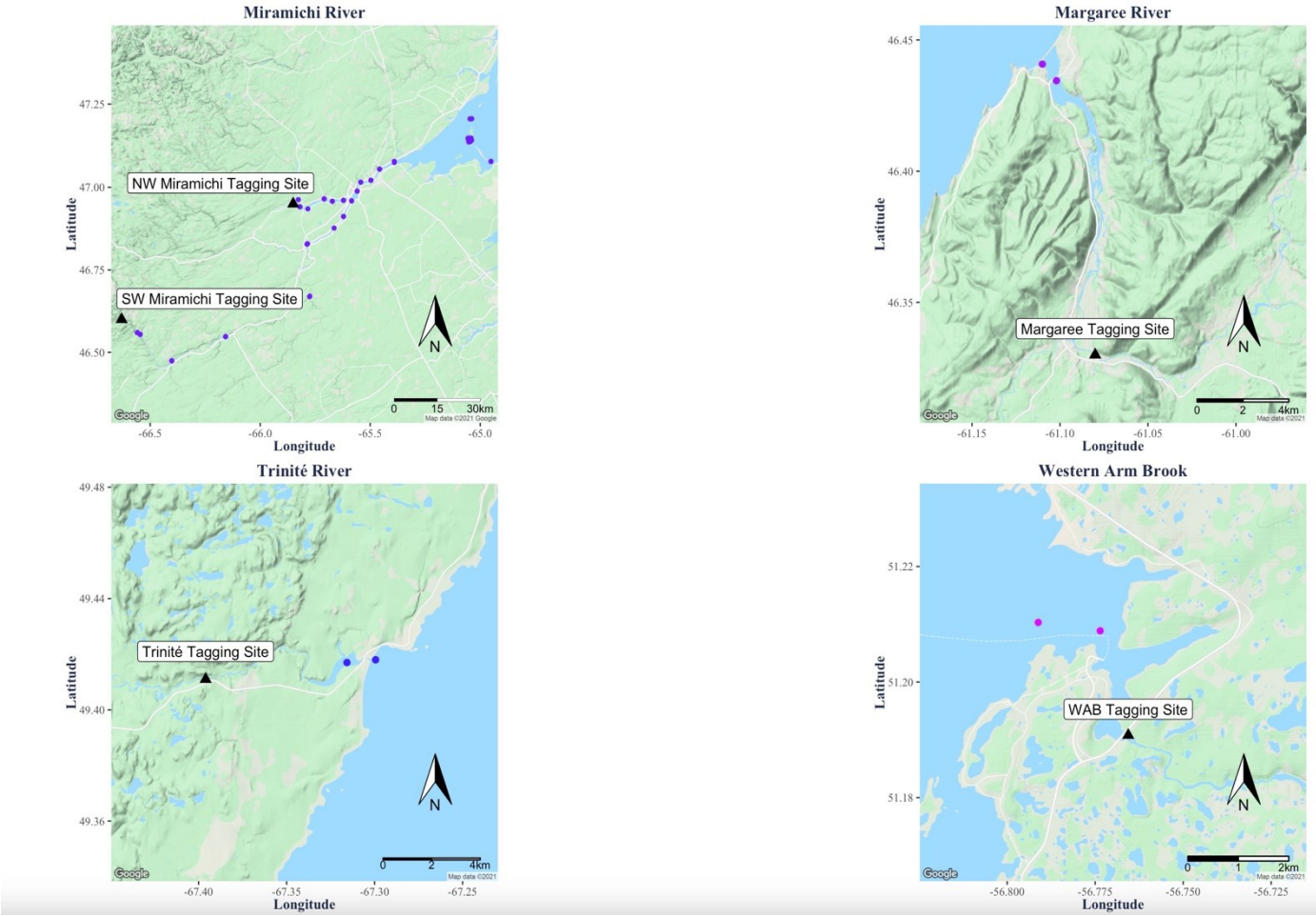

Figure 2: Map depicting the telemetry infrastructure and tagging locations at each river in the present study.

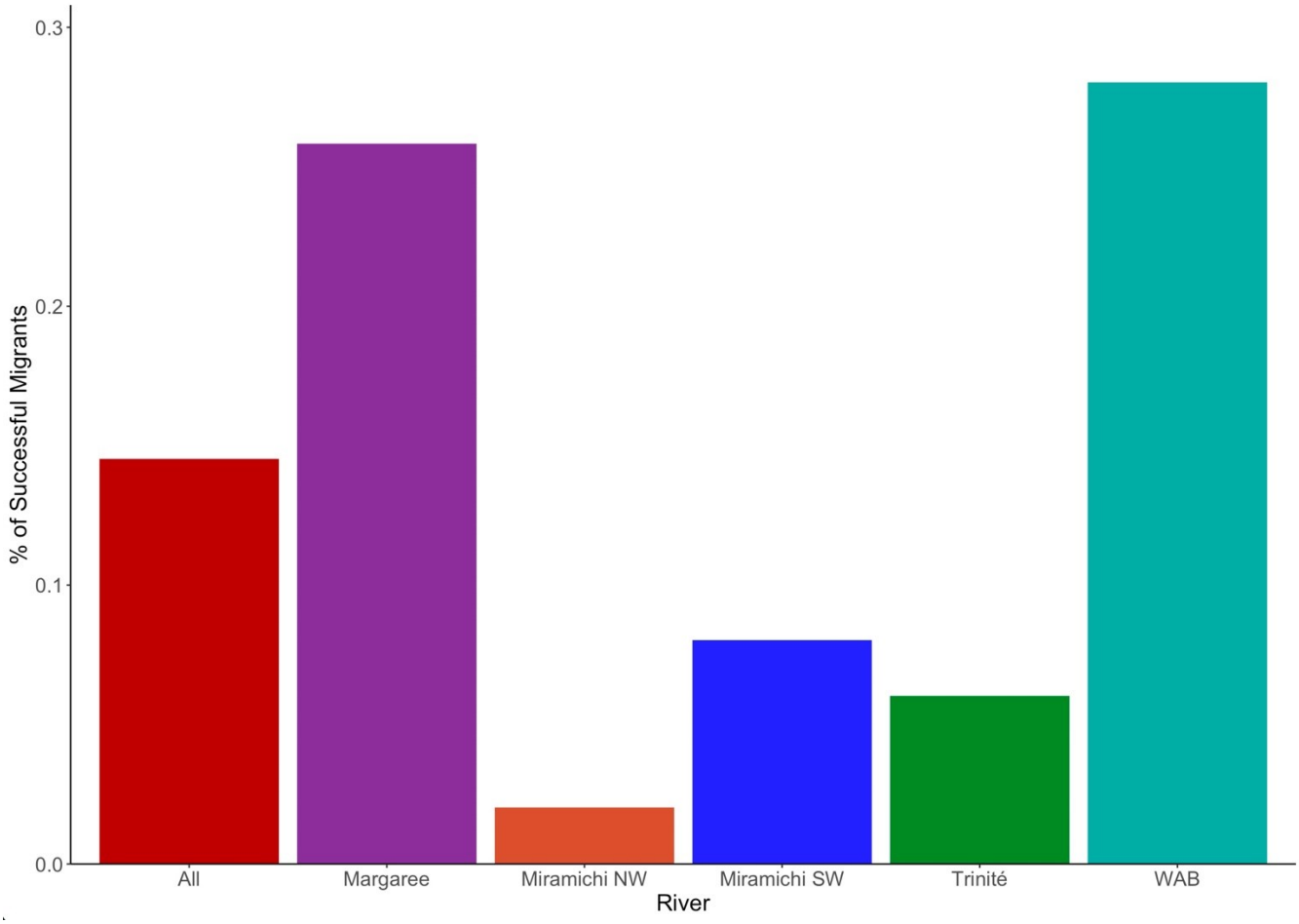

Figure 3: Barplot depicting the percentage of fish that successfully migrated from each river.

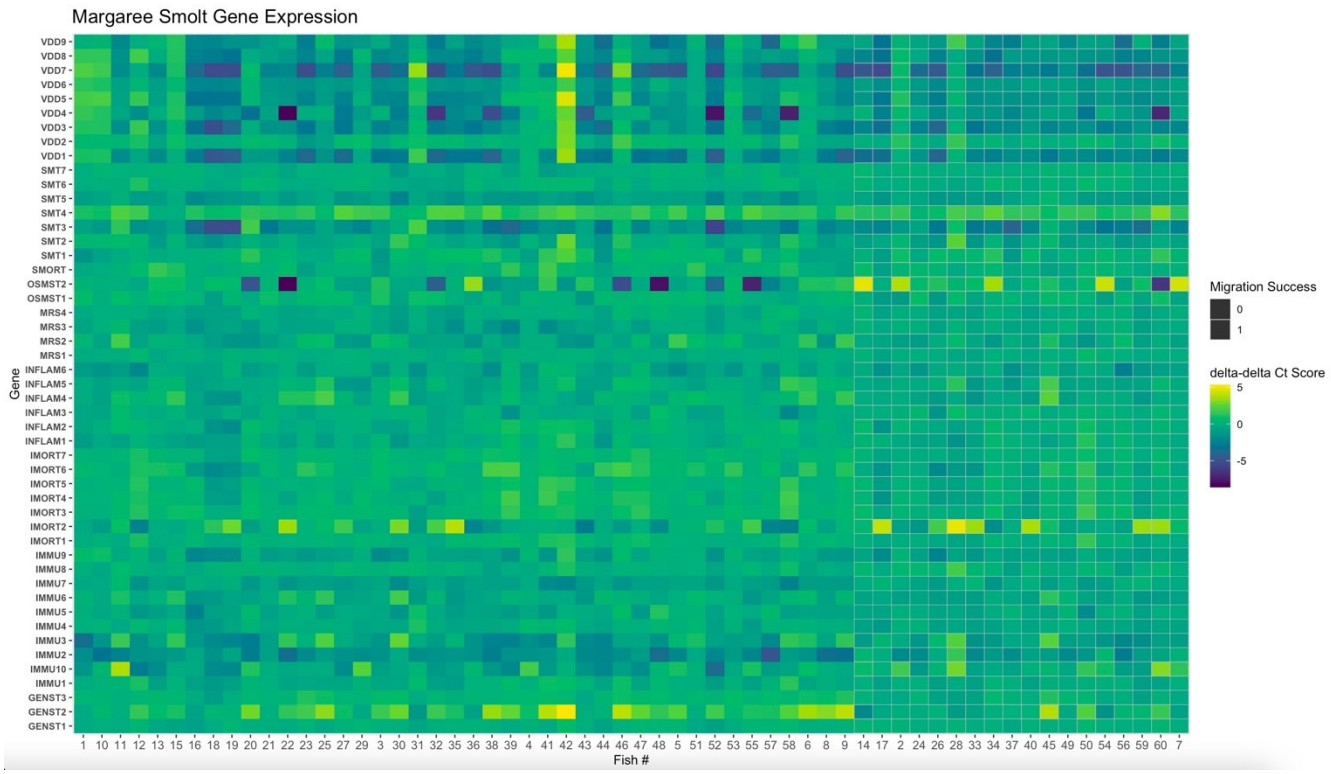

Figure 4: Delta-delta normalized Ct values depicting the relative gene expression of smolts from the Margaree population. Grey outlined tiles, present on the right hand side of each plot, belong to fish that successfully completed their migration. GENST = General Stress, IMMU = Immune Response, IMORT = Imminent Mortality, INFLAM = Inflammation, MRS = Mortality Related Signature, OSMT = Osmotic Stress, SMORT = Stress Mortality, SMT = Smoltification, VDD = Viral Disease Development.

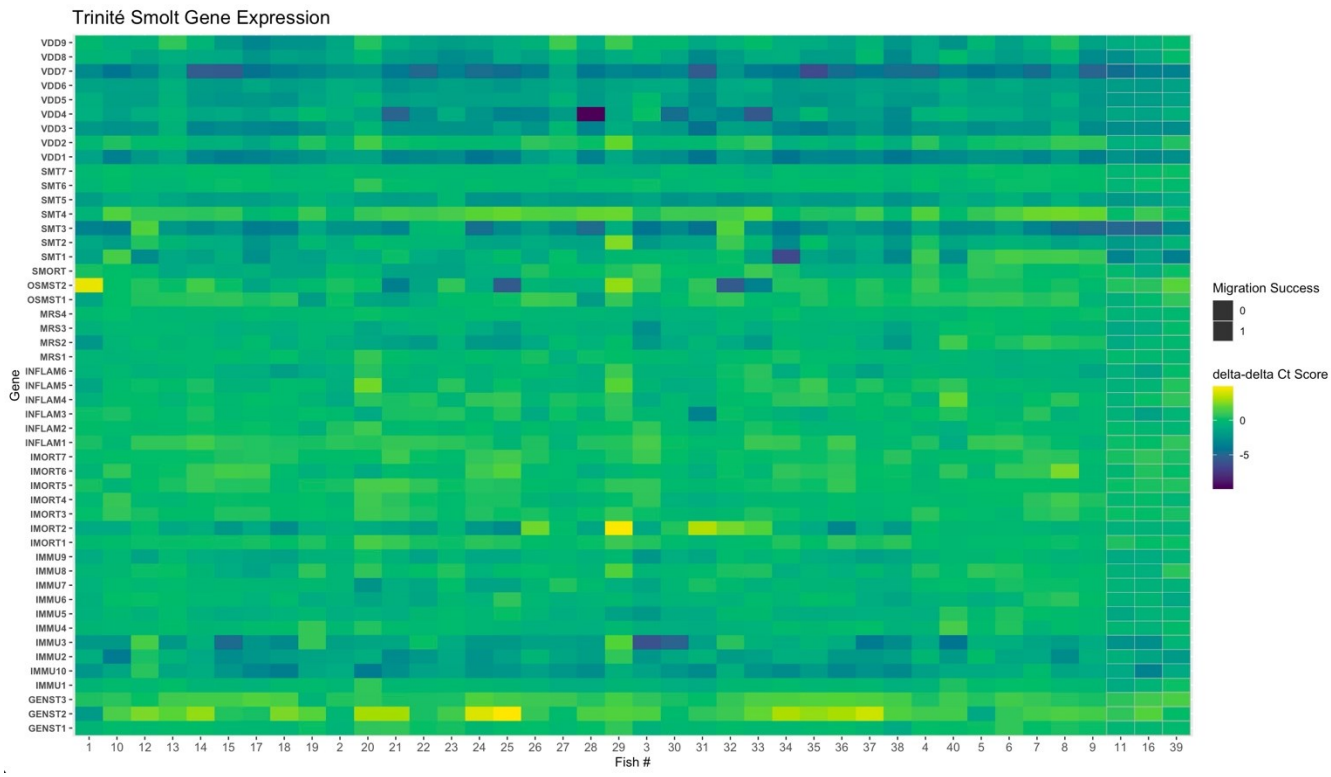

Figure 5: Delta-delta normalized Ct values depicting the relative gene expression of smolts from the Trinité population. Grey outlined tiles, present on the right hand side of each plot, belong to fish that successfully completed their migration. MRG = Margaree, NWM = Northwest Miramichi, SWM = Southwest Miramichi, TRN = Trinité, WAB = Western Arm Brook, GENST = General Stress, IMMU = Immune Response, IMORT = Imminent Mortality, INFLAM = Inflammation, MRS = Mortality Related Signature, OSMT = Osmotic Stress, SMORT = Stress Mortality, SMT = Smoltification, VDD = Viral Disease Development.

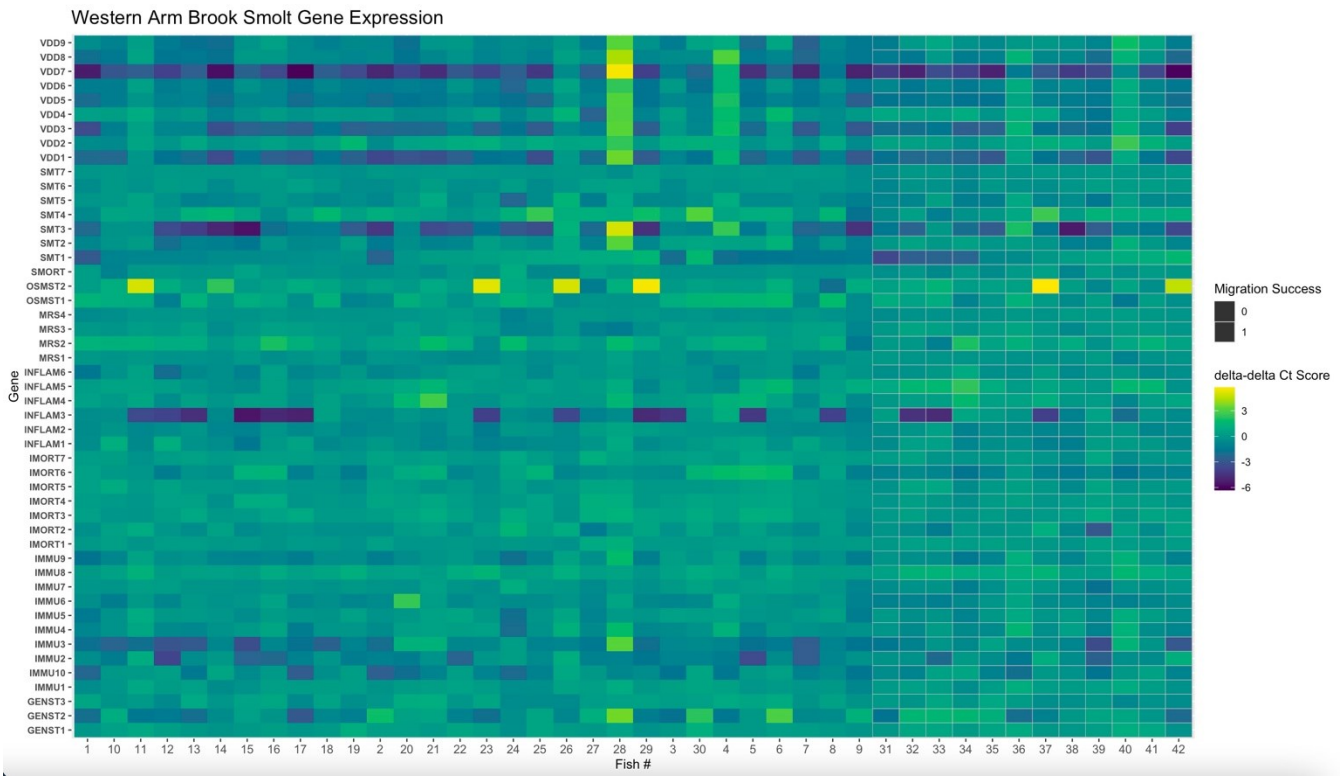

Figure 6: Delta-delta normalized Ct values depicting the relative gene expression of the smolts from the Western Arm Brook population. Grey outlined tiles, present on the right hand side of each plot, belong to fish that successfully completed their migration. MRG = Margaree, NWM = Northwest Miramichi, SWM = Southwest Miramichi, TRN = Trinité, WAB = Western Arm Brook, GENST = General Stress, IMMU = Immune Response, IMORT = Imminent Mortality, INFLAM = Inflammation, MRS = Mortality Related Signature, OSMT = Osmotic Stress, SMORT = Stress Mortality, SMT = Smoltification, VDD = Viral Disease Development.

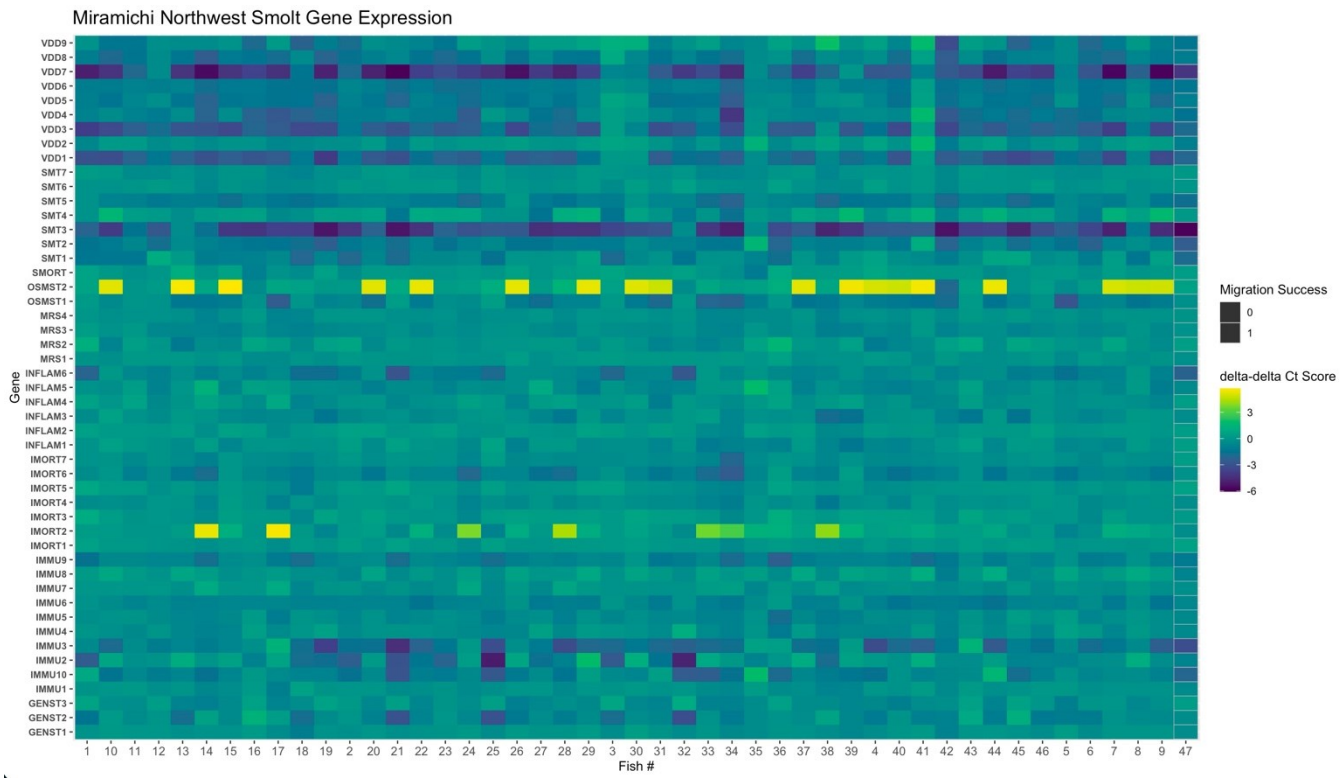

Figure 7: Delta-delta normalized Ct values depicting the relative gene expression of the smolts from the Miramichi Northwest population. Grey outlined tiles, present on the right hand side of each plot, belong to fish that successfully completed their migration. MRG = Margaree, NWM = Northwest Miramichi, SWM = Southwest Miramichi, TRN = Trinité, WAB = Western Arm Brook, GENST = General Stress, IMMU = Immune Response, IMORT = Imminent Mortality, INFLAM = Inflammation, MRS = Mortality Related Signature, OSMT = Osmotic Stress, SMORT = Stress Mortality, SMT = Smoltification, VDD = Viral Disease Development.

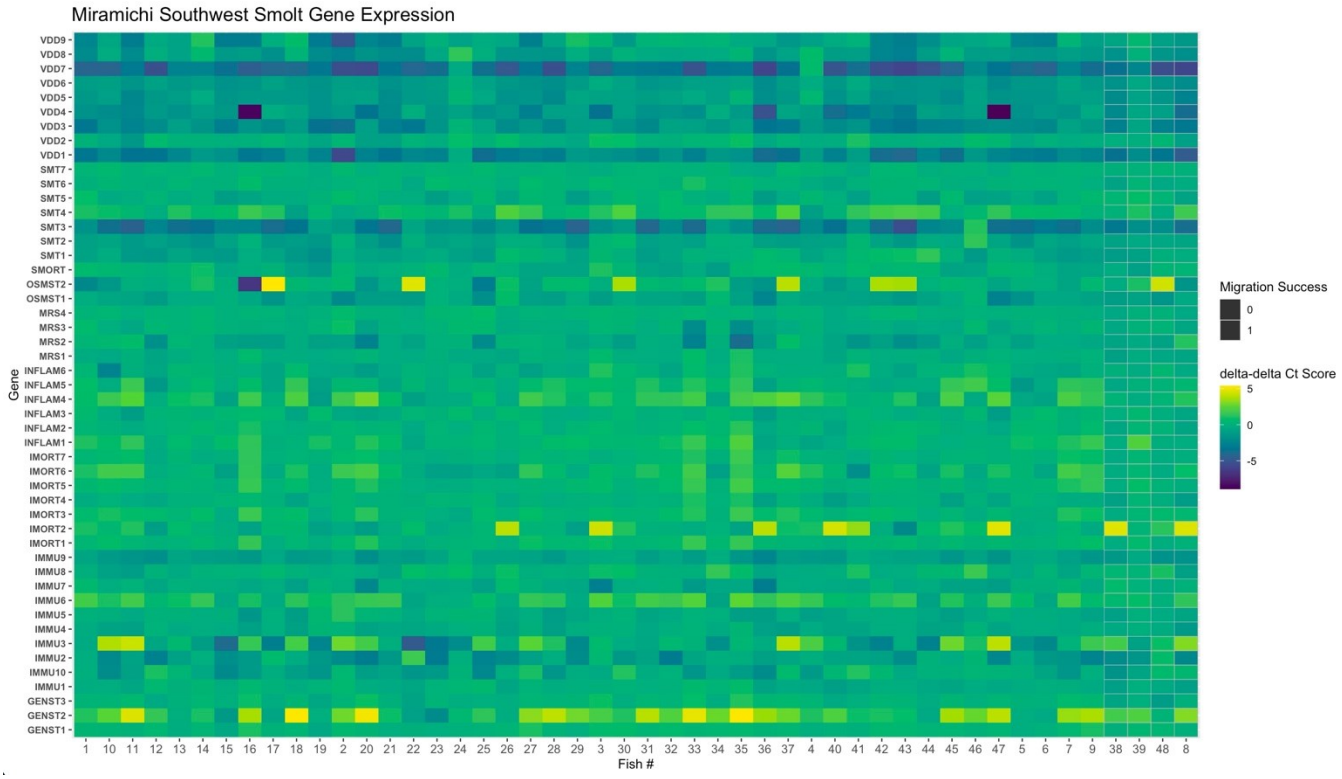

Figure 8: Delta-delta normalized Ct values depicting the relative gene expression of the smolts from the Miramichi Southwest population. Grey outlined tiles, present on the right hand side of each plot, belong to fish that successfully completed their migration. MRG = Margaree, NWM = Northwest Miramichi, SWM = Southwest Miramichi, TRN = Trinité, WAB = Western Arm Brook, GENST = General Stress, IMMU = Immune Response, IMORT = Imminent Mortality, INFLAM = Inflammation, MRS = Mortality Related Signature, OSMT = Osmotic Stress, SMORT = Stress Mortality, SMT = Smoltification, VDD = Viral Disease Development.

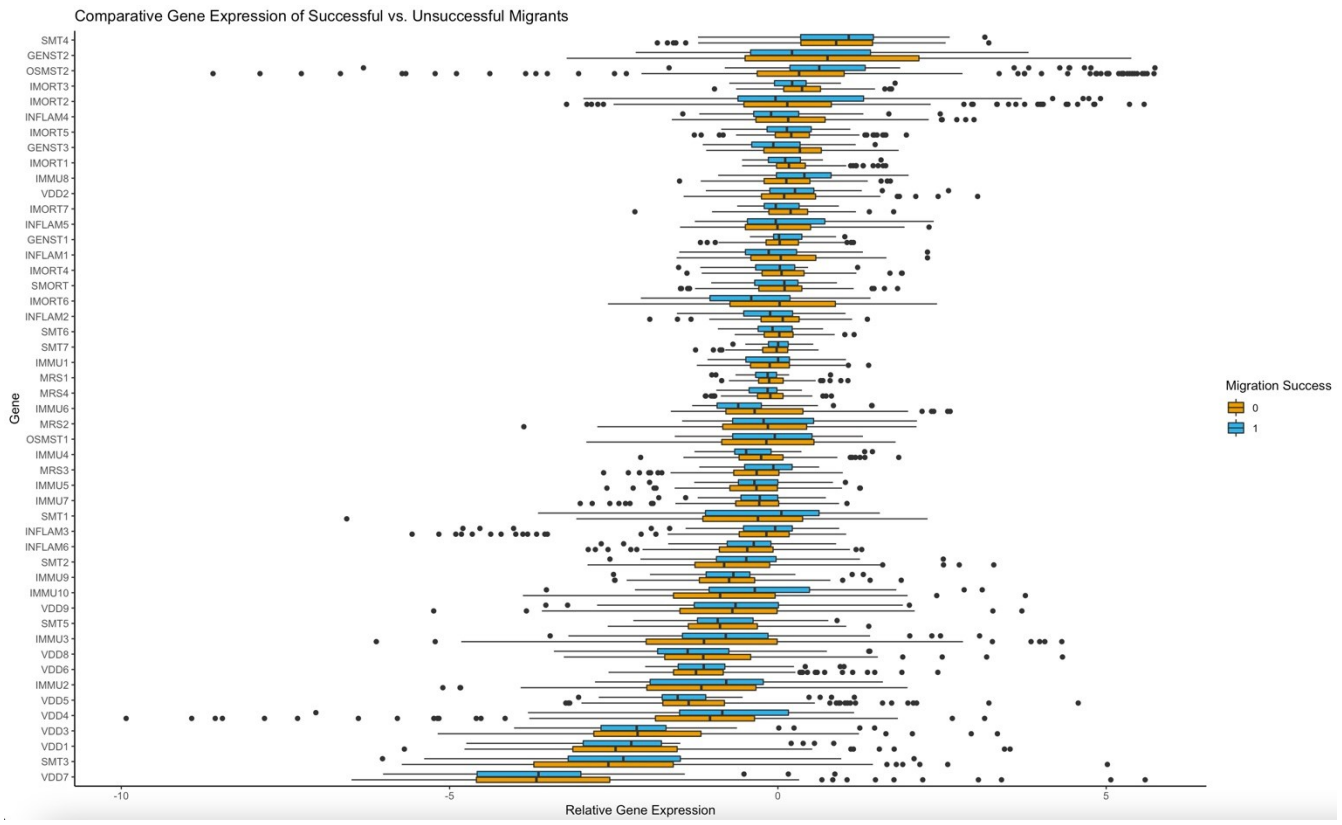

Figure 9: Comparative relative gene expression using delta-delta normalized Ct values between all successful (blue) and failed (orange) migrants in the dataset.

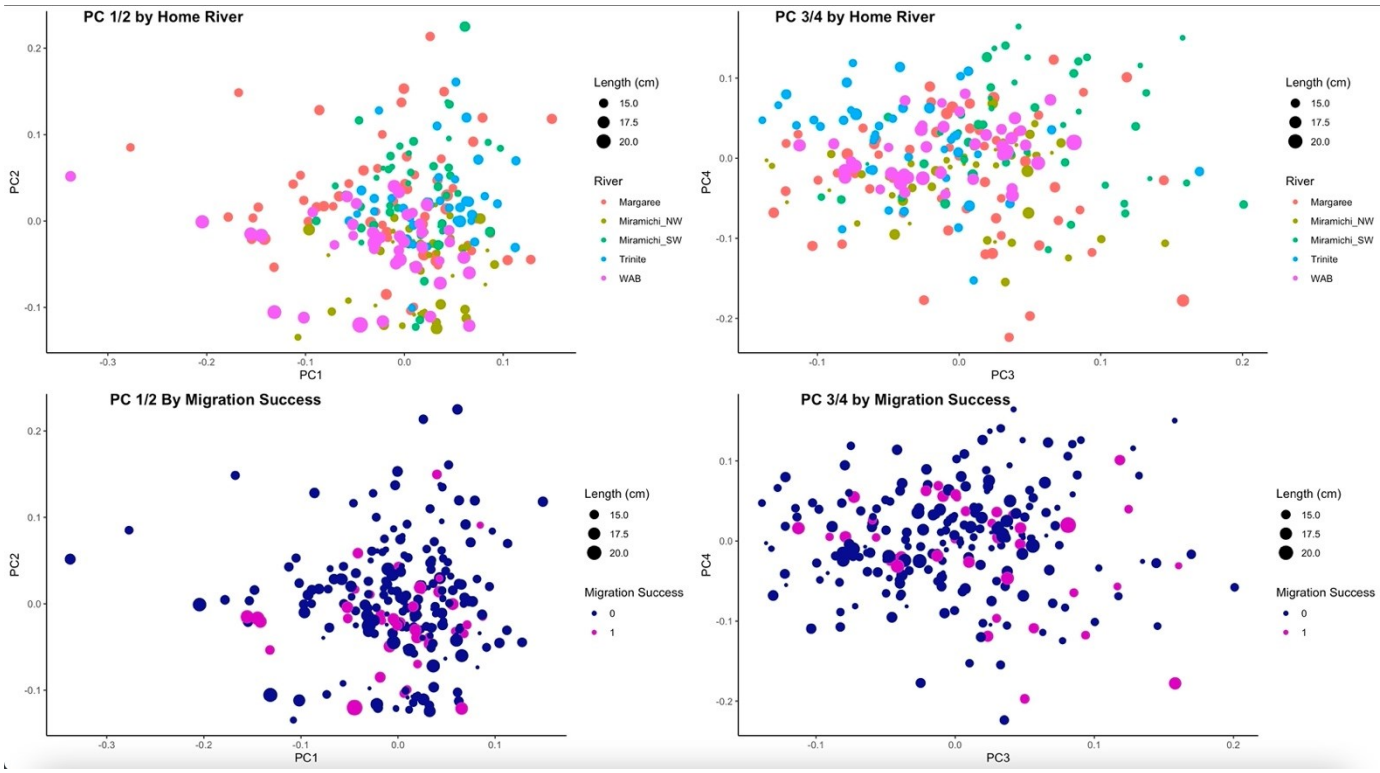

Figure 10: Plot of the first four PCA axes created from the transcriptome data. The left column contains plots of the first two axes, and the right column contains plots of the third and fourth axes. The graphs in each row only differ in how they are coloured; the top graphs are coloured by stock to show the different profiles, and the bottom graphs are coloured by successful versus failed migrations. Point size corresponds to fish fork length in centimeters.

Figure 11: The results of K-means clustering with the first two axes constructed through PCA. The centre of each ellipse represents the cluster which best represents that population with one standard deviation of values. Note that Northwestern Miramichi and Trinité each only have one ellipse because there were too few points to properly calculate two. Also note that each ellipse overlaps with the rest.

Figure 12: The results of K-means clustering with the third and fourth axes constructed through PCA. The centre of each ellipse represents the cluster which best represents that population with one standard deviation of values. Note that Northwestern Miramichi and Trinité each only have one ellipse because there were too few points to properly calculate two. Also note that each ellipse overlaps with the rest.

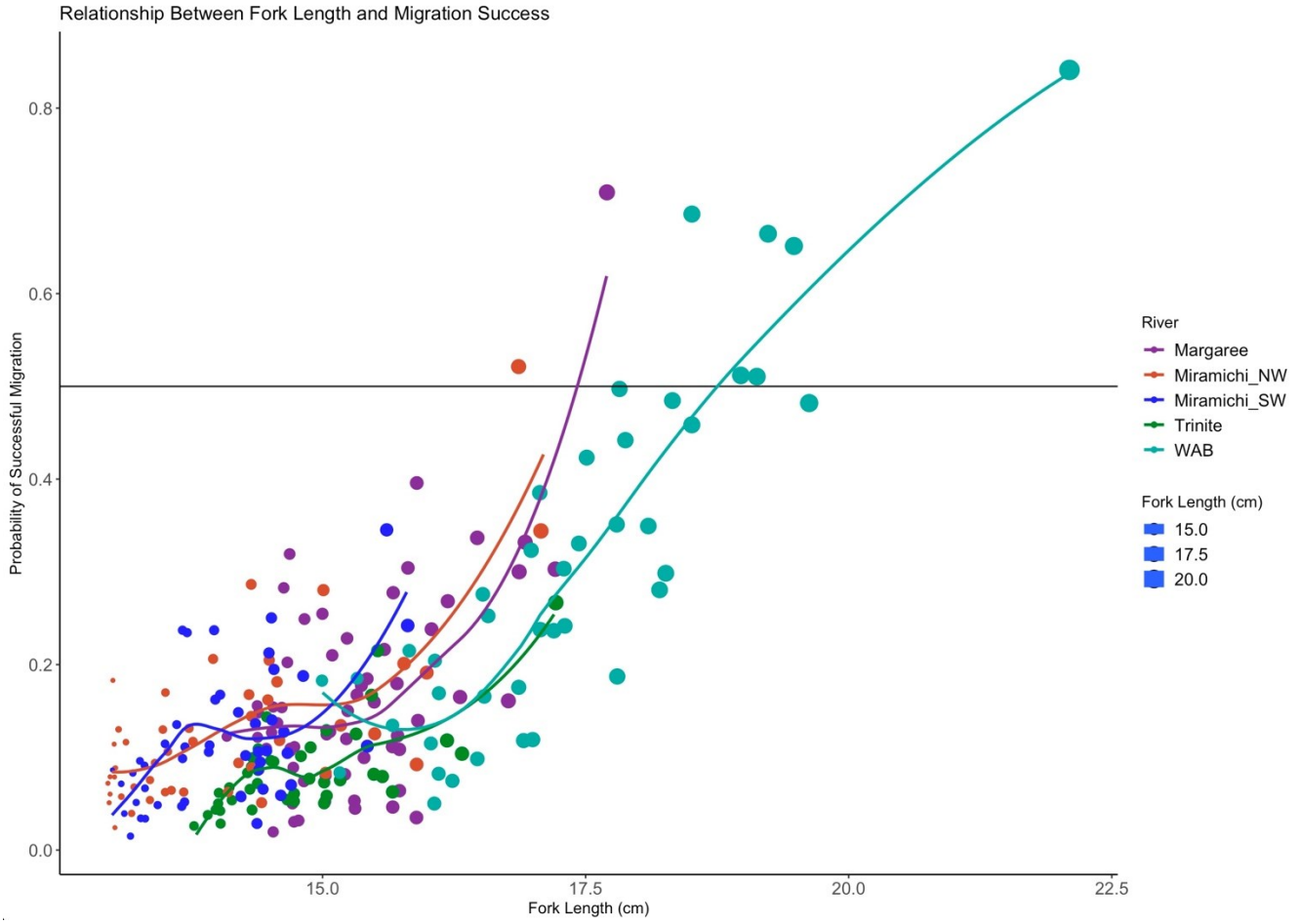

Figure 13: Plot depicting the relationship between increasing fork length and the probability of a successful migration. The size of each point also corresponds to the fork length in centimeters of an individual fish.

Chapter 3: Conclusion and Significance

My study investigated whether transcriptomic profiles could be used to predict migration success in wild Canadian Atlantic salmon smolts. I combined acoustic telemetry with transcriptomics to shed light on possible physiological mechanisms behind Atlantic salmon smolt mortality during the marine migration within the Gulf of St. Lawrence, hypothesized to be a period of high mortality repressing the recovery of Atlantic salmon populations (Thorstad *et al*., 2012; ICES, 2017). By creating transcriptomic profiles for each tagged smolt, I was able to glimpse a "snapshot" of the internal state of an individual smolt and link differentially expressed genes to an ecological endpoint.

This study highlighted that different populations of salmon are facing different physiological stressors from one another. Despite the absence of a multi-gene signal in the transcriptomic profiles that identified successful migrants from unsuccessful migrants, I found that the differential expression of one or more genes exhibited statistically significant effects on the odds of an individual smolt surviving its migration. The gene(s) in question could be different from one population to the next.

Notably, the genes that influenced migration success were linked to immune system function. This suggests that for at least some populations, pathogens may be exerting a strong influence on migration success. The smolt life stage is known to be more vulnerable to some pathogens (Pickering, 1994; Miller *et al*., 2014). Yet, because of the already high mortality rate of this life stage and the inability to track smolts once they exit their natal river system it is hard to quantify pathogen related mortality. I hypothesize that pathogens do not directly cause mortality, but contribute indirectly by using energy reserves needed for migration, making an individual slow to react to predator presence, or

by inducing some other detrimental behavioural change in the smolt. The pressure exerted by existing and novel pathogens of Atlantic salmon will only increase as the Northern hemisphere warms (Altizer *et al*., 2013; Semenza & Suk, 2018).

Limitations and future considerations

The study included here was confounded by some methodological limitations. In an ideal experiment, we would have tagged fish in their rivers a standard distance from the head of tide in order to compare freshwater survival and any other behavioural differences between the populations. This would have also helped to standardize the conditions experienced by each fish post-tagging, as some populations could then quickly proceed to exit the river while others had several kilometers to swim through before entering the ocean. However, because this study was conducted alongside standardized sampling by our collaborators, the location was out of our control.

I was also confounded by the available telemetry infrastructure. While I was able to adequately detect fish crossing our receiver gates, I calculated an array efficiency of just 66.7%, meaning it is highly likely that some fish did succeed in their migration, but went undetected. With respect to my study, this is detrimental for two reasons: first, I was already under sample size constraints because so few fish did successfully migrate, so each missed fish leads to a weaker analysis. Second, misclassifying a fish as deceased would cause it to dilute signatures between successful and unsuccessful migrants.

While ample care was taken not to overtly stress sampled fish during the tagging procedures, it is impossible to wholly eliminate effects caused by the tagging surgery and handling. During fish sampling, myself or our collaborators fished the trap used to collect fish daily, but some fish could be held for as long as 24h which would subsequently

stress the fish in question. This could have muddied stress-related transcriptomic data since all of the fish were trapped and handled similarly, thereby inducing stress responses in every sampled fish.

The data I present here can be used a baseline for further transcriptomic studies. In particular, this study presents a blueprint for researchers to follow and refine as they carry out their own transcriptomic analysis. A long-term dataset showing transcriptomic profiles from different populations and linking them to environmental variables could prove to be very useful in assessing how Atlantic salmon are responding and adapting to stressors over time. Going forward, other biological characteristics such as age (from scales) and sex could be incorporated and add further strength to the resulting dataset.

Significance

The present study has shown that transcriptomics is a powerful tool that can illuminate otherwise hard-to-detect stressors on the internal state of Atlantic salmon smolts, but it requires careful planning and a large sample size. The most interesting result to arise from my study was the lack of differences in the overall gene expression between the populations included in this study. Because of the different environmental conditions experienced by the smolts in the days leading up to their sampling and the known genetic distance between the populations included here (King *et al*., 2001; Spidle *et al*., 2003)., I expected to see some of the populations cluster separately from one another, but this was not the case. This result shows that despite living in different environmental conditions, being separated by several hundred kilometers of ocean, and exhibiting genetic distinctness, different populations of Atlantic salmon physiologically respond to stressors at a similar transcriptomic level.

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Appendix A: Supplementary Tables

Table A 1: The current status of the COSEWIC Designatable Units of Atlantic salmon in Canada.

UNIT NAME	NOT AT RISK	THREATENED	ENDANGERED	EXTINCT	SPECIAL CONCERN	DATA DEFICIENT
Nunavik (DU 1)						X
Labrador (DU 2)	X					
Northeast NFL (DU 3)	X					
South NFL (DU 4)		X				
Southwest NFL (DU 5)	X					
Northwest NFL (DU 6)	X					
Quebec Eastern North Shore (DU 7)					X	
Quebec Western North Shore (DU 8)					X	
Anticosti Island (DU 9)			X			
Inner St. Lawrence (DU 10)					X	
Lake Ontario (DU 11)				X		
Gaspe-Southern Gulf of St. Lawrence (DU 12)					X	
Eastern Cape Breton (DU 13)			X			
Nova Scotia Southern Upland (DU 14)			X			
Inner Bay of Fundy (DU 15)			X			
Outer Bay of Fundy (DU 16)			X			

Appendix B: Supplementary Figures

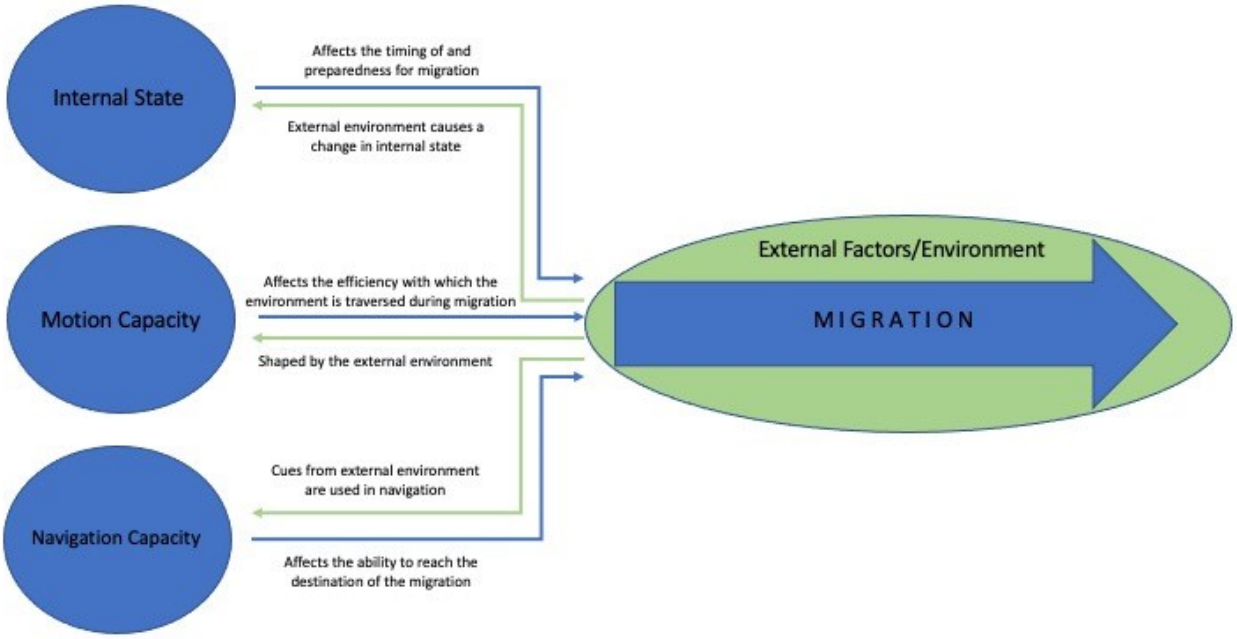

Figure B 1: Diagram showing the connectivity between the four pillars of the Movement Ecology framework proposed by Nathan *et al*. (2008).

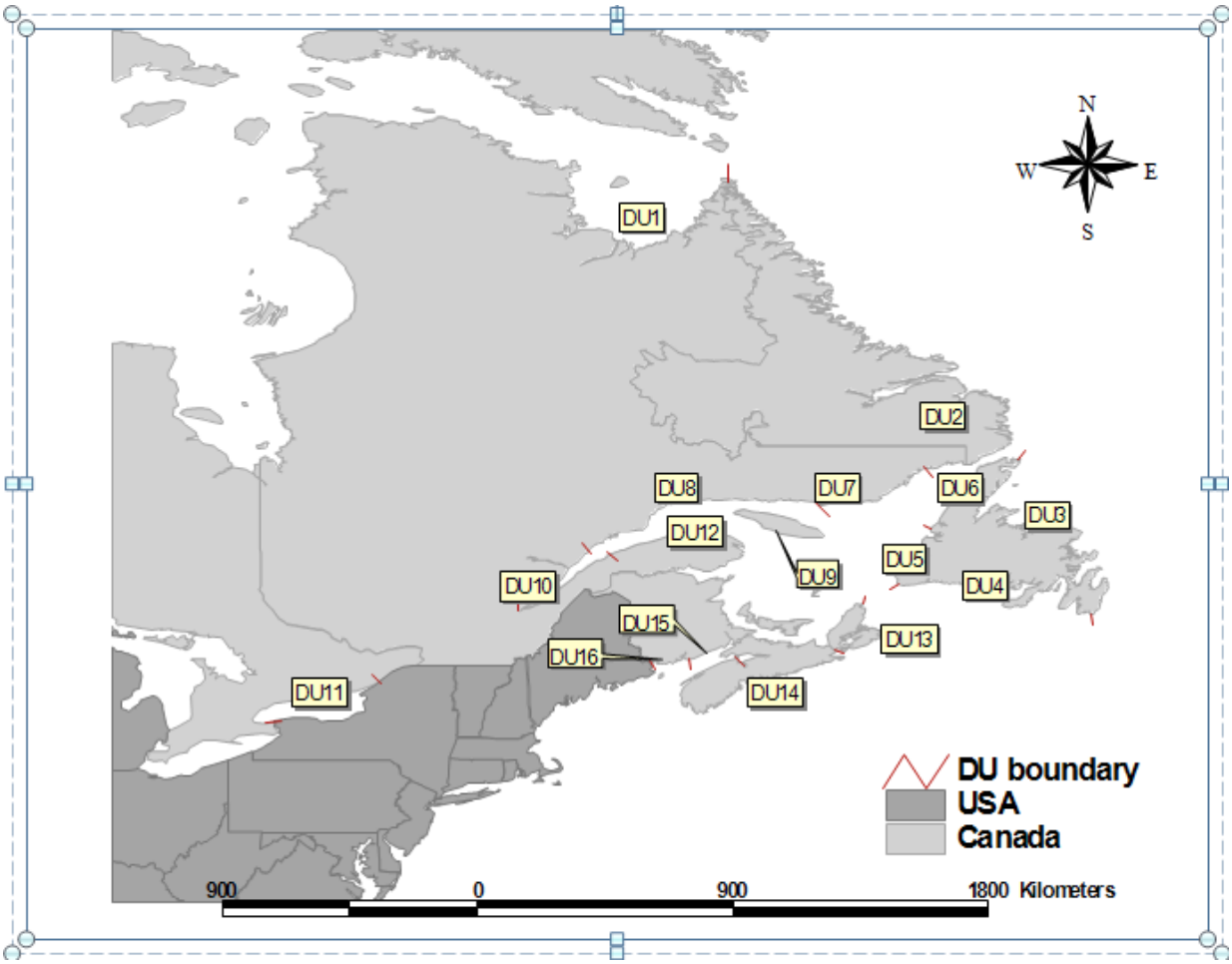

Figure B 2: Map depicting the different Designatable Units of Atlantic salmon as proposed by COSEWIC (2010). From COSEWIC (2010).