INVESTIGATIONS OF INSTREAM INCUBATION CONDITIONS ON THE DEVELOPMENT OF ATLANTIC SALMON (*SALMO SALAR*) EMBRYOS IN A NORTHERN NEW BRUNSWICK RIVER

by

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ABSTRACT

The spatial distribution of landscape characteristics within a watershed can influence instream water quality conditions and impact the growth and development of incubating Atlantic Salmon (*Salmo salar*) embryos as they incubate within substrates (i.e., redds). With globally declining Atlantic Salmon populations it is becoming increasingly important to foster greater freshwater resilience, especially at very early stages of development when embryos are immobile and most sensitive to subtle changes in water quality conditions. However, relatively little is known on the prevalence of Atlantic Salmon embryo deformities, especially within Jordan/Scotty incubators, which represents an important data gap that could have important implications for the future viability of certain populations.

It is my aim to investigate how landscape characteristics vary at multi-spatial scales at six incubation sites within the Serpentine River, New Brunswick and how this variation may influence water quality conditions that incubating embryos rely upon for proper growth and development. Performing a principal component analysis (PCA) of landscape characteristics and water quality conditions I explored what environmental factors were influencing variation in incubation habitat conditions. Using a combination of one-way ANOVAs and simple linear regressions, I also explored what site-level water quality conditions and bundle-level incubation conditions had a statistically significant influence on the mortality and deformity status of the 18 000 developing embryos. My PCA results indicate that natural landscape characteristics at the catchment-scale, such as relative percent forested area are primarily influencing changes in water quality conditions and substrate composition, such as fewer heavy metals (i.e., Fe) and finer substrates (i.e., proportion gravel) within downstream sites of the Serpentine River.

Overall, the proportion deformities we observed (~2.6%) did not reflect the elevated proportions (~80%) observed within a previous study conducted in 2013, and although the proportion of mortalities we observed (~45%) were quite high, these findings were consistent with other similar in-situ incubation studies. ANOVA results indicated that there were no between field and control site differences in the proportion of either mortalities or deformities per incubator. Further investigation of between field site differences in the total number and proportion of embryo mortalities and deformities using site level water quality conditions (i.e., cumulative degree days and coefficient of variation in dissolved oxygen concentrations) and bundle-level incubation conditions (i.e., distribution of sand particles within incubators) were not statistically significant. Therefore, these findings suggest no environmental factors within the Serpentine River are influencing embryo survival or reducing their overall fitness and suggest in-stream incubation is an alternative to rearing within hatchery environments.

DEDICATION

I dedicate this thesis to my fiancée Kendra DeWitt who has supported me through this entire academic journey and taught me the importance of celebrating my accomplishments.

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CHAPTER 1: GENERAL INTRODUCTION

Atlantic Salmon *(Salmo salar)* is a ray-finned fish species with a complex life cycle and species range encompassing the entire North Atlantic Ocean. They are found in the Northern hemisphere, with populations found in the United States, Canada, Faroe Islands, Greenland, Iceland, Ireland, Norway, Sweden, Finland, France, Spain, and the UK (Reddin et al., 2012). Atlantic Salmon use both freshwater and marine environments (i.e., anadromous) (Reddin et al., 2012), return multiple times to spawn as adults (iteroparous) (Fleming & Reynolds 2004; Bordeleau et al., 2020), and regulate their internal body temperature by the surrounding environment (i.e., poikilothermic) (McGinnity et al., 2009). Atlantic Salmon begin their life cycle as fertilized embryos incubating within river substrates (called redds) and within the Outer Bay of Fundy (OBoF) region they typically spend 2 - 4 years in the freshwater environment as juveniles and 1-3 years in the marine environment as adults (Gibson et al., 2016; Fisheries and Oceans Canada, 2016). Atlantic Salmon embryos are deposited in redds by sexually mature females in the fall, are fertilized, buried, and left to develop throughout the winter months. These fertilized eggs undergo various stages of pre-hatch development (cleavage, blastulation, gastrulation, somitogenesis, vascularization of yolk sac, formation of caudal fin rays), and will hatch in the spring as yolk-sac alevins. After emerging from the gravel, fry stay within the freshwater environment throughout the summer months to grow into parr. When Atlantic Salmon parr are large enough, they undergo morphological and physiological changes in preparation for migrating out to sea and become smolts. Once the smolts successfully navigate the estuarine environment and reach the mouth of the river, their marine life cycle stage begins. As Atlantic Salmon feed and grow in the marine environment they eventually reach sexual maturation and return to the same rivers where they were born (i.e., natal rivers) to spawn as grilse, thereby fulfilling their life cycle and ensuring the production of the next generation (i.e., fertilized embryos). After spawning, Atlantic Salmon can immediately return to the marine environment to continue to feed and grow or opt to overwinter within the freshwater environment and migrate to marine environment in the spring.

Given that Atlantic Salmon are anadromous, iteroparous, and poikilothermic they can occupy a variety of different aquatic habitats, which exposes them to a myriad of pressures such as disturbance from clear-cutting activities or pollution, and the associated responses to these pressures, such as increased water temperatures, sedimentation, and reduced dissolved oxygen (DO) concentrations. With the cumulative impact from multiple environmental pressures and stressor, Atlantic Salmon populations across the globe and within Canada have been declining, especially since the 1990s (NASCO, 2019). Many populations within the Canadian Atlantic provinces are currently listed as either threatened or endangered, with the Outer Bay of Fundy (OBoF) population currently undergoing review of endangered status (COSEWIC, 2010; Fisheries and Oceans Canada, 2016; NASCO 2019). Poor marine survival is one of the most common cited factors contributing to the decline of Atlantic Salmon (Hansen et al., 2012; Jonsson & Jonsson, 2004; Peyronnet et al., 2008). As Atlantic Salmon survival decrease (especially within marine environments), it is possible that a positive correlation between population density and the individual fitness of the population (i.e., reduced population growth) can occur (Drake et al., 2011). If Atlantic Salmon survival continues to decrease it is possible that it will give rise to a critical population size below which the population cannot persist and therefore impact its density dependency (Stephens et al., 1999).

Specifically, the Outer Bay of Fundy (OBoF) population of Atlantic Salmon have been steadily declining (i.e., ~64% net decline in all sexually mature individuals over past 3 generations – 15 years) (Jones et al., 2014), with only 509 returning adults observed in 2018 within Wolastoq | Saint John River at the Mactaquac Generating Station fishway (Fisheries and Oceans Canada 2020). These more recent declines in abundance mirror previous trends observed over the last 6 – 9 generations (i.e., 30 – 45 years) with the estimated abundance of returning North American Atlantic Salmon collapsed from 700,000 to 800,000 before 1980 to 110,000–150,000 during the 1990s, an 85% decline (Kocik & Brown 2002; Mills et al., 2013).

Given the uncertainty surrounding marine survival, conservation efforts can be made to increase the number of strong, healthy, and resilient Atlantic salmon smolt emigrating from freshwater environments (Thorstad et al., 2021). There is great scope for freshwater conservation efforts, such as water quality enhancements, modified river regulation, removal of migration barriers, and physical river habitat improvements (Thorstad et al., 2021). It is anticipated that this focus on freshwater environments is an optimal approach to reduce impacts of changing ecosystems and low marine survival (Thorstad et al., 2021), which can potentially increase the number of returning adults. Furthermore, the very early life cycle stages of Atlantic Salmon (i.e., pre-eyed stage), when embryos are incubating within river substrates over winter months, are extremely vulnerable to stressors, such as thermal stress from rapidly changing water temperatures and the associated variations in DO concentrations (Gibson et al., 2013). As abundance of juvenile Atlantic Salmon (i.e., parr) is linked to availability and access to high-quality habitat, greater survival could be achieved by restoring and reconnecting these freshwater habitats (Cunjak et al., 1998; Cunjak & Newbury, 2005; Thorstad et al., 2021).

Additionally, as impacts experienced at earlier life cycle stages can have an influence on survival and fitness at later life cycle stages (Clarke et al. 2016), greater freshwater resilience can be achieved by ensuring adequate incubation habitat for embryos, thereby increasing abundance at post-hatch life cycle stages.

There is an innate connection between landscape composition and spatial and temporal distributions of aquatic habitats within rivers and streams. As water flows across the landscape, the resulting erosion, runoff and chemical leaching from natural landscape features such as forest cover and bedrock geology, produce predictable patterns in habitat formation. There is scientific debate on what scales (i.e., site, reach, catchment) anthropogenic land uses have the greatest impact on aquatic habitat and survival of freshwater fishes, because it is often influenced by the wider spatial context of land use specific to each watershed and the scale of analysis used. Some recent research within New Brunswick, Canada indicates that catchment- and reach-scale impacts from anthropogenic land uses, such as clearcutting, are detrimental to in-stream water quality conditions and aquatic biota, especially when analyzing headwater catchments within intensively managed forest landscapes (Erdozain et al., 2018). Other studies have shown that these spatially discreet anthropogenic impacts can be attenuated at larger drainage basin scales, where natural landscape features predominately drive in-stream water quality conditions and aquatic biota (Tang et al., 2020). Therefore, selecting the proper spatial scale of analysis and placing the potential impact of various land use types within the wider context of downstream landscape composition is important in understanding the unique pressures and stressors Atlantic Salmon face as a result of habitat fragmentation and degradation.

Habitat fragmentation within the freshwater environment such as in-stream anthropogenic physical barriers like dams or reservoirs, and improperly installed culverts or road crossings can impede or prevent upstream and downstream access to good quality habitat. Alternatively, existing habitat can be degraded by anthropogenic land uses throughout the watershed, such as clearcutting, which can contribute to reduced water quality conditions (Forseth et al., 2017). The large rivers of the OBoF population of Atlantic Salmon, which include the Wolastoq-Saint John River, St. Croix River, and Magaguadavic Rivers, have experienced over a century or more of industrial development which has had a negative impact on their population abundance during freshwater life cycle stages (Marshall et al., 2013). Within the Wolastoq-Saint John River Basin there are over 200 dam or water control structures, which are reducing habitat connectivity, while over 100 sources of municipal wastewater effluent and another 70 non-municipal effluent sources (i.e., pulp and paper mills, sand/gravel pits, food processing and aquaculture facilities) are degrading the quality of freshwater habitat (Kidd et al., 2011). Although water quality conditions have been slowly improving between 1960 – 2000, it is likely due to better treatment of municipal and industrial wastewater (Kidd et al., 2011). However, a study conducted by Cunjak & Newbury (2005) concluded that hydroelectric dams and industrial pollution within the mainstem Wolastoq-Saint John River probably represent the worst case of fragmentation within eastern Canada. Furthermore, the habitat requirements of juvenile Atlantic Salmon are diverse, varying both spatially and temporally depending on the needs of different life cycle stages (i.e., embryo - smolt).

The very early embryonic life cycle stages of Atlantic Salmon are the only time during their life they are unable to relocate to more favourable conditions. This lack of mobility during incubation highlights their increased sensitivity to subtle changes in water quality conditions such as water temperature, fine sediments, or dissolved oxygen concentrations (Bisaillon et al., 2007; Jones & Flanagan, 2007). Additionally, any impacts experienced during the very early stages of embryonic development, such as deformity, can negatively influence fitness at later life cycle stages and often results in mortality. Thus, by monitoring the water quality conditions within river substrates that incubating embryos experience over winter, we can gain insight into environmental factors that may limit survival and increase the prevalence of deformities. It is important to study incubating Atlantic Salmon embryos as they provide a unique opportunity to observe their response to changing habitat conditions over the winter months at discrete spatial locations. By identifying potential limiting factors, we can aim to alter land management practices and develop conservation strategies that improve water quality conditions and incubation habitats, therefore contributing to greater resilience of all freshwater life cycle stages.

Juvenile stocking, which is the release of hatchery-reared smolts has been a conservation strategy used in Canada to bolster depressed wild Atlantic Salmon populations for over 100 years. However, the effectiveness of hatchery-reared smolts to improve wild population abundances has resulted in variable success, and has highlighted reduced fitness (i.e., lower growth and/or reproduction) when compared to wild stocks (Araki et al., 2008; Houde et al., 2010; Morrison, 2012, Clarke et al., 2016). The fact that hatchery fish are not exposed to the same natural variability in habitat and water quality conditions as their wild counterparts has led some researchers to examine whether the expression of some genes are being modified by their captive exposure, especially at the very early life cycle stages which can shape behaviour once released (Burgerhout et al., 2017). In a recent study conducted in British Columbia, Canada, researchers found evidence that the epigenetic modifications induced by hatchery rearing conditions provided a potential explanation for reduced fitness of hatchery-reared Pacific salmon species (Luyer et al., 2017). It has been demonstrated that similar epigenetic modifications are present within hatchery reared Atlantic Salmon fry and parr (Wellband et al., 2021), with similar research being conducted on Atlantic Salmon embryos (Roth et al., unpub.). Therefore, to maximize freshwater survival of Atlantic Salmon and improve their fitness and overall resilience as a species there is a growing scientific consensus that exposure to the hatchery environment should be minimized as much as possible (Araki et al., 2008; Houde et al., 2010; Morrison, 2012, Clarke et al., 2016).

In-stream incubation of salmonid embryos is becoming more common within North America, since it is a low-cost alternative to hatchery rearing for biologists and other fishery managers attempting to bolster depressed populations (Conley et al., 2020). In-stream incubation equipment, such as Jordan/Scotty incubators are hard plastic containers which can protect individual embryos from physical disturbances, reduce the spread of fungal infections and predation by fish, while enabling the retrieval and subsequent study of Atlantic Salmon at pre-hatch life cycle stages (i.e., pre-eyed embryos). However, relatively little research has been conducted on the in-stream incubation conditions of pre-eyed Atlantic Salmon embryos within Jordan/Scotty incubation boxes, specifically the prevalence of deformities as they overwinter within river substrates.

In-stream incubation attempts to maximize exposure of embryos to more naturally variable water quality conditions, which is thought to increase individual fitness and survival that is sometimes only evident at post-hatch life cycle stages (Araki et al., 2008).

There are a multitude of embryonic deformities that can negatively influence the survival and overall fitness of hatchery reared embryos within post-hatch life cycle stages. Skeletal deformities such as lordosis and corkscrew spinal malformations have been observed in triploid Atlantic Salmon which can negatively influence their post-hatch swimming ability, while ocular deformities, such as small eyes or partial blindness can impact navigation and feeding behaviour, as observed in aquaculture settings (Powell et al., 2009). Additionally, some embryonic deformities experienced during incubation, such as small yolk sacs can have immediate impacts on growth and development while others exhibit latent effects, such as blue sac disease (i.e., swelling of yolk sac due to exposure to cold incubation temperatures or toxic substances) which can contribute to mortality during post-hatch life cycle stages (Finn, 2007). Additionally, given the potential implications of embryonic deformities on the population structure and overall freshwater resilience of Atlantic Salmon, quantifying the prevalence of deformities represents an important knowledge gap that must be filled.

Atlantic Salmon embryos are sensitive to rapidly changing water quality conditions such as water temperatures or dissolved oxygen (DO) concentrations. As both water temperatures (i.e., number of degree days) and DO decrease the metabolic activity of embryos tend to decrease causing them to develop slower, with mortality occurring at extremely low water temperatures (i.e., ice crystal formations when <0 °C) or at extremely low DO concentrations (i.e., hypoxia when <50% saturation for more than 24hours) (Finn, 2007; Ørnsrud et al., 2004, Levasseur et al., 2006). Likewise, if water temperatures are too warm it can cause them to develop more rapidly and drastically influence the timing of their hatching, which may cause them to emerge when there are insufficient food sources in the surrounding habitat. Additionally, both wild Atlantic

Salmon redds and Jordan/Scotty incubators can experience different levels of fine and coarse sand particles, which can decrease hyporheic flows and contribute to mortalities and/or deformities due to reduced DO concentrations. The impact of fine sediments such as silt, clay and sand within wild redds and their associated linkages to DO concentrations have been previously linked to similar studies on *in-situ* salmonid embryo mortality throughout North America (Louhi et al., 2008). However, relatively little is known on what causes deformities within non-laboratory environments. A 2013 study conducted on the Tobique River, New Brunswick, Canada noted greater than 80% deformities in Atlantic Salmon embryos at certain incubation sites (A. Bartlett, unpub. data). Additionally, there was a previous study conducted on Atlantic Salmon embryo within the Tobique River in 2003, but alternative incubators were used within the experiment and only the influence of fine sediments and variable hydroelectric flow regimes on mortality were explored (Flanagan, 2003). These results provided a foundation for further studies of *in-situ* embryonic growth and development by providing a baseline of prior mortality and deformity rates of Atlantic Salmon embryos and motivated the current study.

The aim of this thesis was to investigate what landscape characteristics and environmental conditions may be influencing the growth and development of incubating Atlantic Salmon embryos within the Serpentine River, a tributary within the Tobique River watershed. Exploring drivers of the deformity phenomena previously observed in 2013 may provide insight into the pressures and stressors impacting freshwater survival and fitness of Atlantic Salmon embryos, especially during the very early embryonic life cycle stages (i.e., fertilization - eyed stage). This thesis explores two key questions to identify the unique pressures and stressors that incubating Atlantic Salmon embryos face, which helps us better understand the current constraints on future population management activities.

First, I wanted to understand how natural and anthropogenic landscape characteristics interact across multiple spatial and temporal scales and contribute to among-site variation in Atlantic Salmon incubation habitat within the Serpentine River (Chapter 2). I hypothesized that anthropogenic land uses and variable water flows were shaping downstream water chemistry and substrate conditions at multiple spatial scales (i.e., site, reach, catchment). I predicted that increased anthropogenic landscape pressures and stressors (e.g., clearcutting, unpaved road networks) would follow a longitudinal gradient and contribute to increased sedimentation (e.g., % fines) within downstream sites which would have detrimental impacts on Atlantic Salmon incubation habitats (i.e., redds). I also predicted that surficial and bedrock geology will influence downstream water quality (e.g., physicochemical characteristics) and impact potential composition of incubation substrates.

Second, I wanted to explore how water quality conditions interacted across multiple spatial and temporal scales and contributed to both among- and within-site variation in incubation water quality and substrate composition and impacted the proportion of embryo mortalities and deformities throughout the winter months (Chapter 3). I hypothesized that changes in both water temperature and dissolved concentrations will contribute to embryo mortalities and deformities, with similar impacts on survival and development caused by fine and coarse sediment within incubators which can reduce hyporheic flows. It is predicted that there will be greater mortalities at sites with lower water temperatures (i.e., lower cumulative degree days) and greater amount of fine sediment within incubators. It is also predicted that there will be greater deformities at sites with lower DO concentration and greater amount of coarse sediment within incubators.

Chapter 4 will explore the broader implications of this research and provide recommendations for future in-stream incubation studies. By examining potential mechanistic linkages between landscape composition, instream water quality conditions and embryo mortalities and deformities within the Serpentine River, we can more accurately identify and mitigate future pressures and stressors in other watersheds with depressed populations.

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CHAPTER 2: LANDSCAPE ANALYSIS AND STUDY SITE CHARACTERIZATION

ABSTRACT

Natural and anthropogenic landscape characteristics can influence instream water quality conditions and alter Atlantic Salmon habitats, especially the incubation habitat of embryos as they grow and develop within river substrates. Here we investigate the differences in landscape characteristics and in-stream habitat features at catchment-, reach-, and site spatial scales to determine how they influence substrate composition and water quality conditions within the Serpentine River, New Brunswick. Using Principal Component Analysis (PCA) we collapsed abiotic data collected in 2020-2021 from six sampling locations within the Serpentine River into a matrix to explore how incubation habitat differ. Our results indicated that natural landscape characteristics, such as forested land cover and bedrock geology at the catchment scale influenced the longitudinal pattern in substrate composition and water quality conditions. Upstream catchments (Site 1-2) tended to have relatively smaller proportions of forested areas (72.6-76.3%) with felsic dominant bedrock geology, which contributed to finer substrate composition and more variable water quality conditions at downstream sites. With a better understanding of how water quality conditions within the Serpentine River, future land use decisions can be made to preserve existing Atlantic Salmon incubation habitat.

INTRODUCTION

Landscape composition and in-stream water quality conditions are inextricably linked with broad natural landcover types, such as forests influencing water levels and flow regimes (Ugedal & Finstad, 2011), with corresponding influences from various forestry land use activities, such as clear-cuts. As water flows across the landscape and into rivers it can transport physical materials from one area via the processes of runoff and erosion, and deposit them within downstream aquatic environments via processes of sedimentation. However, there are also in-stream flow dynamics and complex depositional processes that govern how rivers naturally meander across the landscape and create predictable sequences of micro-habitats (i.e., riffle-pool-run) within rivers as they flow downstream (Clifford, 1993).

As water flows through both the landscape and within rivers certain metals and minerals from the surrounding bedrock and surficial geology can be leached or dissolved into the water via process of chemical weathering. However, freshwater ecosystems are hierarchically nested, meaning the ability of Atlantic Salmon to survive, grow and reproduce (i.e., species fitness) within fine-scale microhabitats are ultimately constrained by coarse scale landscape features that create specific abiotic conditions. Both natural and anthropogenic landscape characteristics can influence instream water quality conditions, including the incubation habitat of Atlantic Salmon embryos. To better understand how the abundance and distribution of freshwater species are influenced by landscape composition across multiple spatial and temporal scales, freshwater ecologists have attempted to describe them in terms of their functional relationships to habitat selective forces and their specific species traits which have evolved over multiple generations. The water quality conditions of aquatic habitats are largely influenced by landscape composition at various spatial scales and can act as a series of “filters” that species such as Atlantic Salmon must pass through to become a part of the local community or population. These filters can range from coarse scale landscape scales, such as entire watersheds or drainage basins, to certain reaches or channels of the river, all the way down to fine scale microhabitats (i.e., incubation habitats). If a species has developed the necessary traits (i.e., thermal or chemical tolerances) that improve their odds of survival within a certain habitat it can pass through each nested filter (i.e., catchment-, reach-, site-scale), often occupying an ecological niche which gives its overall population a reproductive and evolutionary advantage over other species (Poff et al.,1997). These landscape filters are hierarchical (Frissell et al., 1986; Davies et al., 2000) because if a species does not have the required traits necessary to occupy a certain type of habitat at coarse-scales (i.e., catchment-scale), they will most likely not be able to access the habitats at finer-scales (i.e., reach or site-scale microhabitats), especially immobile life cycle stages such as embryos. However, given that water quality condition change temporally throughout the year, especially within the main branches of rivers, and the strength of these landscape filters can vary at different spatial scales, certain species can temporarily occupy different niches at finer spatial scales (i.e., reach or site-scale microhabitat), but this spatial niche variability is often only seen in mobile life cycle stages (i.e., Atlantic Salmon parr) (Heggenes et al., 1999). Therefore, the physical and chemical characteristics, at a specific location within a river reflects both the local and upstream variations in landscape characteristics and the water level and flow regimes they produce during a specific temporal period (i.e., overwinter incubation period).

Understanding how landscape characteristics at different spatial scales are driving among-site variability is key to conducting effective watershed-based management, especially since instream physical and chemical characteristics provide habitat functions for aquatic communities. Freshwater habitats within rivers are naturally complex and diverse, often following a spatial and temporal pattern along a gradient or continuum. Common examples of anthropogenic land uses covarying with natural landcover types include agricultural activities occurring within historic floodplains or silviculture operations occurring within areas with lower topographical relief (Barry & Elith, 2006; Leal et al., 2016; Tang et al., 2020). By disentangling how natural landscape characteristics influence physical and chemical conditions at multiple spatial scales along the longitudinal gradient of a river, we can gain greater insight into what, where, and how anthropogenic disturbances may be contributing to, or accentuating, local freshwater habitat differences.

Within river ecology, coarse spatial scales often refer to large drainage basins or catchments of rivers that flow across landscapes and drain into oceans or larger mainstem rivers at a common location (i.e., confluence). Meanwhile finer spatial scales often refer to specific locations within rivers, such as a specific type of habitat or study site and often aim to capture more local and spatially discrete variables. The intermediate areas between the coarse landscape and fine local spatial scales are known as reaches or meso-scales, and following the approach described in Lento et al. (2013), the reach scale aims to capture both the landscape and riverine variables within 1 km^2 area upstream from the site.

Typical natural landscape characteristics consist of geographic and geological features, such as topography and bedrock geology, which often dominate among-site variation at coarse scales (e.g., catchment level) and help inform the spatial distribution of natural land cover types such as forests and wetlands (Townsend et al., 2003). For example, Nelson et al. (2011) discovered greater concentrations of H+ ions (pH), Ca^{2+}, Mg^{2+}, and NH_4^+ in basins with mixed geology (e.g., crystalline and sedimentary bedrock) rather than in more homogenous geologic composition (e.g., crystalline bedrock). The influence of bedrock geology on water chemistry can influence the spatial distribution of benthic macroinvertebrate (BMI) and fish assemblages (Lento et al., 2019). Bedrock composition can also influence the distribution of river substrates, with surface flows interacting within upstream landscapes and along riparian hillslopes. These landscape surface flows can alter river channel morphology and in-stream flow characteristics depending on the specific erodibility of bedrock and other geologic material (Whitbread et al., 2015). Typical forestry landscape characteristics include large-scale clearcuts or road networks, which can alter natural land cover types, and disrupt finer-scale water temperature and flow regimes, which often explain more among-site variation in aquatic habitat quality, especially within forestry dominated landscapes (Erdozain et al., 2018).

The aim of this research was to investigate how natural and anthropogenic landscape characteristics interact across multiple nested spatial scales and influence among-site variation in water quality conditions and substrate composition. Using geographic information system (GIS) broad geospatial landscape variables were quantified at the catchment- and reach-scales and in-stream physical and chemical characteristics were quantified at site-specific scales using detailed field measurements. I was hypothesized that variation in water levels (a proxy measure for in-stream flow) was the main factor influencing how upstream landscape characteristics shape downstream water chemistry and substrate conditions at multiple spatial scales (i.e., site, reach, catchment). I predicted that the cumulative impact of forestry activities (e.g., clearcutting, forestry road networks) would be greatest within downstream sites and contribute to increased sedimentation (e.g., larger proportions of sand, silt and clay) and influence the overall substrate composition.. I also predicted that surficial and bedrock geology will be more heterogenous in downstream catchments, which will increase concentrations of metals and minerals (i.e., Fe, Mn) within downstream sites.

METHODS

Study watershed

The Tobique River is located in northwestern New Brunswick, Canada, draining 4331 km^2 and is a major tributary to the Wolastoq | Saint John River watershed (Figure 2.1). Water levels and flows within the Tobique watershed are regulated, with four upstream reservoir lakes (Sisson, Serpentine, Trousers, and Long Lakes), supplying water to the Tobique Narrows Dam that is located near the confluence with the mainstem of the Wolastoq | Saint John River. The Serpentine River is a major tributary in the northeastern part of the Tobique River basin, it drains 394 km^2 and its elevation ranges from 321m-766m above mean sea level (AMSL) (Figure 2.1). The Serpentine River watershed spans two ecoregions (Highlands & Central Uplands) and four eco-districts (Serpentine, Sisson, Beadle, and Ganong) (Zelazny et al., 2007). Ecoregions are large geographic areas defined primarily by their similar range in climatic conditions (i.e., temperature, precipitation, and humidity), and are shaped by major landforms, elevation, latitude and other broad aspects that influence distribution of plant and animal species (Zelazny et al., 2007, Omernik, 1987). Eco-districts are sub-components of an ecoregion and are characterized by distinctive groups of landforms, relief, geology, soils, vegetation, fauna and water bodies (Zelazny et al., 2007, Marshall et al., 1999). The Serpentine River watershed has a predominantly cool, wet climate (annual average of 1119 mm of precipitation), with naturally confined steep slopes that support a mixed forest of balsam fir, spruce, and tolerant hardwoods (Zelazny et al., 2007, Environment and Climate Change Canada, 2020). The dominant land cover type within the Serpentine River watershed is forested (76.8% of the watershed), with a large portion classified as forestry roads (9% of the watershed) or clearcut (17% of the watershed harvested within the last 10 years), which is within the top 25% of New Brunswick watersheds in total area of tree cover loss from 2010-2020 (Hansen et al., 2018; NB DNRED, 2020). All roads within this study area are unpaved forestry roads and will henceforth be referred to as roads. Land within the Serpentine River watershed is almost exclusively (96%) provincial Crown land, of which the majority (97.4% or 367.26 km^2) is leased to forestry companies to conduct forestry operations (e.g., timber harvest).

To assess variation in landscape characteristics across multiple spatial scales (catchment, reach and site), six sites were chosen based on spawning habitat criteria for Atlantic Salmon and proximity to various natural land cover types or anthropogenic land uses (Figure 2.1). Generally, Atlantic Salmon prefer to spawn in relatively deep, swift-velocity habitats (20-50 cm, 35-65 cm/s) and prefer using pebbles (16-64 mm) for spawning substrates (Louhi et al., 2008). The selected sites represent a range of water depths, flows and substrate compositions that reflect spawning habitats available throughout the watershed (Table 3.1). Additional sites were ultimately not feasible given the limited availability of Atlantic Salmon embryos as a threatened species and restrictions of installation equipment (200 embryos/plate). The final site selection was also influenced by site access and logistic difficulties associated with winter fieldwork. Upstream sites (Sites 1 – 2) were generally characterized by coarser substrates while the most downstream site (Site 6) tended to have finer substrates with notable exceptions at

Site 5 , and large variability in water flows, especially at Site 3. Substrate particle size distribution was characterized at each site, which followed the Wolman pebble count and record the intermediate axis of 100 randomly selected stones by following a cross-sectional zig-zag pattern (Wolman, 1954; Environment Canada, 2012). Regular water depth and velocity measurements were taken at each site from September – November 2020 at ten equal intervals using a meter stick and Flowatch flow meter (JDC Electronics, Jackson, MS), with six site visit measurements characterizing the cross-sectional variation in flows until river ice began to form. Once ice had formed an additional two depth measures per site were taken at each water pressure transducer, with the coefficient of variation in water levels used as a proxy measure for flow. These six sites were also chosen because they reflected variation in both natural and anthropogenic landscape characteristics along a longitudinal gradient (i.e., upstream reaches to downstream confluence with Campbell River) within the Serpentine River watershed. Upstream sites (Site 1-2) were characterized by narrow and relatively small reaches (upstream drainage area <100 km^2), with a large proportion of natural landscape characteristics in local catchments, such as wetlands and lakes (i.e., 4.4% - 2.2% relative area) (Figure 2.1). Meanwhile, downstream sites (Site 5-6) were characterized by wider and relatively larger reaches (upstream drainage area $\geq 100 < 518$ km^2) with steep valleys and more recent (i.e., < 10 years) anthropogenic activities in local catchments, such as clearcutting (Figure 2.1).

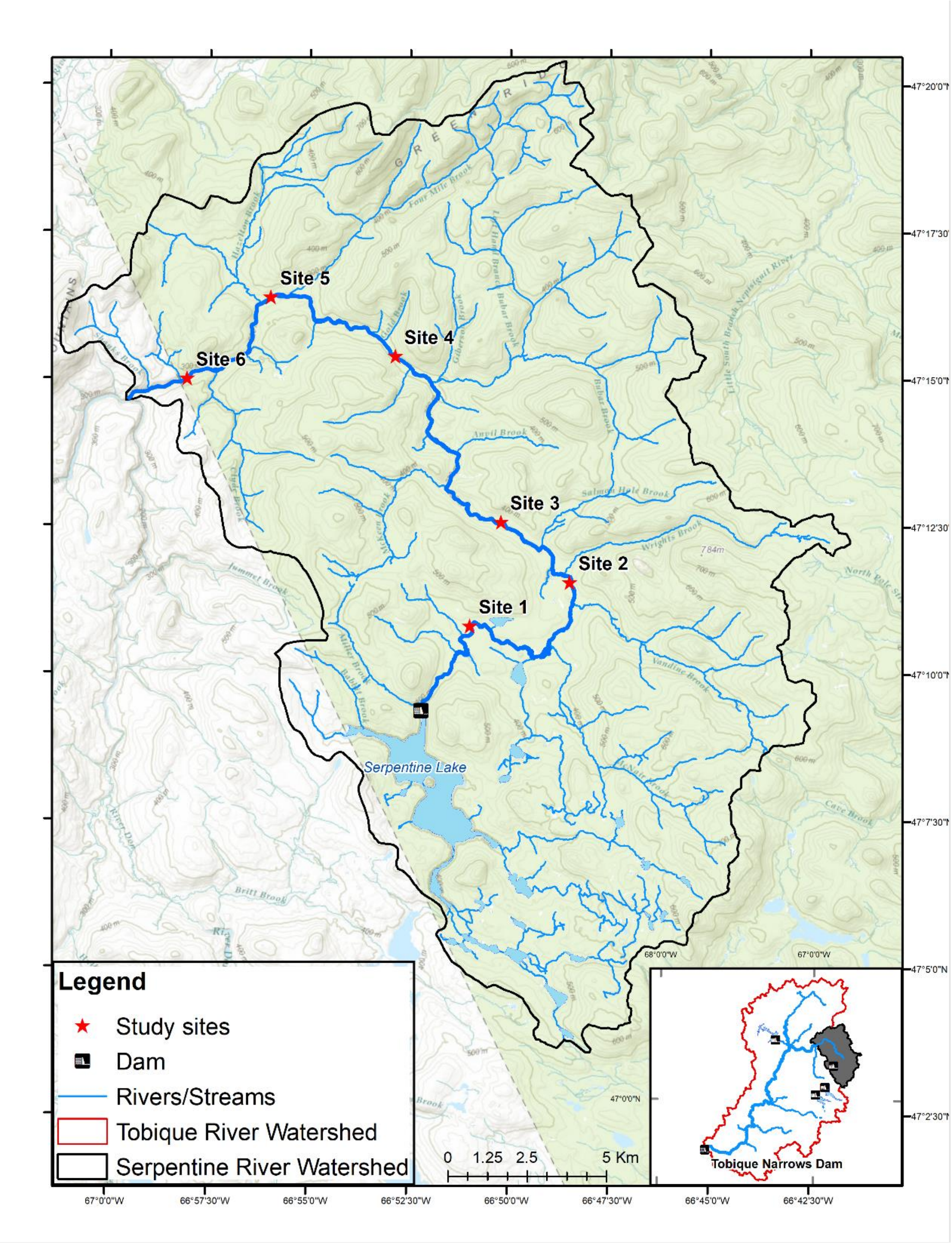

Figure 2.1: Serpentine River watershed with study site locations indicated. The inset map shows the location of the Serpentine River within the Tobique River watershed in northwestern New Brunswick, Canada.

Table 2.1: Spawning site selection criteria (water depth, velocity, and substrate compositions).

Site	Mean Recorded Depth (cm) ± SD	Mean Recorded Velocity (cm/s) ± SD	Percentage Gravel Substrate (2-16mm)	Percentage Pebble Substrate (16-64mm)	Percentage Cobble Substrate (64-256mm)
1	29.9 ± 0.04	25.1 ± 0.01	1.0	53.0	46.0
2	39.6 ± 0.11	29.3 ± 0.10	3.0	57.6	39.4
3	49.6 ± 0.19	41.3 ± 0.16	1.1	69.2	29.7
4	32.4 ± 0.06	23.4 ± 0.05	1.0	61.9	37.1
5	35.1 ± 0.09	23.1 ± 0.07	--	49.0	51.0
6	37.5 ± 0.08	25.1 ± 0.02	2.0	70.0	28.0

Site measurements

Water temperature and dissolved oxygen (DO) were recorded at 15-minute intervals at each site between the 9 September, 2020 and 11 January, 2021 using a Onset HOBO U26 Dissolved Oxygen Data Logger (Onset Computer Corporation, Bourne, MA). Water levels were also recorded at 15-minute intervals at each site during the same sampling period using two Onset HOBO U20-001-02 Water Level Data Loggers (Onset Computer Corporation, Bourne, MA). At each site, two data loggers (DO and water temperature) were secured to a cinder block and placed in the riverbed and tethered to shore with a cable. Meanwhile an additional data logger (water level) per site was secured to a nearby tree and recorded the atmospheric barometric pressure, which was subtracted from the in-stream water level data logger and allowed us to calculate the changes in water levels during the entire sampling period.

A grab water bottle sample was collected at each site during the fall 2020 embryo incubation and early winter 2021 embryo retrieval site visits, to be analyzed for trace metals and general water chemistry at the Research and Productivity Council (RPC) in Fredericton, NB (Table 2.2). Two water samples were taken to help characterize the pre- and post-incubation water quality conditions and to quantify how parameters varied as embryos were incubating within substrates overwinter. The concentrations of water quality parameters used for analysis represent a mid-point average of the two samples. A representative bulk sediment sample (~50-60 g) was also taken at each site during fall 2020 and winter 2021 site visits and submitted to Agriculture and Foods Laboratories (AFL) in Guelph, ON to test for glyphosate (and its metabolite aminomethylphosphonic acid (AMPA)) (0.05 ppm limit of detection).

Landscape characteristics

For this study, the catchment-scale was defined as the drainage area upstream of each study site (Figure 2.2a). Following Lento et al. (2013), the reach-scale represented the area within a 1km-radius catchment buffer upstream from each sampling site (Figure 2.2b). The reach-scale of analysis was chosen because it represented the combined influence of instream geomorphological features found within river reaches (i.e., riffle, pool, run), and the topological and geological features within the upland and riparian areas. Alternatively, the site-scale represented the immediate area and reflected the physical substrate and water chemistry conditions sampled at each site (Figure 2.2c). It is important to note that catchment-scales are nested within each other, meaning that each successive downstream catchment is larger than its upstream neighbour and includes landscape characteristics within the entire upstream drainage area. To help account for the potential influence of local versus upstream landscape characteristics at the catchment-scale, relative percent area metrics were calculated (Tables B.1-B.3).

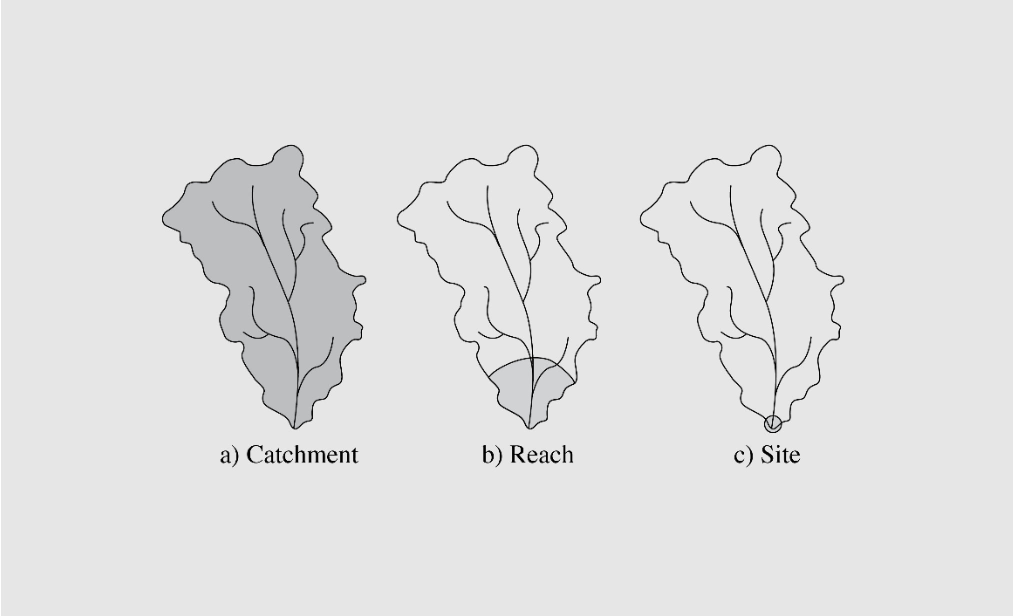

Figure 2.2: Examples of the three spatial scales used for analysis, as indicated by grey shaded area for (a) catchment-, (b) reach-, 1-km upstream radius buffer, and (c) site-scales.

Using ESRI ArcMap 10.5.1 (ESRI, 2017), six different land use classes (i.e., Forest, Wetland, Barren, Urban, Clearcut, and Open Water) were summarized by percent (%) cover composition at the catchment-scale using a 2019 DataBasin dataset from Nature Conservancy of Canada (NCC) (Millar et al., 2019). These land cover and land use types were chosen because they can influence the stability of riparian slopes and have been previously demonstrated to impact Atlantic Salmon habitat quality (Zeimer, 1989; Deschênes et al., 2007). The original 2019 land use raster was updated with coarse-scale 2010-2019 forest cover loss data from Global Forest Watch (Hansen et al., 2018), which was defined as removal or mortality of tree cover (i.e., any vegetation >5m in height) due to a variety of factors, including mechanical harvesting, fire, disease, or storm damage. Meanwhile, fine-scale geospatial data of 2020 proposed clearcut forest block data obtained from the New Brunswick Department of Natural Resources and Energy Development (NB DNRED, 2020) was appended to the original 2019 land use raster. Geospatial data on herbicide applications within the watershed were also collected for each clearcut forest block from NB DNRED for 2019 and 2020, and the area sprayed was summarized by percent (%) area per catchment. It was important to quantify landscape characteristics by percent area to capture scale specific fluctuations in the proportion of natural landcover or forestry activities, especially as nested downstream catchments had successively larger drainage areas.

Other key landscape characteristics summarized at the selected spatial scales included road density and geomorphic attributes (e.g., flood zone, riparian wetlands, material contribution areas, safety buffers), which were also downloaded from DataBasin (Nussey et al., 2020). It is important to note that all roads within the study area were unpaved and their impact on incubation habitat varies depending on their age, use, and extent of revegetation. However, due to incomplete datasets we were only able to quantify the location of forestry roads, not their condition, so we attempted to differentiate their variable impact with the distance to nearest main forestry road metric (Table 2.2). Additionally, material contribution areas are areas with steep slopes (>15% grade) that provide sediment and organic matter to river channels, while safety buffers represent the land adjacent (60m) to rivers that provide similar sediment inputs and help regulate water temperatures (Nussey et al., 2020). Bedrock geology and forest soils acquired from GeoNB were summarized at the catchment and reach spatial scales respectively, along with basic geographic attributes (i.e., elevation, drainage area, and slope), which complement site-scale variables (Service New Brunswick, 2019; Table 2.2). To avoid issues of circularity during data analysis we quantified site-scale differences in substrate composition as an alternative metric to catchment-scale bedrock geology or reach-scale forest soils.

Table 2.2: Summarized natural and anthropogenic landscape characteristics within the Serpentine River.

Abiotic Variables	Scale	Predictor Variables	Description	Abbreviation
Drainage Area	Catchment	Amount of area (km^2) drained per catchment	Total upstream drainage area above each site	Drain_Cat
Land cover/Land use	Catchment	Percent forested (%) Percent wetland (%) Percent barren (%) Percent clearcut (%) Percent herbicide application (%) Road density (km/km^2)	Percent landcover (forest, wetland, barren) and land use (clearcut, sprayed, road) type per catchment (%)	For_Cat Wet_Cat Bare_Cat CC_Cat Spray_Cat Rd_Dens
Bedrock Geology	Catchment	% mafic bedrock % deep water clastic bedrock % felsic intrusion bedrock % felsic volcanic bedrock	Amount of bedrock geology within each catchment	Mafic_Cat DWC_Cat FI_Cat FV_Cat
Proximity to silviculture operations (e.g., clearcut, herbicide applications, and forestry roads)	Catchment	Distance from nearest upstream clearcut block (km) Number of years remaining as clearcut (#) Distance from nearest upstream herbicide application (km) Number of years since last upstream herbicide application (#) Distance to nearest main forestry road (km)	Proximity (km) and temporal (# of years remaining as clearcut classification) variation between to anthropogenic disturbances	Dist_CC Yr_CC Dist_Spray Yr_Spray Dist_2_Rd
Slope	Reach	% slope of mainstem river reach within 1km buffer	Percent slope of mainstem river (%) reach	MSlope_Rch
Geomorphic attributes	Reach	% riparian wetland % flooded % steep slope material contribution area	Percent geomorphic attributes per reach (%)	RipWet_Rch Flood_Rch MCA_Rch

		% riparian buffer (60m)		Rbuff_Rch
Surficial Geology	Reach	% PLPM5 soil (metamorphic dominant) % PLPM9 soil (granitic dominant) % PLPM10 soil (felsic dominant) % PLPM11 soil (metasedimentary dominant) % PLPM12 soil (igneous dominant)	Amount of forest soil types grouped by primary lithology of parent material (PLPM)	PLPM5_Rch PLPM9_Rch PLPM10_Rch PLPM11_Rch PLPM12_Rch
Elevation	Site	Elevation (m) at each site	Elevation above sea level (ASL) at each site	Site_Elev
Water Quality	Site	Potassium (K; mg/L) Calcium (Ca; mg/L) Magnesium (Mg; mg/L) Alkalinity (mg/L) Conductivity (µS/cm) Aluminium (Al; µg/L) Iron (Fe; µg/L) Manganese (MN; µg/L)	Mid-point concentrations of water quality parameters (i.e., nutrients, metals, and minerals) at each site	K Ca Mg ALKY Conduct Al Fe Mn
Water Temperature	Site	Coefficient of variation of mean daily water temperature (°C) Cumulative degree days	Variation in water temperature (°C) Number of thermal input units (calculated using water temperature measurements)	CV_Temp CDD
Dissolved Oxygen	Site	Coefficient of variation of dissolved oxygen (mg/L)	Variation in dissolved oxygen concentration (mg/L)	CV_DO
Water Level	Site	Coefficient of variation of water level (m)	Variation in water level (m)	CV_Lvl

Substrate	Site	Proportion gravel Proportion small pebbles Proportion large pebbles Proportion small cobble Proportion large cobble Proportion boulder Proportion bedrock Median substrate size (cm)	Proportion of substrate type at each site and median substrate size (cm)	Propn_Grav Propn_SPeb Propn_LPeb Propn_SCob Propn_LCob Propn_Bould Propn_BedR WD50
Physical Site Attributes	Site	Wetted width (m)	Wetted width (m) at each site	WetWidth

Data analyses

A fundamental goal of landscape ecology is to understand how and at what spatial scales, species and populations respond to habitat composition and vary at multiple scales in space and time using geospatial data (Turner, et al 2015)., The data were analyzed at both the combined and individual spatial scales (i.e., site, reach, and catchment) using a principal component analysis (PCA) approach to determine which landscape characteristics were driving the majority of among site habitat variability within the Serpentine River watershed. However, how and where these variables differ between sites ultimately depends on their locations within the hierarchy or nested spatial structure of the river and is determined by their interaction with variables at multiple spatial scales. Given that certain landscape characteristics within upstream catchments can influence downstream catchment-, reach-, and site-scale variables it is important to determine what variables are driving these site differences.

A PCA approach was used because it is a multivariate statistical tool that attempts to maximise the variation explained across all six sites at both individual and combined spatial scales. The main assumption of PCA is linearity, which we tested and met using a series of scatterplots to visualize the data. PCA can filter out the noise introduced by other variables and determine the most influential variables and display them across the top two or three dimensions. Additionally, a PCA approach was the most appropriate statistical tool to use because it can handle multi-collinearity which is often associated with nested multi-spatial datasets, such as our catchment scale variables, thereby avoiding issues of pseudo replication often encountered by other statistical approaches. Although PCA can handle large multivariate datasets of predictor variables, highly correlated variables (using a correlation coefficient threshold of >0.8) were removed to help support the interpretation of axes scores (Table C.1). It is important to acknowledge that there are risks of among site differences being masked when analyzing nested data, with any potential outliers influencing axis loading values, therefore caution must be used when interpreting results. All input variables were standardized by scaling to z-scores (i.e., mean = 0, SD = 1) to allow variables with large ranges (i.e., Drain_Cat measured in km^2) to be comparable to variables with very small ranges (i.e., Fe measured in mg/L) and the number of meaningful axes was estimated using scree-plots and the broken stick rule criterion (MacArthur, 1957). All analyses were run in R (version 4.0.3; R Core Team, 2020) in the RStudio (version 4.0.3; RStudio Team, 2020) environment using *prcomp* (v3.6.2; Venables & Ripley, 2002), *corrplot* (v0.84; Wei & Simko, 2017), and *ggbiplot* (v0.55; Vu, 2011) packages.

RESULTS

Our six study sites represent a range in the basic characteristics required for suitable spawning habitat and were selected based on variables such as water depth, water velocity, and substrate compositions (Table 2.1). Upstream sites (Sites 1 - 3) were characterized by a wide range of water depths (30-50cm), relatively coarser substrates (greater % cobble and %boulder), and faster water velocities (25-40cm/s), while the most downstream site (Site 6) had a moderate water depth (37.5cm), relatively finer substrates (greater % pebbles), and a lower water velocity (25 cm/s). However, there were other abiotic factors that influenced the suitability of Atlantic Salmon habitat beyond just water depth, velocity and substrate composition and contributed to hierarchical differences between sites. Since upstream catchment-scale variables are nested and interact with downstream catchment-, reach-, and site-scale variables, they can alter certain water quality conditions and influence landscape characteristics depending on their locations within the river.

The PCA analysis was complimented by bubble plots (Figure 2.3-Figure 2.5), which illustrated how and where certain variables differed at specific sites, but they only looked at site differences at individual scale levels. The catchment-scale bubble plot (Figure 2.3) illustrated less anthropogenic land uses at upstream sites (Site 1-3), such as relatively lower road densities and percent of catchments clearcut. The reach-scale bubble plot (Figure 2.4) illustrated more natural hydrogeomorphic features at upstream sites (Site 1-3), such as the percent flooded areas. Meanwhile, the site-scale bubble plot (Figure 2.5) also illustrated greater variation in incubations habitat conditions, such as relatively coarser substrates (% cobble) and greater variation in water temperatures (CV_Temp). The bubble plots discussed above (Figure 2.3-2.5) highlight how and where sites are different for water quality and landscape characteristics at multiple spatial scales. Upstream catchments (Site 1-2) were found to have relatively less anthropogenic land uses, such as lower road densities and percent area clearcut. Upstream reaches (Site 1-2) were found to have more hydrogeomorphic features, such as greater percentage of flooded areas, while upstream sites (Site 1-2) had greater variation in habitat characteristics, such as greater water temperature variations (CV_Temp) and coarser substrates (% cobble).

Alternatively, our PCA analysis attempted to explain which variables were driving site variation at both individual and combined spatial scales. Our PCA analyses, when combined at all spatial scales, show that the majority of the variation among sites was primarily being driven by geophysical and anthropogenic landscape characteristics at the catchment scale, which was reflected by differences in substrate and water chemistry variables at the site-scale (Figure 2.6). When variables from all spatial scales (i.e., catchment-, reach, site-scales) were analyzed, PC1 and PC2 explain 67.2% of the variance in the landscape characteristics and in-stream physical and chemical characteristics (Figure 2.6).

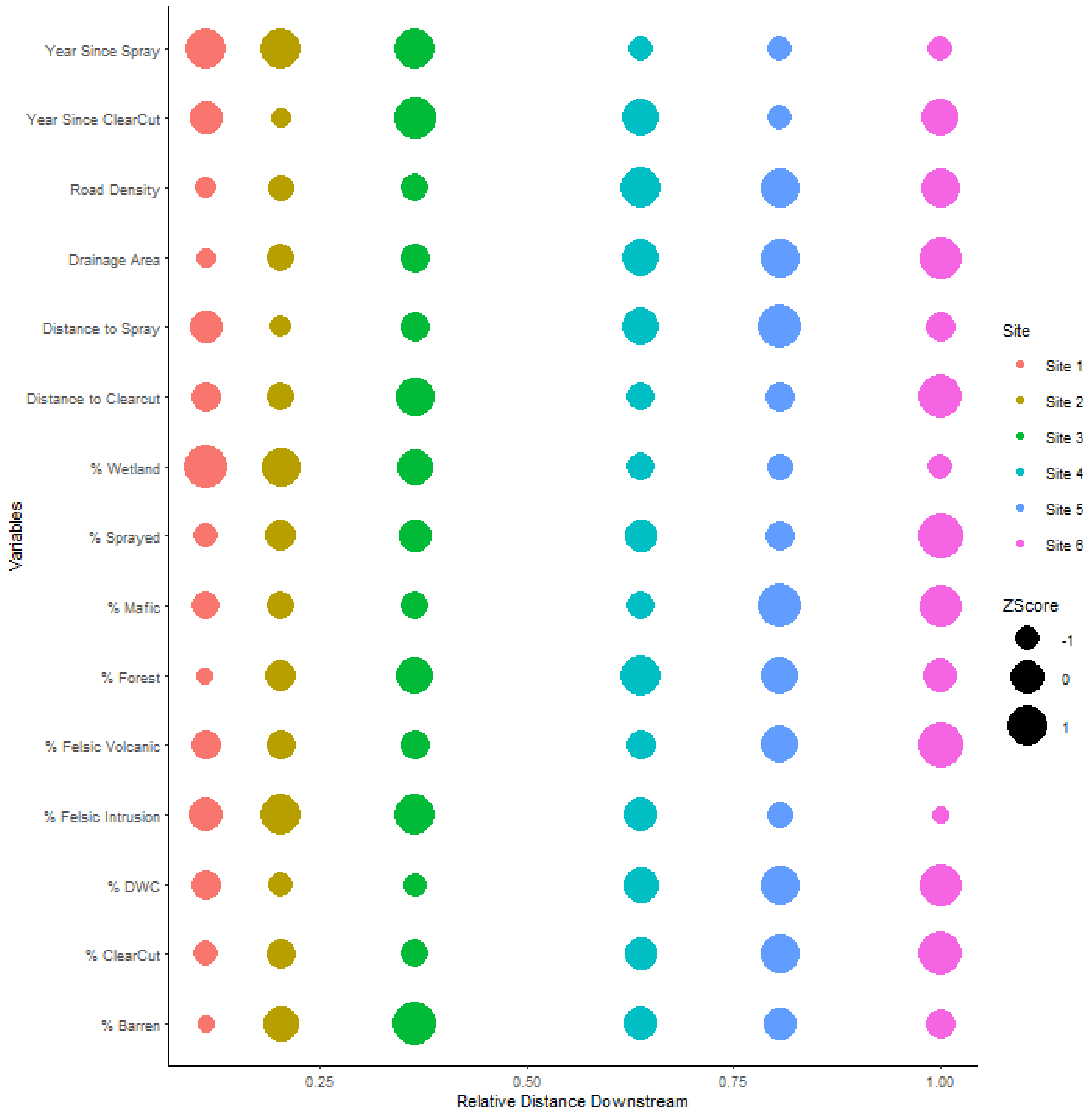

Figure 2.3: Bubbleplot of catchment-scale variables with proportional z-score values showing how environmental variables differ at sites along Serpentine River.

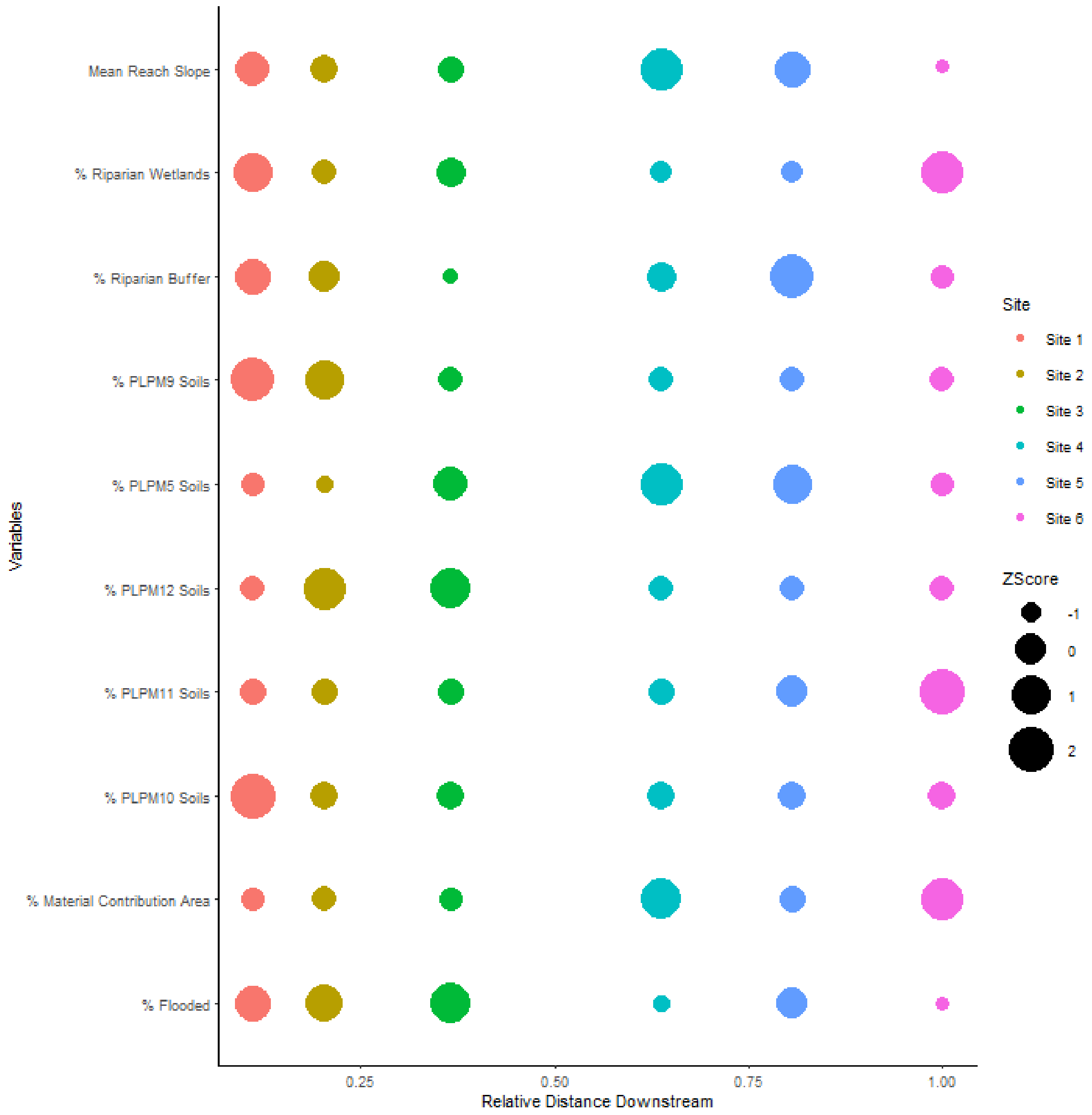

Figure 2.4: Bubbleplot of reach-scale variables with proportional z-score values showing how environmental variables differ at sites along Serpentine River.

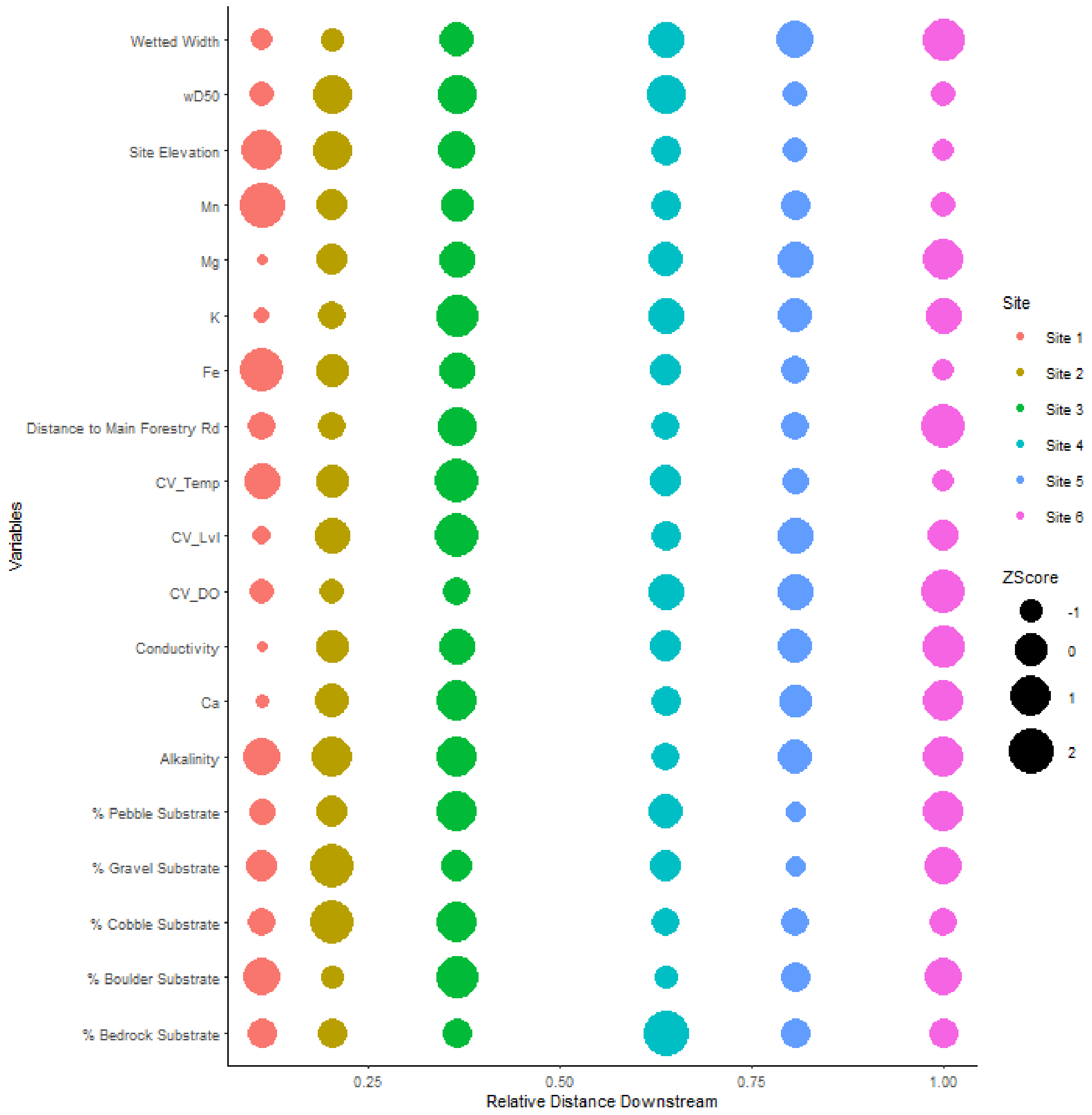

Figure 2.5: Bubbleplot of site-scale variables with proportional z-score values showing how environmental variables differ at sites along Serpentine River.

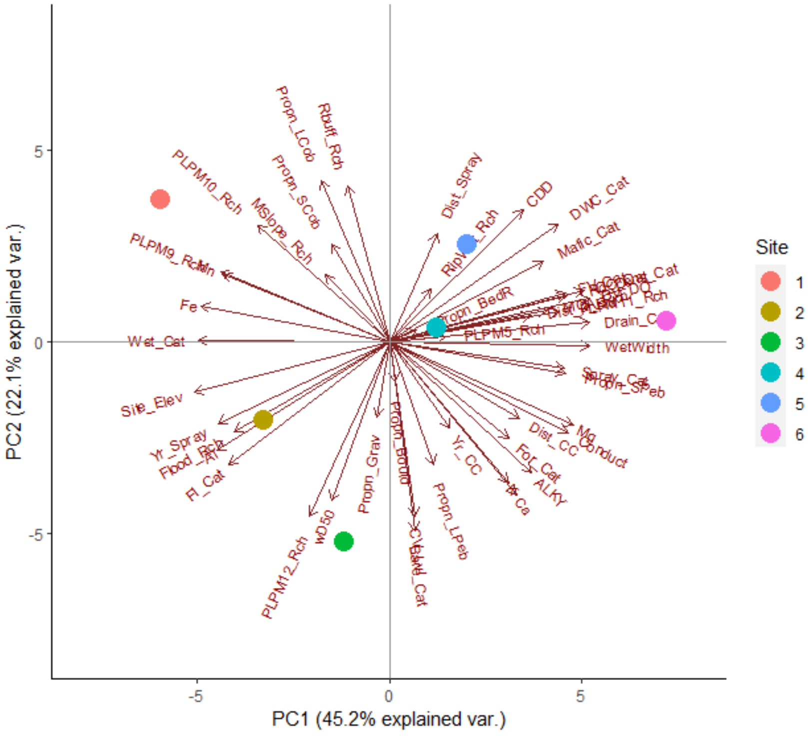

Figure 2.6: Principal Component Analysis (PCA) biplot of catchment-, reach- and site-scale land use, land cover, water quality and substrate variables.

Overall, for PC1 (45.2% of the variation), sites were characterized by loadings from geophysical variables, such as drainage area and site elevation; water chemistry variables, such as the amount of variation in dissolved oxygen concentrations, and both natural and anthropogenic landscape characteristics, such as percent area wetlands or clearcuts. Upstream sites (Site 1 and 2) were positively correlated and align with PC1 given greater percent area of wetlands, higher site elevations and elevated iron (Fe) concentrations while the most downstream site (Site 6) was positively correlated with greater percent area clearcut, larger drainage area and wetted widths, and greater variations in dissolved oxygen. Sites 1 and 2 were both characterized by a greater proportion of wetlands within their local catchments, with granite/felsic dominant forest soil types (PLPM9_Rch/PLPM10_Rch) at the reach-scale influencing Site 1 and relatively greater site elevation and proportion riparian flooded areas (Flood_Rch) at the site- and reach-scale influencing Site 2. Alternatively, Site 6 had the greatest wetted width, metasedimentary forest soil types (PLPM11_Rch) and longest distance from main forestry roads (Dist_2_Rd).

Overall, for PC2 (22.0% of the variation), sites were characterized by loadings from local substrate and water chemistry variables with some minor influence from land cover and land use types at the catchment and reach spatial scales. The variables primarily contributing to the variation in PC2 were substrate size classes, such as proportion gravel (Propn_Grav) and large pebbles (Propn_LPeb), variation in water levels (CV_Lvl), and amount of calcium (Ca), with percent barren (Bare_Cat) and percent riparian buffers (Rbuff_Rch) at the catchment- and reach-scales also influencing among-site variability. Site 3 was positively correlated with proportion gravel substrates (<16mm) at the site-scale, amount of igneous dominant forest soil types (PLPM_12) at the reach-scale and is negatively correlated with the percent area of riparian buffers (i.e., 30m on either side of river). Meanwhile, Sites 4 and 5 were influenced by their relatively greater percent area of clastic bedrock (DWC_Cat) and greater distances from nearest herbicide spray blocks (Dist_Spray) at the catchment-scale, with relatively weak influence from their metamorphic dominant forest soils (PLPM_5) at the reach-scale.

When only catchment-scale variables were analyzed, PC1 and PC2 explained 77.9% of the variance in the landscape characteristics (Figure 2.7). Overall, sites tended to vary based on their drainage area, anthropogenic land use, and natural land cover, which follow a very clear upstream-downstream gradient. For PC1 (61% of the variation), sites were characterized by loadings from geophysical variables, such as drainage area, anthropogenic land use, natural land cover and bedrock geology. The key variables driving variation in PC1 were drainage area (Drain_Cat), percent area of upstream clearcut (CC_Cat), percent deepwater clastic bedrock (DWC_Cat), percent upstream wetlands (Wet_Cat), years since herbicide application (Yr_Spray), and road density (Rd_Dens_Cat). Upstream sites (i.e., Sites 1 and 2) tended to be characterized by natural landscape characteristics, such as greater amounts of wetlands, while downstream sites (i.e., Sites 5 and 6) were increasingly characterized by anthropogenic disturbances, such as greater percent area clearcut, and more recent herbicide applications with greater road densities. In comparison, PC2 (16.9% of the variation) primarily separated sites based on relative natural land cover. The key variables driving variation in PC2 were relative percent area forested (For_Cat) and relative percent area barren (Bare_Cat). Midstream sites (Sites 3 and 4) were characterized by different land cover types, with Site 3 having the most amount of barren land cover (Bare_Cat) and Site 4 having the greatest relative percent area of upstream forested area.

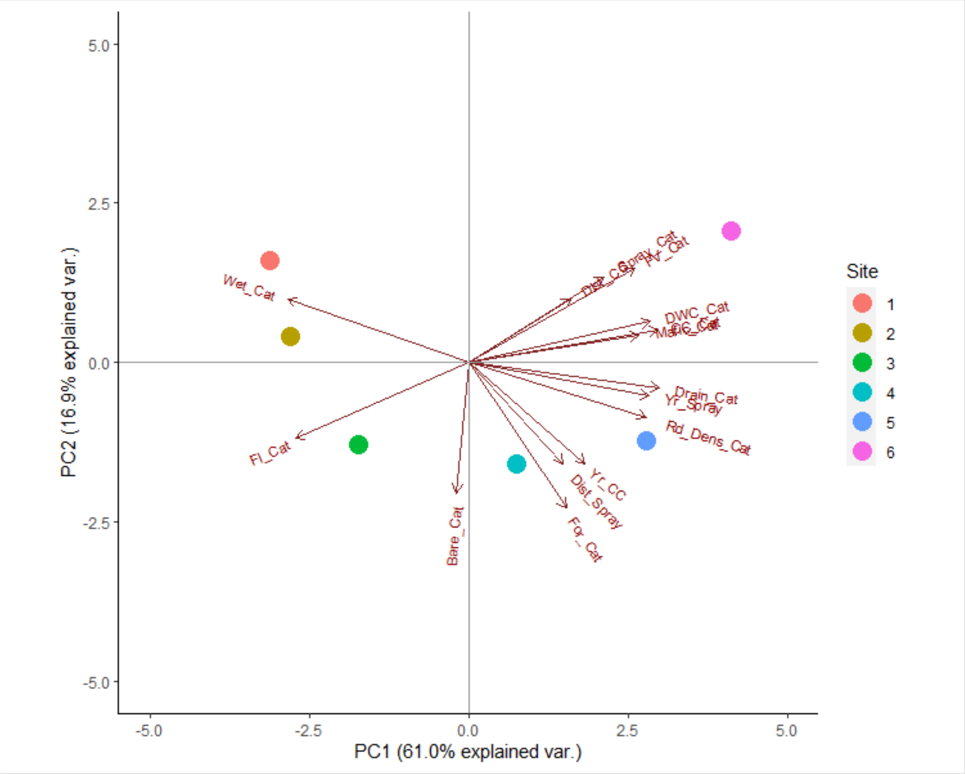

Figure 2.7: Principal Component Analysis (PCA) biplot of catchment-scale land cover and land use variables.

When analyzing only reach-scale variables, PC1 and PC2 explain 66.5% of the variance among sites (Figure 2.8). Overall, for PC1 (35.9% of reach-scale variation), sites varied based on geomorphic and geologic attributes. The variation in PC1 was primarily driven by percent steep slopes (MCA_Rch), flooded area (Flood_Rch), and granitic or metasedimentary dominant forest soils (PLPM9_Rch/PLPM11_Rch). Upstream sites (Sites 1-2) were characterized by greater percent flooded areas and granitic dominant forest soils within their local reaches and riparian areas, while the most downstream site (Site 6) was characterized by the greater percentage of steep slope area and metasedimentary dominant forest soils. Overall, for PC2 (30.6% of reach-scale variation), sites also varied based on geomorphic and geologic attributes. The main variables driving PC2 axis were percent riparian wetlands (RipWet_Rch), mainstream slope gradients (MSlope_Rch), and metamorphic dominant forest soil types (PLPM5_Rch) with a weaker association to metasedimentary dominant forest soil types (PLPM11_Rch). Midstream and downstream sites (Sites 4 and 5) were characterized by higher mainstem slope gradients and greater metamorphic dominant forest soils, while the most downstream site (Site 6) was characterized by a greater percentage of riparian wetlands and greater metasedimentary forest soils.

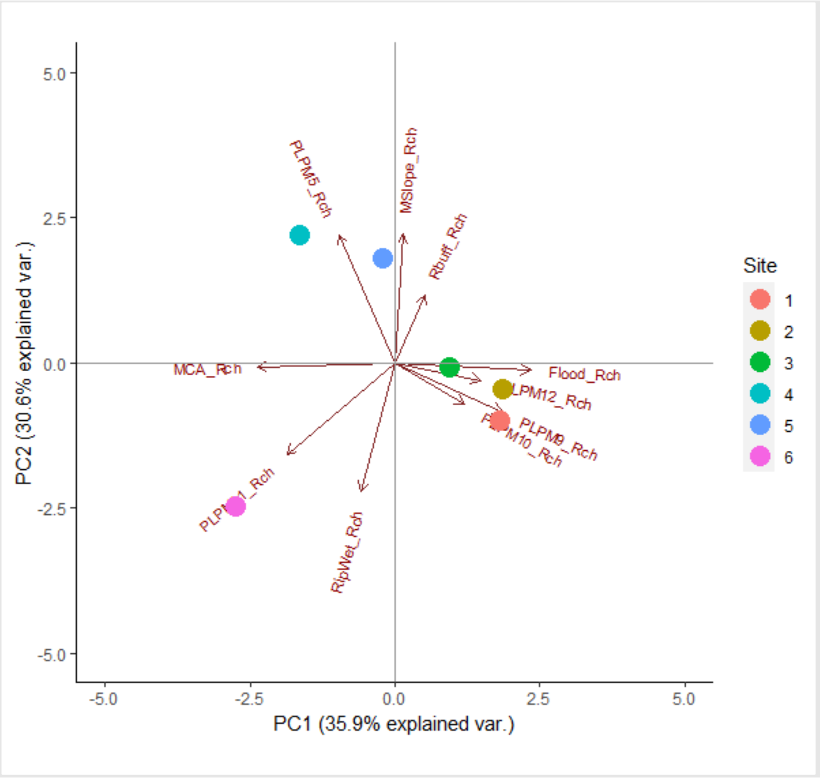

Figure 2.8: Principal Component Analysis (PCA) biplot of reach-scale geomorphic and geologic (i.e., forest soil) variables.

When analyzing only the site-scale physical and chemical characteristics, the outputs from our scree plots and the broken stick criterion suggested that we retain three principal components. PC1-PC3 explained 83.77% of the variance among sites (Figure 2.9). Overall, for PC1 (48.3% of site-scale variance), sites vary based on water chemistry and geographic attributes. The most upstream site (Site 1) was characterized by greater concentrations of certain water minerals and metals, while the most downstream site (Site 6) was characterized by more generalized water chemistry and geographic parameters. PC1 is primarily driven by wetted width (WetWidth), elevation (Site_Elev), variability in dissolved oxygen (CV_DO), conductivity (Conduct) and various water minerals and metals, such as magnesium (Mg) and iron (Fe). Site 1 had higher elevations and amounts of manganese (Mn) and iron, while Site 6 had larger wetted widths, and variation in dissolved oxygen, with concentration of magnesium and greater proportion of small pebble substrate (Propn_SPeb). Alternatively, for PC2 (22.3% of variance) sites varied based on their underlying substrate compositions and specific water chemistry attributes. PC2 was predominantly driven by loadings from substrate variables, such as proportion gravel (Propn_Grav) and large pebble (Propn_LPeb), with variation in water levels (CV_Lvl) and water temperatures (CV_Temp). Sites 3-5 were best characterized by PC2, Site 3 had greater proportion gravel and large pebble substrates with greater relative variation in water levels, while Sites 4 and 5 had greater amounts of bedrock (Propn_BedR) and small cobble (Propn_SCob) substrates.

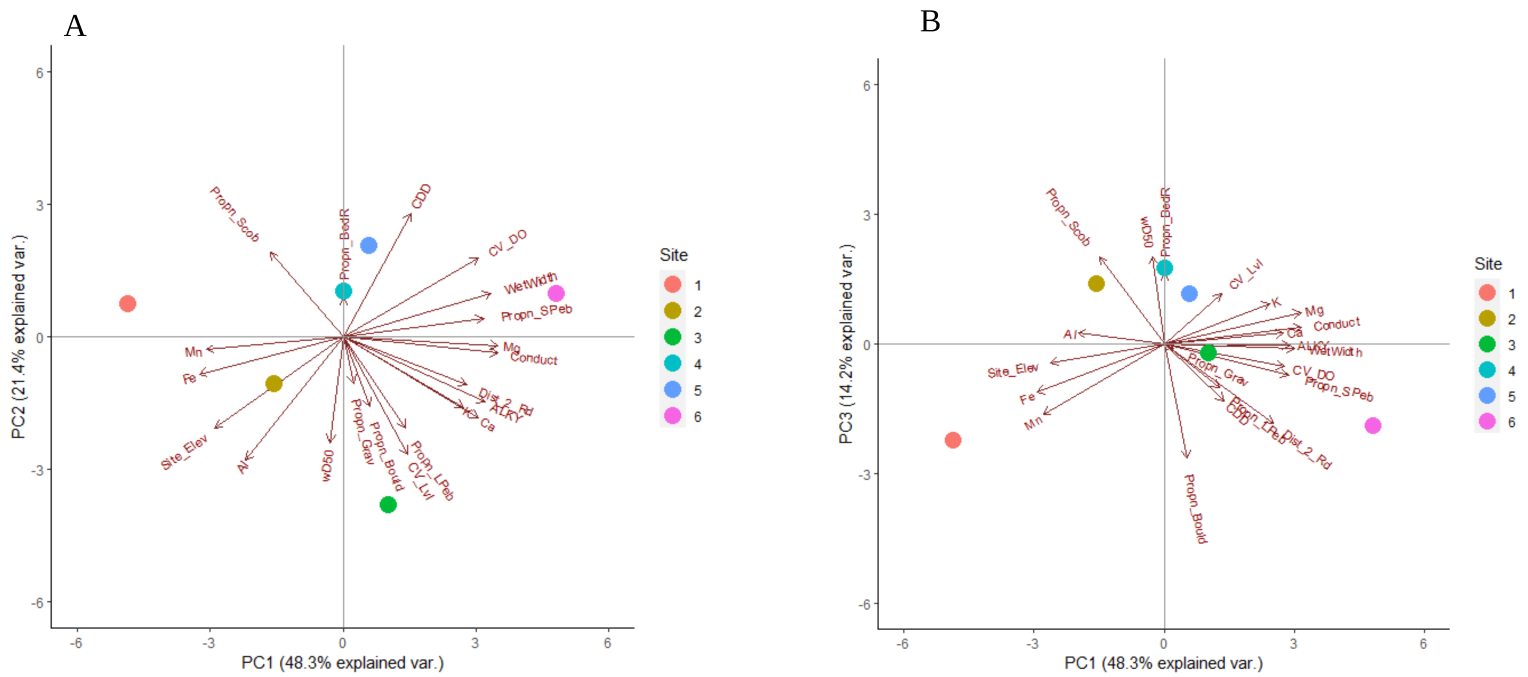

Figure 2.9: Principal Component Analysis (PCA) biplots of site-scale water quality and substrate variables A) PC1 and PC2; B) PC1 and PC3.

Similarly, PC3 (13.2% of site-scale variance) also differentiated sites based on substrate variables, but it also highlighted a potential weak interaction with anthropogenic disturbances. PC3 was most strongly driven by distance to main forestry roads (Dist_2_Rd), variation in water levels and proportion of small cobble (Propn_Scob), and gravel (Propn_Grav) substrates. Site 1 and Site 6 had greater proportion of gravel substrates, with Site 6 having the greatest relative distances from main forestry roads. Meanwhile, Sites 2-5 had larger mean substrate sizes (wD50), proportion of small cobble (Propn_SCob) substrates and variations in water levels, while Site 2 had slightly greater variations in water temperature and Sites 4 and 5 had relatively greater concentrations of potassium (K).

DISCUSSION

Landscape characteristics inherently have spatio-temporal hierarchies, whose organization can influence the variability of instream physical and chemical characteristics and alter the freshwater habitats of Atlantic Salmon (Johnson & Host, 2010; Peterson et al., 2006; Poff et al., 1997). When investigating among-site variability within the Serpentine River, New Brunswick, Canada our findings describe how our sites vary across a longitudinal gradient when analyzed at all combined spatial scales and explain how natural landscape characteristics at the catchment-scale influence downstream variability in site-scale water quality conditions and substrate composition. It is important to understand at what spatial scale upstream landscape characteristics are influencing this among site variation because it highlights how subsequent differences in downstream conditions, such as variable substrate composition or water quality conditions can affect the strength of hierarchal landscape filters (Poff et al., 1997) and the suitability of incubation habitats for Atlantic Salmon embryos.

As seen within the bubble plots (Figures 2.3-2.5) and scale specific (i.e., catchment-, reach-, site-scale) PCA plots (Figures 2.6-2.9) there are clear differences between upstream and downstream sites with multiple environmental variables either increasing or decreasing depending on their relative position within the watershed and the interactions at nested spatial scales. Spatial correlations often exist within stream chemistry data at relatively coarse spatial scales (Peterson et al., 2006), but it is important to explicitly test for spatial autocorrelation to assess the validity of the patterns and confirm proper study design and site selection. Using a Euclidean distance spatial matrix and water chemistry matrix (i.e., variation in dissolved oxygen concentrations) we tested the degree of spatial correlations between sites and given statistical output from a Mantel test we were able to confirm that the six sites were not spatially autocorrelated and therefore not acting as a confounding factor within our analysis. Although variables at individual spatial scales may differ, it is important to understand that freshwater ecosystems are hierarchically nested, meaning the geophysical characteristics (i.e., drainage area) in upstream catchments can influence the geomorphic characteristics (i.e., amount of flooded areas or steep riparian slopes) and geographic characteristics (i.e., elevation and wetted width) within downstream reaches and sites. For example, the watershed ratio area has previously been linked to the variances in upstream and downstream flows estimation methods on ungauged watersheds, which provides useful information on the underlying transport mechanisms that variable flows (i.e., variation in water levels used as proxy measure) have on both water chemistry and substrates (Gianfang et al., 2015). This nested spatial structure of upstream catchments within downstream catchments and the distance between sites means that the drainage area of each downstream site is going to be inherently larger than that of upstream sites. While these variations in physical gradients (i.e., drainage area) may appear obvious given the nested design of our PCA analysis it is still necessary to include them because they highlight important ecological processes. Quantifying changes in downstream watershed topography is important because is has an inherent influence on forest structure and composition (Jucker et al., 2018), which in turn can also influence in-stream water quality conditions and substrate composition. Jucker et al., (2018) found that subtle changes in elevation are linked to changes in soil chemistry and hydrology, therefore it is likely that this variation in physical gradients (i.e., site elevation) within our study produced similar influences on in-stream conditions. Furthermore, the importance of including obvious abiotic variables (i.e., Drain_Cat, Site_Elev, Wet_Width) within or analysis is that it helps illustrate how natural landcover types can sometimes covary with anthropogenic land uses (i.e., forestry operations), which may influence the accuracy of regional bioassessments. When variables from all spatial scales (i.e., catchment, reach, and site) are combined and analyzed it becomes evident how site scale geographic characteristics and reach scale geomorphic characteristics respond to both catchment scale geophysical characteristics and the changing landscape composition (Figure 2.6).

Our findings suggest natural landscape characteristics at the catchment-scale, such as the amount of relative forested area and composition of bedrock geology are intrinsically influenced by this nested spatial hierarchy with the successively larger downstream drainage areas explaining 45.2% of among-site variance (i.e., PC1) when analyzed at all spatial scales (Figure 2.6). These findings are comparable to Tang et al., (2020), who investigated the relative importance of watershed-scale human and natural factors on in-stream physicochemical conditions and how they affected biological conditions (using diatom multimeric index) in streams at ecoregional and national spatial scales in the United States. Tang et al., (2020) found natural factors such as topography, geology, soils, climate and hydrology explained the largest individual proportion of among-site variance of in-stream water quality conditions, which often covary with human factors (i.e. land use) and in regions with high level of human disturbances or small ranges in human disturbances, which can contribute to inaccurate ecological assessments. This among-site variation across a longitudinal gradient is likely due to the nested spatial structure of catchment-scale variables, which incorporate successively larger downstream drainage areas, which can either exacerbate or alleviate the impact of upstream landscape characteristics depending on their relative position within the river (Townsend et al., 2003). Our upstream sites (Site 1-2) are primarily dominated by greater proportions of felsic geology (60-67%) rich in metals such as iron and manganese, while downstream sites (Site 5-6) have more heterogeneous compositions with lower proportions of felsic bedrock geology (52-45%). It is predominantly these variations in bedrock geology and the topographically influenced interactions with different flow regimes at different spatial scales that can influence key water chemistry and substrate characteristics in downstream freshwater habitats.

Historically, cumulative impacts from anthropogenic landscape characteristics at the catchment- and reach-scale, such as land use from forestry activities (i.e., large clear-cut area and small riparian buffers), have often explained variation in site-scale physical and chemical conditions and downstream freshwater habitats. Forestry activities in Sweden have previously been linked to changes in water chemistry, mainly at reach-scales, with increased nitrogen leaching found in areas with little or no forested riparian buffers (Löfgren et al., 2009). However, anthropogenic induced sedimentation, such as the increased composition of non-forested land cover at catchment-scales, has also been associated with the alteration of substrate compositions and fish assemblages in the streams of North Carolina, USA (Sutherland et al., 2002). Generally, catchments with increasing levels of non-forested land cover were shown to have increased suspended sediment concentrations, greater bedload transport capacities and overall greater streambed instability, which can persist across long distances and influence the suitability of downstream freshwater habitats for fishes requiring clean cobble/gravel substratum for spawning (Sutherland et al., 2002). Our upstream sites (Site 1-2) had relatively lower amounts of clearcut areas (11-12%) that are often associated with forestry related disturbances within non-forested landscapes, while our midstream sites (Site 3-4) only had marginal increases (11.6-13.1%). Meanwhile, within our downstream sites (Site 5-6) we observed the greatest amount of clearcut area (15-16.6%) per catchment but given that we did not directly measure suspended sediments and the limited temporal period of our analysis (Nov 2021-Jan 2022) its direct impact on substrate composition remains unclear. Additionally, although there was a large range in the amount of riparian buffers at between upstream and downstream reaches (0.3%-17.4%), they did not explain the same amount of variation as catchment scale land use did (Figure 2.7 and Figure 2.8). Despite the presence of non-forested land and marginal riparian buffers at the catchment- and reach-scales within the Serpentine River, we did not observe similar alterations in physical and chemical conditions from anthropogenic landscape characteristics alone.

Although other upstream anthropogenic landscape characteristics (i.e., road density) can also impact site-scale physical and chemical characteristics these impacts can be mitigated, especially if subsequent downstream catchments are dominated by natural landscape characteristics (i.e., increasing forested area). Multiple studies have highlighted that anthropogenic landscape characteristics, such as percent urban or agriculture at smaller reach and site- scales accounted for the most among site variance of in-stream conditions (Ou et al., 2016; Shi et al., 2017; Su et al., 2015; Sun et al., 2010; Wu et al., 2021). However, the differences in regional settings and water quality variables of interest between these studies raise doubt regarding at which spatial scale (i.e., reach- vs. site-scale) they have the greatest influence on instream physical and chemical characteristics (Zorzal-Almeida et al., 2018). Given the regional history of forestry operations within the Serpentine River watershed, we were expecting a strong association with anthropogenic landscape characteristics at the catchment-scale, especially road density and percent area clearcut to influence water quality conditions and substrate composition, as other New Brunswick researchers have previously observed (Erdozain et al., 2018). Our sites vary along a longitudinal gradient in road density, with upstream (Site 1 -2) catchments having relatively low densities (1.65 – 1.74 km/km2), which increase within mid-stream (Site 3-4) catchments (1.78-2.21 km/km2), but slightly decrease within downstream (Site 5-6) catchments (2.16-2.17 km/km2). However, the explanation of why we did not observe a similar impact from anthropogenic landscape characteristics as other researchers is likely due to differences in study and sampling design, our relatively low ranges of percent land use composition (i.e., only 5.6% difference in area clearcut and 6.6% difference in forested area between upstream and downstream sites), and potential covariation with natural landscape characteristics. It appears that the increasing amount of anthropogenic activities (i.e., increasing road densities and percent area clearcut) within downstream catchments are offset by the coinciding increase in natural landscape characteristics. Our upstream catchments (Site 1

-2) and mid-stream catchments (Site 3-4) reflect an increasing amount of forested areas along the longitudinal gradient, which increase from 72.6% at Site 1 to 79.3% at Site 4. Conversely, the amount of forested area within downstream (Site 5-6) catchments slightly decreases, from 77.9% at Site 5 to 76.8% at Site 6, but still remains quite high.

Where the focus of the Erdozain et al. (2018) study was exclusively on headwater streams (i.e., stream order 1-3), our study was more focused on the differences along a more longitudinal gradient, ranging from upstream sites to downstream sites along the main stem of the Serpentine river (i.e. stream order 4 -5). As previous research has indicated, freshwater habitats and by extension the physical, chemical, and landscape characteristics that shape them, are organized along a continuum and nested within hierarchies that interact with each other as they flow downstream (Frissell et al., 1986; Poff et al., 1997; Vannote et al., 1980). Given the results of our analysis the forestry activities within this landscape do not seem to translate into any measurable effect on Atlantic Salmon incubation habitat from November 2020-January 2021. However, the small range (i.e., 11-17%) of percent area clearcut and potential covariance with natural landscape characteristics, such as topography and forested landcover make it difficult to interpret (Tang et al., 2020). Therefore, to gain greater insight into what is driving among-site variation within the Serpentine River we must instead focus on natural landscape characteristics and investigate how their spatial arrangement facilitates the exacerbation or amelioration of key physical and chemical characteristics.

It has also been argued that site substrates are influenced by reach-scale hydrogeomorphic characteristics, such as instream channel slope, which has the potential to influence streamflow and alter streambank bank erosion rates, especially within anthropogenically modified landscapes (Prosser et al., 2001). However, in-stream channel slope within rivers, and the transport of sediment and woody debris that influences channel morphology are ultimately mediated by upstream processes, such as landscape and streambed erosion rates. As Whitebread et al. (2015) highlighted when investigating postglacial streams in the Scottish Highlands, the geometry or shape of the instream channels, and the underlying substrates of which they are composed of control the erosion rate. Typically, substrates within rivers generally follow a predictable pattern, with headwaters characterized by coarser substrates and more downstream areas characterized by finer substrates (Church, 2015). Whitbread et al. (2015) demonstrated that bedrock channels are often narrower and deeper than alluvial channels and do not covary with slope, but rather are associated with increased discharge (or flow) and sediment flux. These findings are consistent with our own where an increased proportion of gravel substrates were observed at Site 3 with a corresponding increase in the amount of small cobble substrates at Site 4, which seems to suggest a potential flow and sediment flux between these two sites.

The differences in site substrate composition within the Serpentine River are scale-dependent and are influenced by its location along the longitudinal gradient, which reflects the relatively homogenous landscape characteristics at headwater sites and more heterogeneous landscapes at downstream sites. In Buendia et al. (2013) when investigating fine sediment loads in Spanish rivers, the runoff from hillslopes was a significant source of sediment, and although reach-scale hydraulics influenced freshwater habitats and biotic assemblages, they were ultimately governed by upstream catchment landscape characteristics. These findings are similar to our own, where Site 3 had the lowest percentage of riparian buffers and largest amount of upstream flooded areas (i.e., at Site 2) which could be contributing to locally elevated proportions of gravel (2-16mm) substrates due to increased erosion potential and disturbance of upstream substrates during water level fluctuations (i.e., episodic floods).

Within the Serpentine River watershed natural landscape characteristics appear to better explain among-site variation, with the differences in site substrate and water chemistry variables reflecting coarse catchment-scale variations in topography, bedrock geology and land cover. These findings are consistent with similar studies done in France (Tudesque et al., 2014), China (Zhang et al., 2019) and South Africa (Mwaijengo et al., 2020) where they found catchment-scale landscape characteristics better explained among site variation in water quality than at the riparian scale. Another explanation of why our reach-scale variables were not as influential at the combined spatial scale analysis (Figure 2.6) is due to our lack of traditional anthropogenic landscape characteristics, such as different land use types, which were necessary to remove during the feature selection process to avoid issues of circularity with catchment-scale variables. With catchment-scale landscape characteristics established as influencing the majority of among-site variation within the Serpentine River, future research should focus on the use of biotic data to verify these findings. It is also important to note that due to conservative and reactive solutes dynamics many water chemistry parameters can be influenced by dilution from upstream drainage areas or persist within the water column (Baker & Webster, 2017), especially from resuspension of sediments via ice scour during winter ice freeze and thaw cycles (Turcotte et al., 2019). With a better understanding of how catchment-scale landscape characteristics influence downstream freshwater habitats within the Serpentine River we can begin to predict how future changes to natural landscape characteristics, via climate change or increased silviculture operations, may impact the organisms that inhabit them.

Overall, natural landscape characteristics at the catchment-scale are driving the most amount of variability in physical and chemical characteristics between sites, with reach-scale variations reflecting upstream topography and geologic characteristics. We were able to confirm our hypothesis that catchment-scale topography and varying water levels (i.e., proxy measure for flow regimes) were likely the primary mechanism influencing how upstream landscape characteristics shape downstream water chemistry and substrate conditions. It is this interaction of topography and surficial/bedrock geology at the reach- and catchment-scales that reflect the accumulation of minerals and dilution of harmful metals (Fe) in downstream sites. Although we did not observe a strong influence of anthropogenic influences on sedimentation and substrate composition it is likely because of differences in study and sampling design, relatively low ranges of landcover types (i.e., 5.6% difference in percent area clearcut and 6.6% difference in forested areas between sites) and potential covariation with natural landscape characteristics. With variations in the physical and chemical characteristic between our sites within the Serpentine River future research should expand the temporal analysis to investigate interannual variations, especially within the lens of climate change and further explore how living organisms (i.e., fish embryos or benthic invertebrates) respond to these changes at different times of the year.

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CHAPTER 3: EMBRYO MORTALITIES & DEFORMITIES

ABSTRACT

In-stream incubation of Atlantic Salmon (*Salmo salar*) embryos within Jordan/Scotty incubation boxes is a promising alternative to traditional stocking methods, which often rely on hatchery reared smolts or adults to bolster depressed wild populations. However, there is a significant knowledge gap on the development of Atlantic Salmon embryos, and more specifically the prevalence of mortalities and deformities within the pre-eyed stages of field reared embryos. Embryonic mortalities have a detrimental impact on recruitment and overall population structure, but deformities at the pre-eyed stage can also negatively influence their growth and development at later life cycle stages, which has the potential to reduce overall freshwater resilience and negatively impact their survival. Here I aim to investigate what environmental factors might be responsible for previously documented embryo mortalities and deformities within the Serpentine River watershed, a major tributary to the Tobique River. During November 11th,2020 and January 9th, 2021, I incubated a total of 19200 hatchery-fertilized Atlantic Salmon (*Salmo salar*) embryos within river substrates at six field sites along the Serpentine River using Jordan/Scotty incubation boxes, with 4 bundles per site, 3 plates per bundle, and 200 egg per plate. Overall, the proportion of embryo mortalities within our Jordan/Scotty incubators were high, but still within the natural range observed in wild Atlantic Salmon redds and other incubation experiments (8096/18000=44.9%). The remaining alive embryos (n=9904) were assessed for deformities, of which only a very small proportion were classified as deformed

(265/9904 = 2.7%). Our observations of embryonic deformities do not support the findings from previous research conducted in 2013 (up to 86% deformity) at similar incubation sites within the Serpentine River, which confirms the field deformity phenomena was not occurring during 2020-2021. However, given the results of the chi-square test of independence some of the 32 random (1 male x 1 female) crosses used for this experiment had statically significant ($p<0.001$) high proportions of mortality when raised within traditional hatchery flow through troughs. It is therefore recommended that any future *in-situ* incubation experiments investigate how hatchery rearing may influence the survival and development of incubated embryos as it could have important implications for increasing their freshwater resilience and stabilizing population structure of wild Atlantic Salmon.

INTRODUCTION

Atlantic Salmon *(Salmo salar)* is considered an indicator species, meaning their presence is directly related to the overall health of the ecosystems they inhabit (Rodriguez, 2019). Atlantic Salmon populations across the globe have been rapidly declining in the last few decades, with many populations in Atlantic Canada being listed as threatened or endangered under by COSEWIC (COSEWIC, 2010; NASCO, 2019). As Atlantic Salmon are anadromous, meaning they use both freshwater and marine environments, and are iteroparous, meaning they can return multiple times to spawn as adults, it causes them to face a myriad of stressors and pressures (Reddin et al., 2012). A pressure is defined here as any disruption from the natural environmental conditions, such as landscape disturbance from clear-cutting activities, while a stressor is defined by its response to pressures, such as increased water temperatures, sedimentation, or reduced dissolved oxygen (DO) concentrations.

Globally declining Atlantic Salmon populations have often been linked to poor marine survival, with rising ocean water temperatures, acidification, increased predation, and prevalence of sea lice cited as common pressures (Vollset et al., 2019). Conversely, pressures and stressors to the freshwater environment are more well-known and studied, such as influence from aquatic barriers such as dams and in-stream flow alterations, or pollution and reduced water quality (Forseth et al., 2017). However, there are still some emerging unknown pressures within freshwater environments, such as the potential toxicity of new herbicide formulas, such as glyphosate or its metabolite AMPA (aminomethylphosonic acid), and it potential to leach into river sediments and influence the development of Atlantic Salmon embryos. There is an innate ecological and biodiversity value of preserving Atlantic Salmon, which the international community recognized in 1983 by the North Atlantic Salmon Conservation Organization (NASCO). There are also important social, economic and cultural values of Atlantic Salmon. In the Miramichi River watershed, located in New Brunswick, Canada the economic value of Atlantic Salmon from recreational fisheries alone was $19.1 million in 2011, which represents an important sector of the provincial economy (Gardner Pinfold., 2011). There is also an ongoing debate within the scientific community on how best to ensure the survival of Atlantic Salmon, but some researchers suggest the best strategy to minimize the impact of marine mortality is to maximize freshwater (i.e., embryo - smolt) survival and resilience (Thorstad et al., 2021).

Atlantic Salmon begin their freshwater life cycle stage as eggs (i.e., deposited in redds) and typically spend 2 - 4 years in the freshwater environment as alevin, fry, parr and smolt, and 1-4 years in the marine environment before returning to natal rivers to spawn as adults. There have been numerous studies done on the juvenile and adult life cycle stages of Atlantic Salmon (Bisaillon et al., 2007; Pardo et al., 2021), but relatively little is known about the very early embryonic life cycle stages (i.e., pre-eyed stage) of incubating embryos, especially in non-aquaculture settings. There are, however, some notable exceptions, Lavery (2017) investigated the cumulative impacts of abiotic factors on the instream incubation success of embryos buried within substrates of the Miramichi River, New Brunswick using Jordan/Scotty incubators. Similar *in-situ* incubation studies were also conducted by (Flanagan, 2003), who investigated how the survival of embryos was impacted by fine sediment, intergravel water temperatures, and variable flow regimes associated with hydropower generation activities within the Tobique River, New Brunswick.

An in-stream incubation experiment conducted in 2013 observed greater than 80% deformities in embryos collected from Jordan/Scotty incubators at sites within the Tobique River watershed (A. Bartlett, unpub. data). However, the contributing extraneous factors for the embryonic deformities were not determined during that study but such a deformation rate could have a drastic impact on the overall viability of the larger population structure if that is an ongoing phenomenon. The different types of embryonic deformities observed during both previous (2013) and current (2021) incubation experiments include skeletal, ocular, and yolk sac types of malformations. Embryonic skeletal deformities can include lordosis, which is an abnormal ventral curvature of the vertebral column (i.e., spine is almost folded in half), which can negatively impact swimming ability and create increased metabolic costs. Ocular deformities, such as small eye cups or partial blindness (i.e., missing eye cusps) were observed during 2013 study, which can negatively impact navigation, feeding, and prey avoidance behaviour (Powell et al., 2009). Additionally, some embryonic deformities experienced during incubation, such as small yolk sacs can have immediate impacts on growth and development (i.e., less available yolk to absorb during incubation). Meanwhile other yolk sac deformities can exhibit latent effects, such as blue sac disease (i.e., abnormal swelling of yolk sac due to exposure to cold incubation temperatures or toxic substances) which can negatively impact respiration and feeding behaviour and can contribute to mortality during post-hatch (i.e., alevin or fry) life cycle stages (Finn, 2007).

Quantifying the current extent of embryonic deformities within Atlantic Salmon embryos is important because the reduced swimming ability and associated metabolic costs can reduce their overall fitness, especially at later life cycle stages (i.e., smolts) (Powell et al., 2009), and potentially negate the benefits of in-stream incubation efforts if deformity rates are abnormally high. Therefore, further investigation and understanding of the environmental factors that contribute to deformities within the Serpentine River is needed to ensure future viability of Atlantic Salmon populations within the Tobique River watershed.

Winter in Atlantic Canada is a dynamic season often associated with low air and water temperatures, variable ice conditions and changing channel flows which can pose significant challenges for incubating embryos within river substrates. As climate change intensifies there are likely to be more freeze-thaw cycles that have the potential to further alter river habitats (Turcotte et al., 2019). As both the physical and thermal characteristics of river incubation habitats become disrupted it may have corresponding impacts on the embryonic life cycle stages of Atlantic Salmon (e.g., disrupt growth, development and timing of hatching or swim-up stages), which can further imperil the sustainability of wild populations (Rooke et al., 2020). Therefore, it is increasingly important to understand how these overwinter incubation conditions may be contributing to embryonic mortalities and deformities.

Atlantic Salmon embryos are especially sensitive to water temperature and dissolved oxygen fluctuations because these variables directly influence their metabolism and how fast they can grow and develop, with extreme fluctuations often contributing to deformities or mortalities (Levasseur et al., 2006). There is an inverse relationship between water temperature and dissolved oxygen concentration, warmer water temperatures are less able hold dissolved oxygen concentrations while cooler water temperatures have a greater capacity hold dissolved oxygen concentrations. Incubating embryos exposed to extreme water temperature fluctuations or prolonged low dissolved oxygen concentrations (>24hr) at very early life cycle stages have previously been linked to mortality and skeletal or yolk sac deformities, which only become discernable at the eyed stage of development or manifest at later life cycle stages (Finn, 2007; Ørnsrud et al., 2004, Levasseur et al., 2006). Hyporheic interstitial flow, or the flow of water through the sand, gravel, and streambed sediments, is also a contributing factor to incubating embryos as it is the primary mechanism that delivers DO and periodically flushes sediments within redds, which are an important factor whose disruption can also indirectly contribute to embryo mortalities and deformities.

DO concentration within redds (natural or artificial) can also be influenced by accumulation of fine sediments (<2.0mm), especially as it can disrupt hyporheic flows during a time of the year when the influence of water temperature on DO is somewhat muted (i.e., impact of solar radiation on water temperature is reduced due to the high albedo of ice cover). As fine sediments fill in the interstitial spaces within redds, they can alter hyporheic flows which can reduce delivery of DO to incubating embryos and indirectly limit the availability of dissolved oxygen available within the surrounding substrates. Dissolved oxygen concentration within substrates is naturally variable and occur along a gradient which can fluctuate hourly, daily, and throughout the entire incubation period based on several factors, including changes to local flow regimes or altered regional precipitation patterns (Reeder et al., 2018; Sear et al., 2014; Birkel et al., 2013). In extreme cases when DO concentrations remain depressed for prolonged periods of time (>24h) there is a potential for incubating embryos to suffocate, which can result in mortality. Additionally, the spatial location of incubation sites within a watershed (i.e., upstream reaches vs downstream reaches) can also influence DO concentrations within redds, especially as upstream reaches typically have larger substrates and less fine sediments facilitating greater hyporheic flows. It is also important to consider how certain upstream landscape characteristics, such as forested or non-forested land cover (i.e., clearcut area) can introduce additional sediments into water column and disrupt hyporheic flows.

Fine sediments such as silt, clay and fine sand have been known to cover the outer layer of the embryo (i.e., chorion) and limit the DO exchange across the membrane regardless of DO availability within surrounding substrates. Sediment accumulation within the hyporheic environment can vary based on the type of streambed sediment (and their specific erodibility characteristics), potential inputs from surface waters, and localized variations in hyporheic flows (Tonina, 2013). In extreme cases when sediments are not periodically flushed from redds by flood pulses or changes in flow regimes, there is a potential for silt, clay, and fine sand to accumulate on the embryo chorion and smother them, which can result in mortality. However, quantifying hyporheic flows and DO concentrations within substrates during winter months remains difficult due to the complex spatial and temporal variations within and across streams, as well as the challenges ice formation event pose for monitoring equipment. Likewise, it can also be difficult to quantify both the temporal and spatial variation of accumulation of fine sediments within redds throughout the winter months given the unique depositional characteristics within and across sites and similar challenges posed by ice formation events on monitoring equipment. Understanding the direct or indirect disruption of dissolved oxygen to incubating Atlantic Salmon embryos via sediment and water temperature influence are vital to their survival because as they consume their yolk sac (via oxidative processes) metabolic wastes such as carbon dioxide can build up within the egg and must be expelled or diffused across the chorion (Danner, 2008). When incubating embryos are exposed to low concentrations of DO, either directly or indirectly, it is a condition known as hypoxia, which reduces their metabolism and ability to expel waste (Danner, 2008; Shang et al., 2004). Atlantic Salmon can tolerate periodic exposures to hypoxic incubation conditions, but as the magnitude, frequency, and duration of these events increase it will have a negative influence on their growth and development and may contribute to the development of deformities.

Exposure to naturally variable water temperature and flows promote comparatively greater fitness in offspring that have been incubated within their natural freshwater habitat, rather than in a hatchery setting (Milot et al., 2013). With recent advances in embryo incubation equipment, such as Jordan/Scotty boxes, in-stream incubation projects and supplementation of fish at the egg stage are now becoming a low-cost alternative to traditional hatchery rearing programs, as seen with other salmonid species in Western Canada (Conley et al., 2020).

Here I aim to investigate how site-specific water quality conditions influence the variation in Atlantic Salmon embryo mortalities and deformities during their early winter incubation within the Serpentine River, New Brunswick, Canada. I hypothesized that changes in both water temperature and dissolved concentrations will contribute to embryo mortalities and deformities, with similar impacts on survival and development caused by fine and coarse sediment within incubators which can reduce hyporheic flows. I predicted that there will be greater mortalities at sites with lower water temperatures (i.e., lower cumulative degree days) and greater amount of fine sediment within incubators. I also predicted that there will be greater deformities at sites with lower DO concentration and greater amount of coarse sediment within incubators.

METHODS

Study Site Selection

The Tobique River watershed is located within northwestern New Brunswick and is a major tributary to the Wolastoq | Saint John River drainage basin. The Serpentine River is a major tributary to the Tobique River and is situated within the northeastern part of the watershed, with its confluence located upstream from the nearby community of Nictau, New Brunswick, Canada. Six study sites were selected along the mainstem Serpentine River (Figure 3.1) as *in situ* incubation sites to assess Atlantic Salmon (*Salmo salar*) embryo mortalities and deformities within Jordan/Scotty incubators. Consideration for adding additional sites were made, especially given the small sample size and the potential implications for statistical power. However, adding additional sites were ultimately not feasible given the limited availability of Atlantic Salmon embryos (i.e., threatened species), their sensitivity to excessive handling and restrictions of installation equipment (200 embryos/plate), therefore this was the best available compromise.

The Serpentine River drains 394 km² and ranges in elevation from 321-766m above sea level (ASL). The Serpentine River spans two ecoregions (Highlands & Central Uplands) and four eco-districts (Serpentine, Sisson, Beadle, and Ganong). Ecoregions are large geographic areas defined primarily by their similar range in climatic conditions (i.e., temperature, precipitation, and humidity), which are shaped by major landforms, elevation, latitude and other broad aspects that influence distribution of plant and animal species (Zelazny et al., 2007). Eco-districts are sub-components of an ecoregion and are characterized by smaller more distinctive groups of landforms, relief, geology, soils, vegetation, fauna and water bodies (Zelazny et al., 2007). The Serpentine River which has a predominantly cool, wet climate (i.e., annual average of 1119 mm across 160 days of precipitation), with naturally confined steep slopes that support a mixed forest of balsam fir, spruce, and tolerant hardwoods (Environment and Climate Change Canada, 2020). The dominant land cover type is forested (77%), which is interspersed with relatively high amount of clearcutting (17% of watershed harvested since 2010) and road networks (9%) due to ongoing silviculture operations (Hansen et al., 2018; NB DNRED, 2020). Individual sites were chosen based on spawning habitat criteria for Atlantic Salmon, proximity to various anthropogenic land uses, and logistical considerations for winter site access. Generally, Atlantic Salmon prefer to spawn in relatively deep, swift-velocity habitats (20-50 cm, 35-65 cm/s) and prefer using pebbles (16-64 mm) for spawning substrates (Louhi et al., 2008). When selecting final locations to incubate our embryos within each site (via Jordan/Scotty incubators) we attempted to position them within the tail end of a pool and the crest of a downstream riffle to maximize potential hyporheic flows, thereby encouraging occasional flushing of sediments and ensuring adequate delivery of dissolved oxygen. Additionally, to capture the cross-sectional variation in incubation habitat at each site the Jordan/Scotty incubators were positioned across the wetted width of the river at equal intervals from the thalweg across the channel. The selected sites represent a range of water depths, flows and substrate compositions that reflect the diverse spawning habitats available throughout the watershed (

Table 3.1).

Upstream sites (Sites 1 – 2) were generally characterized by coarser substrates while the most downstream site (Site 6) tended to have finer substrates with notable exceptions at Site 5, and large variability in water flows, especially at site 3. Substrate particle size distribution was characterized at each site, which followed the Wolman pebble count and record the intermediate axis of 100 randomly selected stones by following a cross-sectional zig-zag pattern (Wolman, 1954; Environment Canada, 2012). Regular water depth measurements were taken during site visits (September – October 2020) and during incubator deployment (November 2020), which were used in the calculation of the coefficient of variation in water levels.

Site	Mean Recorded Depth (cm) ± SD	Mean Recorded Velocity (cm/s) ± SD	Percentage Gravel Substrate (2-16mm)	Percentage Pebble Substrate (16-64mm)	Percentage Cobble Substrate (64-256mm)
1	29.9 ± 0.04	25.1 ± 0.01	1.0	53.0	46.0
2	39.6 ± 0.11	29.3 ± 0.10	3.0	57.6	39.4
3	49.6 ± 0.19	41.3 ± 0.16	1.1	69.2	29.7
4	32.4 ± 0.06	23.4 ± 0.05	1.0	61.9	37.1
5	35.1 ± 0.09	23.1 ± 0.07	--	49.0	51.0
6	37.5 ± 0.08	25.1 ± 0.02	2.0	70.0	28.0

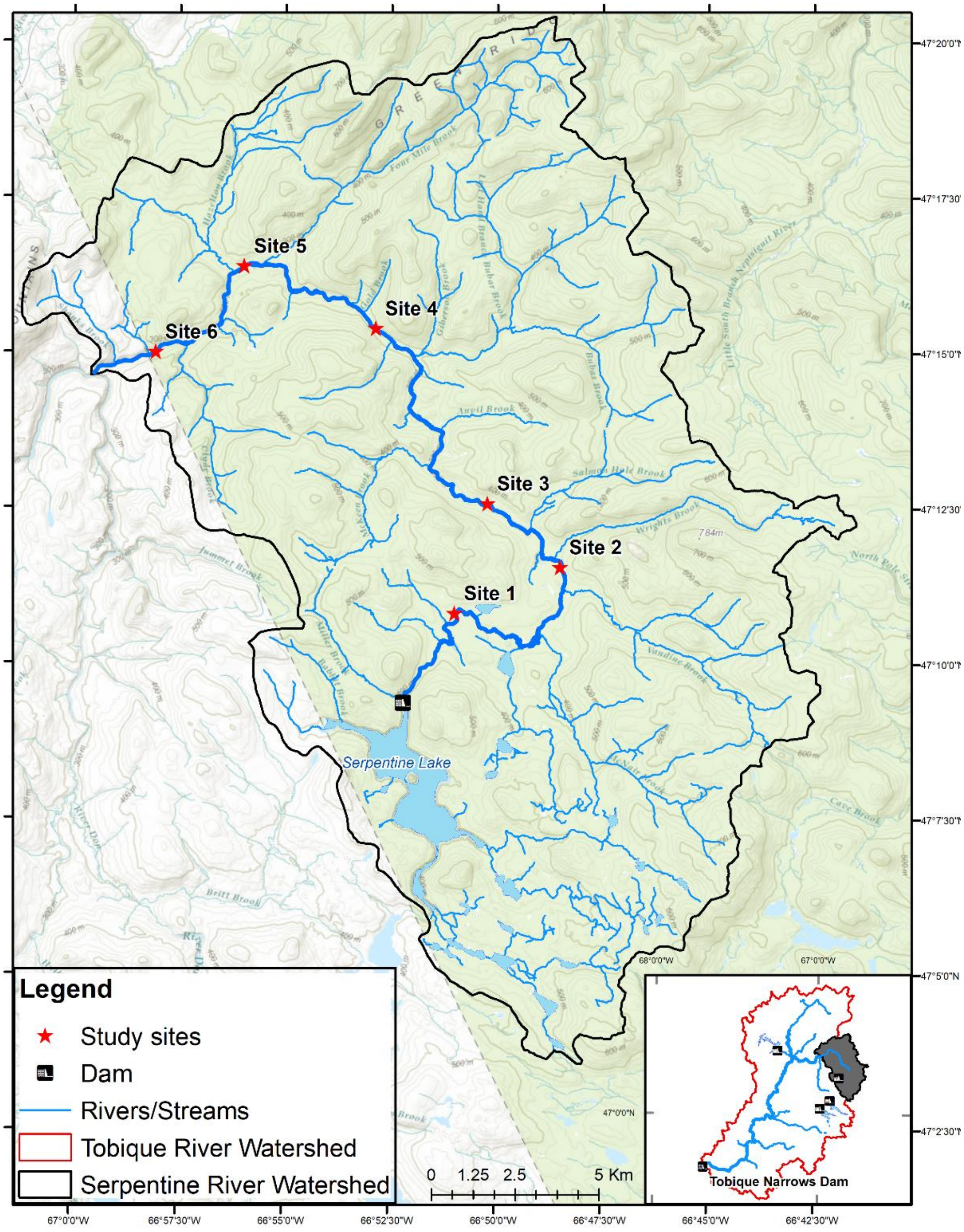

Figure 3.1: Map of the Serpentine River, a major tributary to the Tobique River. The inset map shows the location of the Serpentine River within the Tobique River watershed in northwestern New Brunswick.

Table 3.1: Spawning site selection criteria (water depth, velocity, and substrate compositions).

Site	Mean Recorded Depth (cm) ± SD	Mean Recorded Velocity (cm/s) ± SD	Percentage Gravel Substrate (2-16mm)	Percentage Pebble Substrate (16-64mm)	Percentage Cobble Substrate (64-256mm)
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5	35.1 ± 0.09	23.1 ± 0.07	--	49.0	51.0
6	37.5 ± 0.08	25.1 ± 0.02	2.0	70.0	28.0

Study Design

Jordan/Scotty egg incubation boxes were used for this project (Figure 3.2). Each Jordan/Scotty egg incubation plate holds 200 embryos, with three plates constituting a bundle (200 eggs x 3 plates = 600 eggs/bundle) and four bundles installed at each site (4 bundles = 2400 eggs/site). Three plates per bundle were chosen because each plate installed acted as a replicate for bundle-scale variation, while four bundles per site were chosen because each bundle installed acted as a replicate for site-scale variation. During this study, a total of 24 field bundles (i.e., 14400 embryos) were embedded vertically (~30 cm) into the river substrate. Sites were prepped in September – October 2020 where a bottomless five-gallon bucket was inserted to hold the surrounding substrates in place until pre-loaded Jordan/Scotty incubator bundles were ready to be installed (4 per site; November 2020). Interstitial water temperature and dissolved oxygen concentration were recorded at 15-minute intervals at each site between the 9 September, 2020 and 11 January, 2021 using a Onset HOBO U26 Dissolved Oxygen Data Logger (Onset Computer Corporation, Bourne, MA). A portion of sediment was taken from each incubator during winter retrieval and retained for particle size analysis. Water levels were also recorded at 15-min intervals at each site during the same sampling period using two Onset HOBO U20-001-02 Water Level Data Loggers (Onset Computer Corporation, Bourne, MA). At each site, two data loggers (U26 and U20) were secured to a cinder block and placed in the riverbed substrates directly upstream (<2m) from incubator locations, which was tethered to shore with a cable, while one data logger (U20) was secured to a nearby tree at each site. The data logger (U20) attached to the tree at each site was used to record the reference barometric pressure, which was subtracted from the in-stream water level data logger (U20) and allowed us to calculate the changes in water levels during the entire incubation period. Bulk sediment samples were also collected when the incubator bundle holes were pre-dug and sent to Agriculture and Food Laboratories (AFL) in Guelph, ON for analysis of the presence of glyphosate (0.05 ppm limit of detection) and its metabolites (e.g., AMPA).

Hatchery and transport control replicates were required to account for extraneous factors impacting embryonic development and survival, especially as newly fertilized Atlantic Salmon embryos are extremely sensitive to handling and physical shock (Krise, 2001). Using an exterior substrate pond at Mactaquac Biodiversity Facility (MBF) a total of eight bundles (i.e., 4800 embryos) were embedded vertically (~30 cm subsurface) with half being used for hatchery (four bundles) controls and half being used for transport controls (four bundles). Hatchery controls were installed within the substrate at MBF immediately after incubators were loaded with fertilized embryos, while transports controls travelled to and from study sites within coolers, before being installed within incubation substrates at MBF. The substrate ponds at MBF are concrete fishponds (11m x 11m) filled with various sized substrate (20-50 cm) with an average depth of approximately 30-38 cm depth, to mimic natural lotic environments and are mainly used to hold adult Atlantic Salmon brood stock before their egg are harvested and they are released back into natal rivers. All proper animal care and egg handling protocols were followed when incubating and transporting Atlantic Salmon embryos, with all installation equipment being thoroughly disinfected with Ovadine solution.

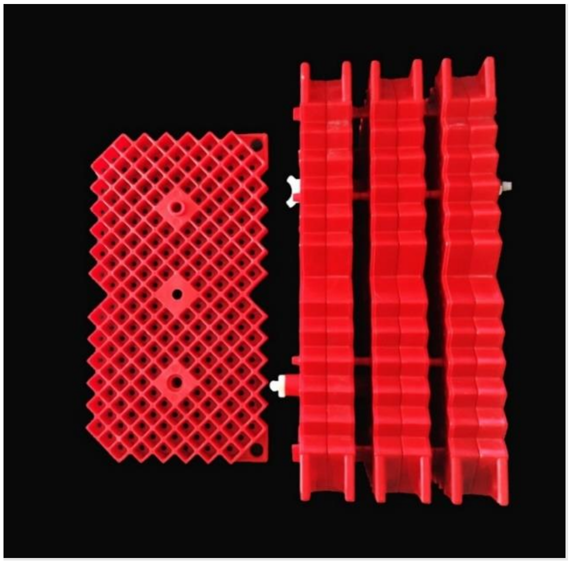

Figure 3.2: Example of Jordan/Scotty incubator bundles, with 3 plates/bundle and 200 embryos/plate (600 embryos total).

Tobique Brood Stock & Hatchery/Transport Controls

The Atlantic Salmon embryos used for this experiment came from mature adults of Tobique River stock that were captured as wild juveniles and raised in captivity at Mactaquac Biodiversity Facility (MBF) until they reached sexual maturity (Jones et al., 2014). During this study, 19 200 fertilized Atlantic Salmon embryos were taken from 32 random crosses (1 male x 1 female), with roughly equal number of eggs taken from each cross (~600 embryos/cross) to adequately reflect the genetic variation within broodstock fish. The remaining embryos from each of the 32 random crosses were retained at MBF within traditional hatchery flow through troughs and placed in egg trays, with the total number of mortalities per cross being quantified to help measure the variable rates of percent survival. Once embryos were water hardened (6-8h post-fertilization) they were pre-loaded into Jordan/Scotty plates at the MBF hatchery and assembled into bundles. Effort was made to reduce the impact of handling effects on embryos by gently pouring them onto loading trays, transferring them to incubation plates, and using spray bottles of water to dislodge them from loading trays, to ensure only one embryo per cell for each plate. Many incubation bundles (24 bundles =14 400 embryos) were installed *in situ* at field sites, while the remaining bundles (8 bundles=4800 embryos) were installed at MBF as hatchery and transport controls. However, during winter embryo retrieval 2 incubation bundles at Site 3 were lost, therefore only 1200 embryos were collected for this site rather than 2400, which brought the total number of field reared embryos to 13200 (Table 3.2).

All hatchery and transport control bundles were installed within pre-dug holes within substrate pond at MBF, which was sub-divided into quadrants (i.e., two redds per quadrant) to capture within pond variability of incubation conditions. The exterior substrate pond at MBF was likewise equipped with the same water quality monitoring equipment, with one Onset HOBO U26 Dissolved Oxygen Data Logger attached to a cinder block with one Onset HOBO U20-001-02 Water Level Data Logger, and placed in quadrant 1 to record water temperature and dissolved oxygen concentrations, while barometric pressure readings from the suspended water level logger was subtracted from the in-situ water level logger and used to calculate changes in substrate pond water levels (Onset Computer Corporation, Bourne, MA). The hatchery control bundles were installed in the substrate pond at MBF at randomized upstream or downstream positions (1 hatchery bundle per quadrant) to provide valuable information on their natural rate of deformity and mortality (Figure 3.3). Meanwhile, the transport control embryos were placed in coolers and transported to and from study sites, and then returned to MBF and installed in the remaining location within the substrate pond (1 transport bundle per quadrant) to determine what proportion of deformities and mortalities were transport induced. When transporting bundles to incubation sites they were secured within tengallon coolers filled with river water to act as a buffer against physical abrasion and each had its own portable aerator to ensure adequate DO concentrations.

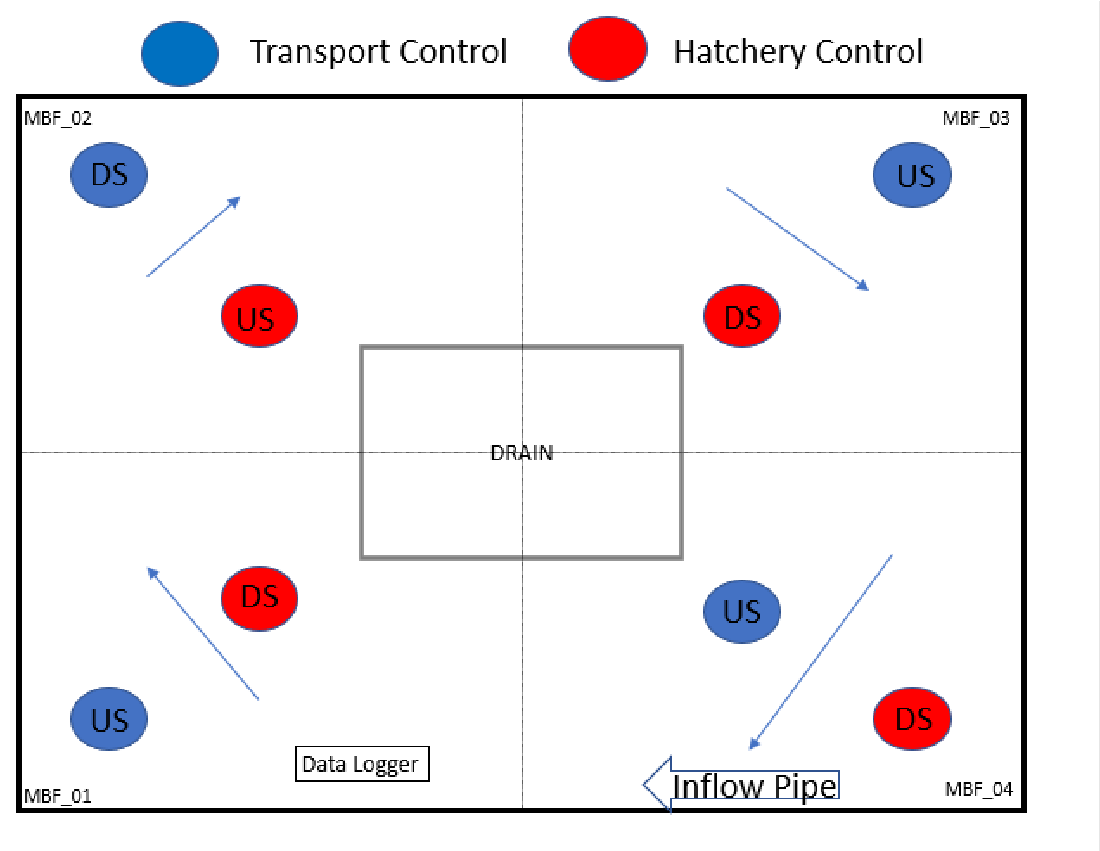

Figure 3.3: Conceptual layout of MBF experimental set up and placement of hatchery and transport control incubation bundles within substrate pond at upstream (US) and downstream (DS) positions**.**

Fall embryo incubation

When installing incubation bundles at field sites they were gradually acclimated to river water temperatures during the installation process (~1-2h) and placed vertically in pre-dug holes (i.e., four holes/site), and oriented parallel to the river flow. Once incubation bundles were securely held in place with rebar, the five-gallon buckets were removed, and the surrounding substrate buried the incubation bundles. One grab water sample per site was collected during incubator installation in November 2020 and sent to Research Productivity Council (RPC) in Fredericton, NB, for analysis of trace metals and nutrients. Due to the time sensitive nature of fall embryo incubation the bulk sediment samples used for glyphosate analysis were collected earlier in the fall 2020 during site visits and in-stream equipment installation.

Winter embryo retrieval

Once the Atlantic Salmon embryos reached the estimated eyed stage of development (i.e., ~26 accumulated degree days), they were retrieved and preserved in scintillation vials containing Stockard's solution. Here we refer to accumulated degree days (ADD) as the number of days where the mean daily water temperatures were greater than 5°C, used to estimate the relative age of different stages of embryonic development for Atlantic Salmon (Gorodilov, 1996). As embryonic metabolism and development is linked to water temperature it is often faster at higher temperatures and slower at lower temperatures. It was assumed that incubated embryos developed more rapidly during fall incubation (i.e., November) when mean daily water temperatures were elevated (~4-6°C), with development gradually slowing down during winter incubation (i.e., December-January) as water temperatures cooled (~1-2°C). Using a combination of periodic site visits and water temperature readings (collected from site 5), flow data from hydrometric stations, and air temperature data from weather stations we were able to approximate when incubated embryos were within eyed stage of development (~26-51 ADD), which we later confirmed with in-situ water temperature measurements. Retrieval at the eyed stage ensured that key morphological attributes were visible (i.e., eyes, head, spine, caudal fins rays) when assigning deformities, and that we were able to collect them before the swim up stages (i.e., alevins). An additional challenge we faced during winter embryo retrieval within the Serpentine River was that we needed to collect our incubation bundles before the hydroelectric discharge regime from the upstream reservoir lake (i.e., Serpentine Lake) increased water levels, which could significantly alter incubation conditions or contribute to ice scour events that could dislodge bundles completely.

We wanted to quantify the distribution of sediment within incubator bundles due to the potential influence of silt and clay particles on embryonic mortalities or deformities, and therefore an important aspect of the sediment sampling design was the use of a hard plastic flow deflection plate. The deflection plate was inserted immediately upstream from each Jordan/Scotty incubator, which helped minimize the amount of fine sediment loss from incubation bundles during retrieval. A representative sediment sample was divided into different particles fraction sizes using a sieve, dried at 60°C for at least 24 h in pre-weighed aluminum pans and weighed to determine total dry mass. As previously discussed, during winter embryo retrieval in January 2021, we were unable to locate two incubation bundles (i.e., 1200 embryos) at Site 3 and therefore they were not included in the analysis. During retrieval each incubation bundle was subjected to a minor mechanical shock (i.e., shook in tote filled with water) to promote the congealing of the chorion (i.e., outermost membrane of egg) of any recently dead embryos, which avoided any inaccurate assessments of mortality. After processing the embryos within each incubator bundle a representative sample of sediment was collected (~50-60g) for grain distribution analysis (Figure 3.9). Finally, it is also important to note that we experienced some issues with our U-26 HOBO data logger at Site 5 reporting inaccurate DO concentrations, especially as water temperatures approached 0 °C, which would suggest potential ice formation events. Therefore, for the purposes of our analysis we calculated the median DO concentrations for site 5, which reflect the upstream (Site 4) and downstream (Site 6) DO measurements.

Laboratory Analysis

Tau-somites (τs) are a unit for measuring the relative age or developmental stage of an Atlantic Salmon embryos since it represents the time it takes to form one somite pair (Gorodilov, 1996). Tau-somite values vary by temperature, with lower water temperatures having higher τs) values because it takes longer to form a somite pair (i.e., development is slower at lower temperatures). After calculating the average number of Tau-somites for embryos incubated within each site, their embryonic developmental state was compared to drawings and morphological descriptions from Gorodilov (1996) before dissecting. Embryos were examined under a dissecting stereomicroscope and any deformities found were classified according to a deformity index (Bartlett et al., 2022) (Figure A.1: Deformity assessment index showing the different deformity types observed in Atlantic Salmon embryos in Tobique River (Bartlett et al., 2022)). When assessing embryos for mortalities, we examined if the chorion of the embryo was clearly ruptured, congealed, or had substantial fungus growth on its exterior. To accurately identify Atlantic Salmon embryo development and assess presence of deformities, it was necessary to remove the chorion (i.e., outer egg membrane), and any excess congealed yolk, and/or vitelline fluid using a scalpel and forceps. Some distinguishing morphological features of the embryos at the eyed stage consist of the presence of gill fissures, beginning of black eye pigmentation, formation of the notochord and vascularization of the yolk sac (i.e., vitelline plexus). Key regions of each embryo were examined for the presence of small or missing eye cusps, curved (i.e., lordosis or corkscrew) or fused (i.e., conjoined) spines, and an assortment of yolk sac abnormalities (i.e., small, enlarged, or interrupted development) (Figure 3.4). All proper lab safety precautions were taken when processing preserved embryos and the Stockard solution was disposed of following established waste disposal procedures.

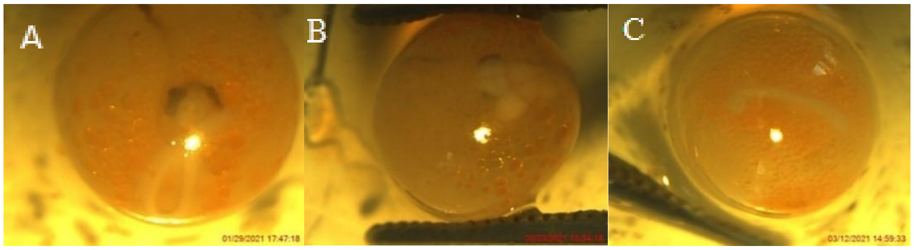

Figure 3.4: Examples of embryonic deformities; A) lordosis, B) twinning, C) corkscrew tail.

Statistical Approach

A Pearson’s chi-square test was performed on the different cross numbers of embryos reared in traditional aquaculture trays (i.e., flow through troughs inside MBF) to determine if their expected vs. observed post-fertilization survival rates were significantly different from each other (alpha = 0.05). By comparing the amount of variation between crosses we were able to quantify if the brood stock genetics had a significant influence on embryo survival. For this analysis the independent variable was cross number and total number of embryos lost (#) per cross was the continuous dependant variable. A two-sample *t*-test was used to investigate if the mean proportion of embryo mortalities and deformities within our hatchery and transport controls were equal. All assumptions for a two-sample *t*-test were met for both mortality and deformity models using quantile-quantile plots (i.e., Q-Q plot), conducting Shapiro-Wilks goodness-of-fit tests, and by examining their *F*-distributions. The proportion of dead or deformed embryos per incubator was used as the dependent variable across the two control types (i.e., hatchery vs transport). A one-way analysis of variance (ANOVA) was conducted on each embryo type to investigate if there were statistically significant differences in embryo mortalities and deformities between hatchery controls, transport controls, and field reared embryos. By comparing the amount of variation between embryo types with the amount of variation within embryo types we were able to quantify if the different pre-incubation treatments (i.e., control vs in situ field incubation) had a significant influence on the total number of embryo mortalities or deformities. For these analyses the factor was embryo type (i.e., 3 levels: hatchery embryo, transport embryo, field embryo) and the proportion embryo mortalities or deformities per incubation bundle were the continuous dependant variable. The established alpha level for all ANOVA models were set at 0.05 and all assumptions were tested by conducting Shapiro-Wilks test of normality, Levene’s tests of equal variance, and by transforming dependent variables or using non-parametric Kruskal-Wallis tests where appropriate.

A one-way ANOVA was also conducted on the total number of dead and deformed embryos at each field site to explicitly test for between site differences (i.e., field sites only). By comparing the amount of variation between sites with the amount of variation within sites we were able to quantify if the spatial location of the incubation sites along the upstream to downstream reaches of the Serpentine River had a significant influence on the total number of embryo mortalities or deformities observed. For these analyses the factor was site number (i.e., Site 1 - 6) and the total number of embryo mortalities and deformities per incubator bundle were the continuous dependant variables. Total mortalities and deformities were resampled at the site level to match observed proportions due to the loss of two bundles at site 3.

Finally, simple linear regression models were constructed to test how environmental conditions between (i.e., site-scale) and within (i.e., bundle-scale) sites could influence the total number and proportion of dead and deformed embryos incubated within the Serpentine River. The environmental conditions used as independent variables included bundle-scale variation of fine-grained sediments (i.e., proportion of fine and coarse sand particles per incubator), or site-scale variables, which included variation in mean daily water temperatures, number of cumulative degree days, and coefficient of variation in dissolved oxygen concentrations and water levels. Meanwhile, the total number (#) of mortalities or deformities per incubator were used as the continuously dependant variables in site-scale linear regressions, while the proportion (%) of mortalities and deformities per incubation bundle were used as the continuously dependant variables in bundle-scale linear regression.

All assumptions of simple linear regression were tested and met for both bundle and site-scale models for embryo mortality and site-scale models for embryo deformity. However, the simple linear regression models for bundle-scale deformity failed to meet the assumptions of normality, and therefore required logit transformation of dependent variables (i.e., proportion of embryo deformities per bundle). Bundle-specific proportions of fine-grained sediment sizes were used as the independent variables within simple linear regression for embryo mortalities and deformities, which includes proportion fine and coarse (0.075-2mm) sand particles. Given the range in the proportion of fine sand (0.075-0.250mm) particles within bundles were extremely small (0-0.08% Propn_fineSand) we simply collapsed them with the proportion of coarse sand (0.250-2mm) particles to represent the total proportion sand (0.075-2mm). Likewise, site-specific water quality parameters were used as the independent variable within simple linear regression for embryo deformity and mortality model, which includes the variation in dissolved oxygen concentrations. The variation in daily average water temperature, coefficient of variation in dissolved oxygen concentrations and water levels were also used as an independent variable within simple linear regression for both embryo deformity and embryo mortality, but all were not statistically significant (p>0.05). All analyses were run using R (version 4.0.3; R Core Team, 2020) in the RStudio (version 4.0.3; RStudio Team, 2020) environment using base statistical packages such as *ggplot2* (version 0.55; Wickham, 2016), *car* (ver3.0-12; Fox & Weisberg, 2019), and *pwr (ver1.3;* Cohen 1988). The simple linear regression models were constructed using the *stats* package (version 4.0.3; R Core Team, 2020).

RESULTS

Of the 18000 embryos retrieved from both field (13200 embryos) and control (4800 embryos) sites, 45% (8096/18000 embryos) died during in-situ incubation, and within the remaining alive embryos, only 2.68 % (265/9904) exhibited any developmental malformations (Table 3.2). Overall, a greater proportion of mortalities per incubation bundle were observed within hatchery control embryos at Mactaquac Biodiversity Facility (MBF) than within either transport controls or treatment embryos within field sites (Figure 3.6A), but results from our one-way ANOVA found these between site differences were not statistically significant ($F_{2,27} = 3.056$, $p = 0.063$). Conversely, a slightly larger proportion of embryo deformities were observed within treatment embryos at field sites along Serpentine River than within hatchery control or transport control embryos at MBF (Figure 3.6B), but results from our one-way ANOVA also found these between site differences were not statistically significant ($F_{2,27} = 3.034$, $p = 0.064$). Furthermore, the two tailed t-test found there was no statistically significant difference in mortality between hatchery control and transport control embryos ($t_6 = 1.13$, $p = 0.30$) nor in the occurrence of deformities between the same groups ($t_6 = 0.82$, $p = 0.43$) (Figure Therefore, since within control type (hatchery vs transport) differences were not significant for either embryo mortalities or deformities, we could rule out any potential transport-induced effects from influencing our field samples. Additionally, when investigating between site differences (i.e., hatchery, transport, field) in the proportion of mortality and deformity per incubation bundle both were not statistically significant, and we could likewise rule out any potential transport-induced or handling-induced effects.

A total of 32 random (1 male x 1 female) crosses were used for this experiment, whose proportion of post-fertilization mortalities had a very large range (1.45% - 71.11% per cross) when placed within egg baskets and incubated within traditional hatchery flow through troughs. The Pearson's chi square test found there was a statistically significant effect of cross number on the total number of embryos that died per cross ($X^2_{(1, 32)} = 9289$, $p < 0.001$), meaning certain crosses experienced greater embryo mortality than others. To help control for this expected variable rate of percent survival among crosses all the embryos were thoroughly randomized before being pre-loaded into incubators at MBF. Furthermore, given these different rates of embryo mortality between crosses we could not rule out any potential genetic effects from influencing base rates of mortality, but best efforts were made to spread this variation out evenly amongst all field and control sites. Unfortunately, the proportion of deformities of each cross within MBF flow through troughs were not measured and we could therefore not make any further inferences on how they differed between crosses.

When further investigating between field site differences in embryo mortalities and deformities we chose to characterize sites by their different water quality conditions, which included the number of cumulative degree days (CDD), the daily average water temperatures (Avg_Temp), the coefficient of variation in dissolved oxygen concentrations (CV_DO), and the coefficient of variation in water levels (CV_Lvl) (Table D.1). Additionally, we tested for glyphosate and its metabolite (i.e., AMPA) at each of the six study sites, as its presence could influence embryonic survival and development, but all samples were well below detection limits (0.05 ppm limit of detection). We also explored how within site differences in incubation conditions could potentially influence embryo mortalities and deformities, which included the proportion of fine and coarse sand particles within each incubator bundle (Prop_Sand). It is important to note that Propn_Sand is a proxy measure for sedimentation within the Jordan/Scotty incubators, which only represents the distribution of sand particles at time of sample collection rather than over entire incubation period.

Table 3.2: Total number of Atlantic Salmon embryo mortalities and deformities.

Site	**Dead Embryos**	**Deformed Embryos**	**% Dead Embryos**	**% Deformed Embryos**	**Total Embryos Deployed**	**Total Embryos Retrieved**
Site 1	1087	15	45.3	1.1	2400	2400
Site 2	1018	29	42.4	2.1	2400	2400
Site 3	698	15	58.2	3.0	2400	1200
Site 4	1080	39	45.0	3.0	2400	2400
Site 5	776	72	32.3	4.4	2400	2400
Site 6	889	56	37.0	3.7	2400	2400
Field sub-Total	**5548**	**226**	**42.0**	**2.95**	**14400**	**13200**
Hatchery Control	1349	22	56.2	2.1%	2400	2400
Transport Control	1199	17	50.0	1.4%	2400	2400
MBF sub-Total	**2548**	**39**	**53.1**	**1.73**	**4800**	**4800**
TOTAL	**8096**	**265**	**45.0**	**2.68**	**19200**	**18000**

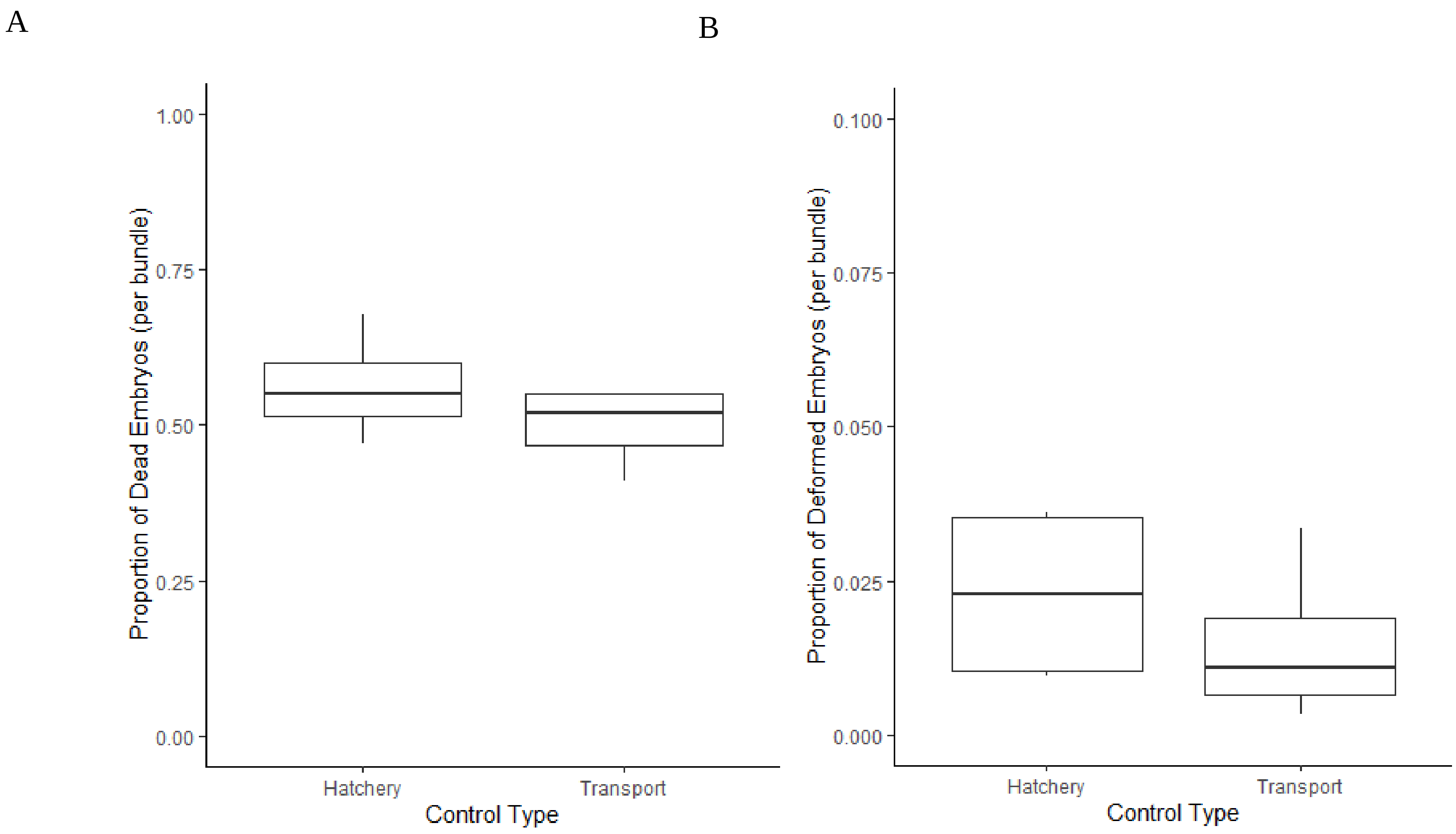

Figure 3.5: A) Proportion of dead embryos per control type, and B) proportion of deformed embryos per control type in each bundle.

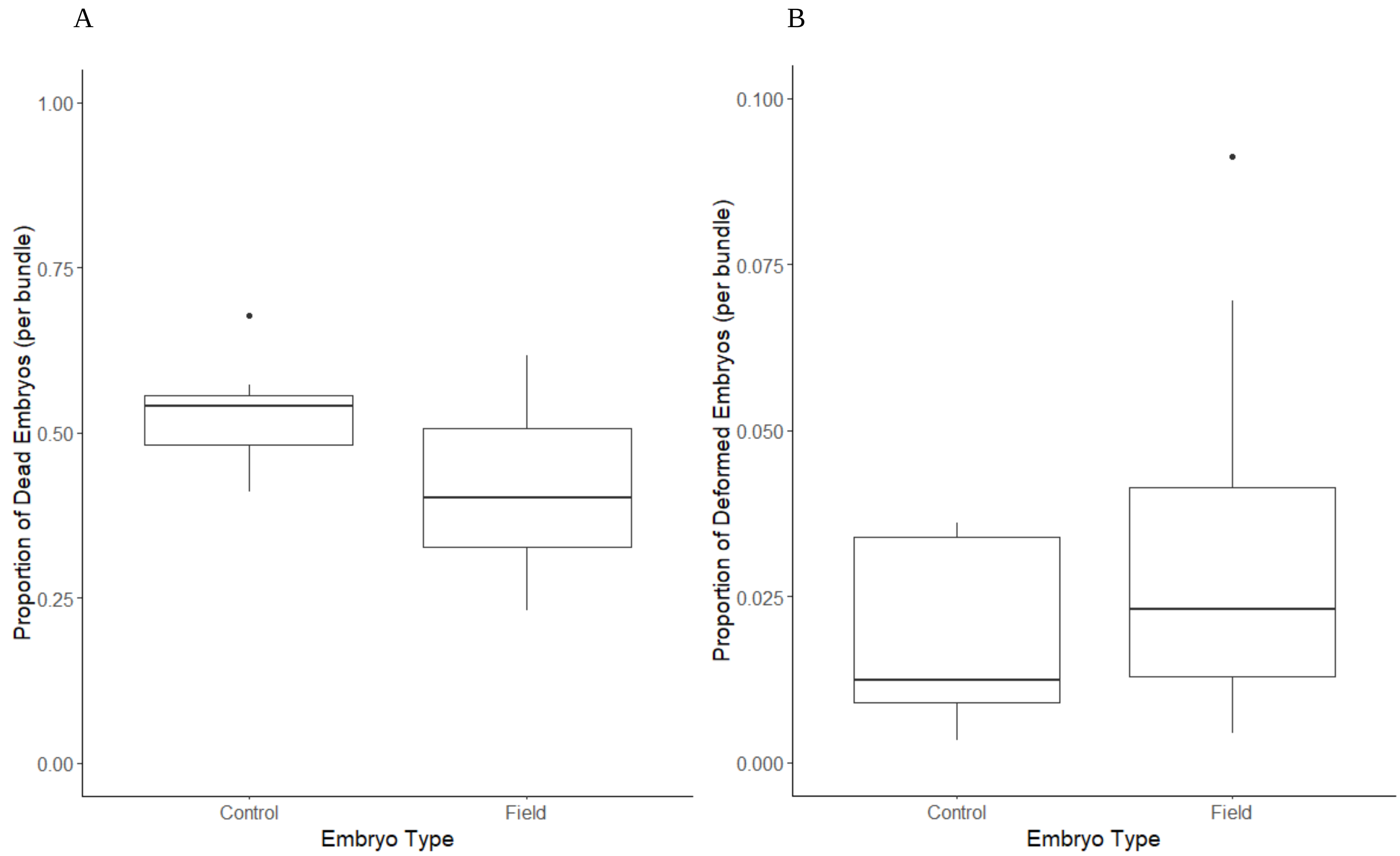

Figure 3.6: A) Proportion of dead embryos per embryo type (y-axis 0-1), and B) proportion of deformed embryos per embryo type in each bundle (y-axis 0-0.1).

Embryo Mortality

Although Site 3 experienced on average a greater proportion of embryo mortalities per incubation bundle than within either downstream or upstream sites (Figure 3.7), results from the one-way ANOVA indicate that these between field site differences were not statistically significant ($F_{5,16} = 1.66$, $p = 0.20$). Simple linear regression between the proportion of sand (0.075-2mm) particles within incubator bundles and total number of mortalities per incubator was not statistically significant ($R^2 = 0.172$, $F_{1,20} = 4.162$, $p = 0.055$). Similarly, the simple linear regression between the number of cumulative degree days (CDD) per site and the total number of mortalities per site (Figure 3.9) was also not statistically significant ($R^2 = 0.56$, $F_{1,4} = 5.091$, $p = 0.087$). Alternative site descriptors were also explored, which include average daily water temperatures (Avg_Temp), coefficient of variation in dissolved oxygen concentrations (CV_DO) and water levels (CV_Lvl) per site as dependant variables to investigate their relationship with observed embryo mortalities (Figure E.1-E.4), but none of the models were statistically significant (i.e., $p>0.05$).

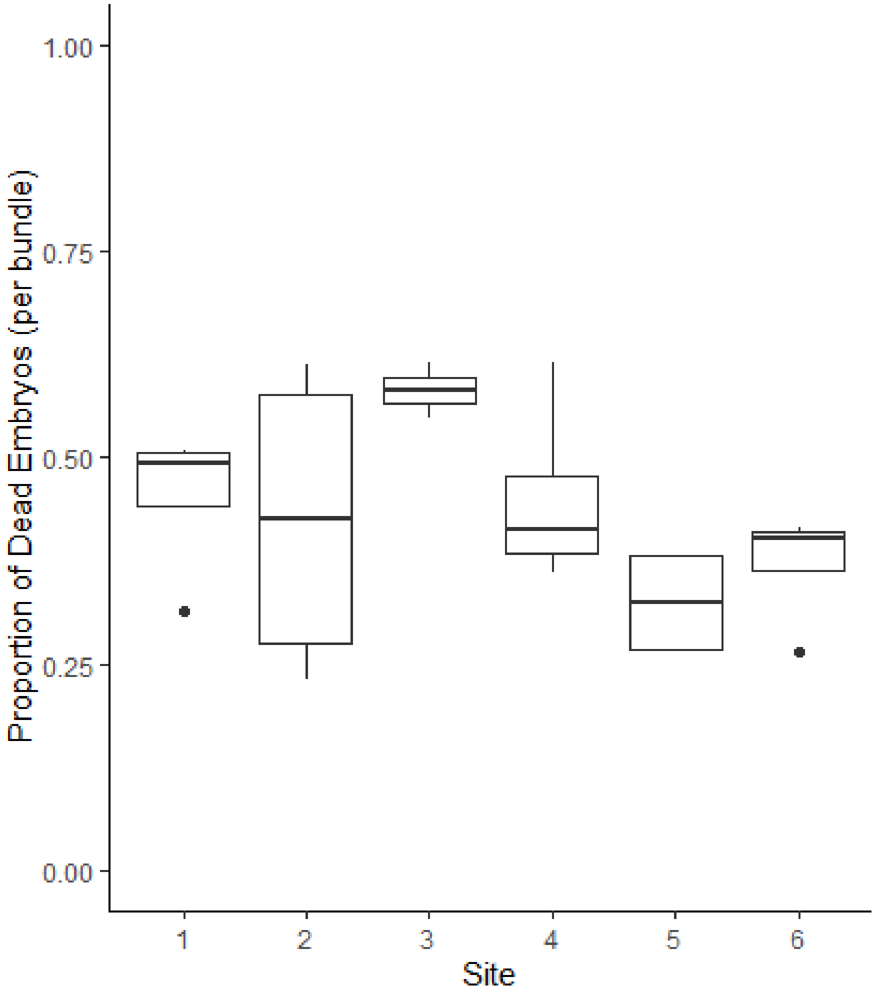

Figure 3.7: Proportion of embryo mortalities within Serpentine River incubation sites.

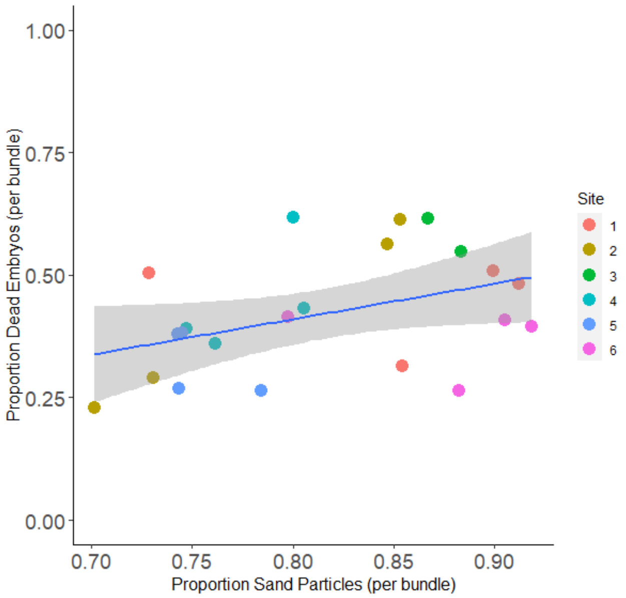

Figure 3.8: Scatterplot of proportion dead embryos and proportion of sand particles per bundle. Proportion Dead Embryos = -0.1709 + (0.7262*proportion of sand particles per bundle).

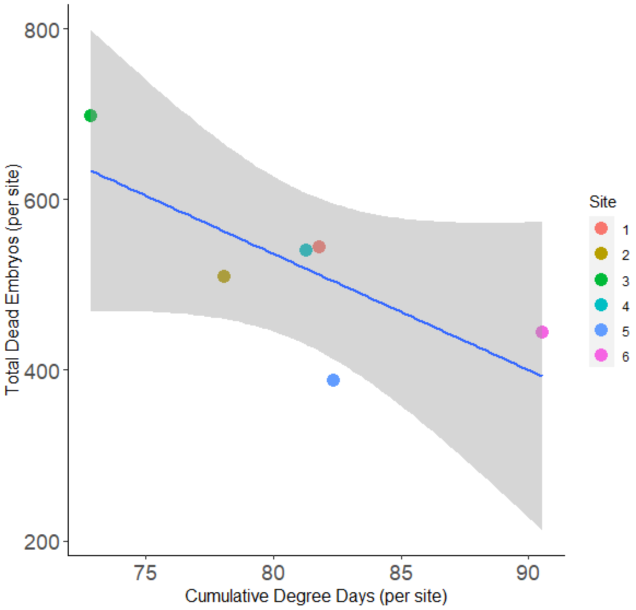

Figure 3.9: Scatterplot of resampled total dead embryos and cumulative degree days per site. Total Dead Embryos = 1625.888+ (-13.620*cumulative degree days).

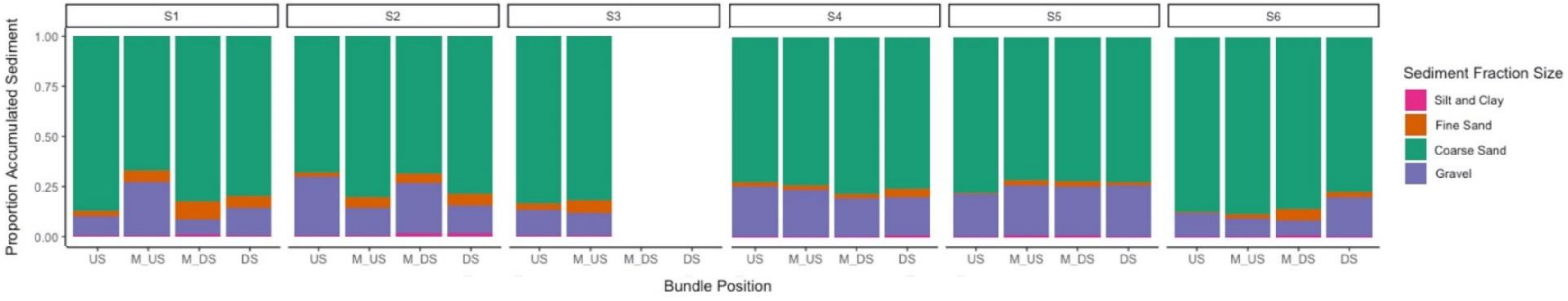

Figure 3.10: Proportion of sand particles within bundles by fraction size (silt/clay < 0.063mm; fine sand= 0.075-0.250mm; coarse sand 0.250-2.0mm; gravel >2.0mm).

Embryo Deformity

Overall, Site 5 experienced on average a greater proportion of embryo deformities per bundle than either upstream or downstream sites (Figure 3.11), but these differences were not statistically significant via a one-way ANOVA ($F_{5,16} = 1.703$, $p = 0.191$). The simple linear regression between the proportion of sand particles (0.075-2mm) per bundle and the observed deformities were also not statistically significant ($R^2 = 0.042$, $F_{1,20} = 0.898$, $p = 0.355$; Figure 3.12). Similarly, the simple linear regression between the coefficient of variation in dissolved oxygen concentrations (CV_DO) per site and total number of deformities were not statistically significant ($R^2 = 0.611$, $F_{1,4} = 6.29$, $p = 0.066$; Figure 3.13). We also explored the coefficient of variation in average daily water temperature (Avg_Temp) and cumulative degree days (CDD) per site as independent variables (Figure E.5-E.8) within a simple linear regression, but neither Avg_Temp ($R^2 = 0.002$, $F_{1,4} = 0.008$, $p = 0.931$) or CDD ($R^2 = 0.401$, $F_{1,4} = 2.687$, $p = 0.176$) were statistically significant.

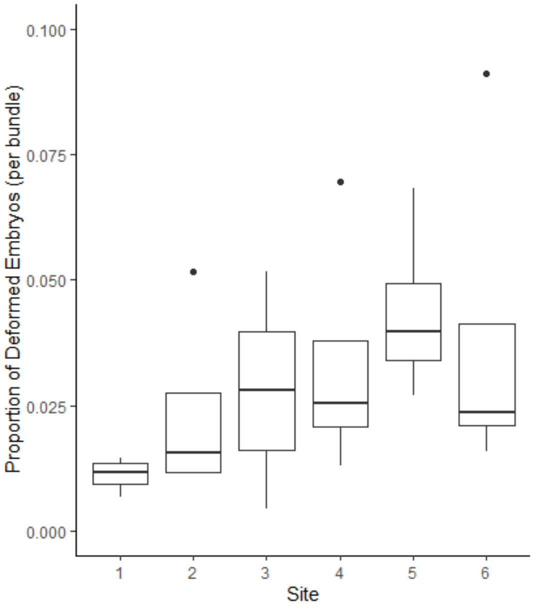

Figure 3.11: Proportion of deformed embryos (per bundle) by site within Serpentine River.

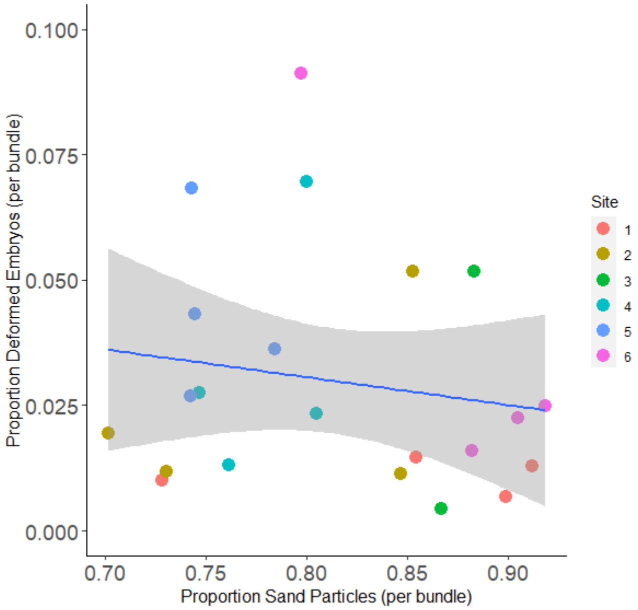

Figure 3.12: Proportion of deformed embryo and proportion of sand particles per bundle. Proportion Deformed Embryos = -1.800 + (-2.416*proportion of sand particles per bundle).

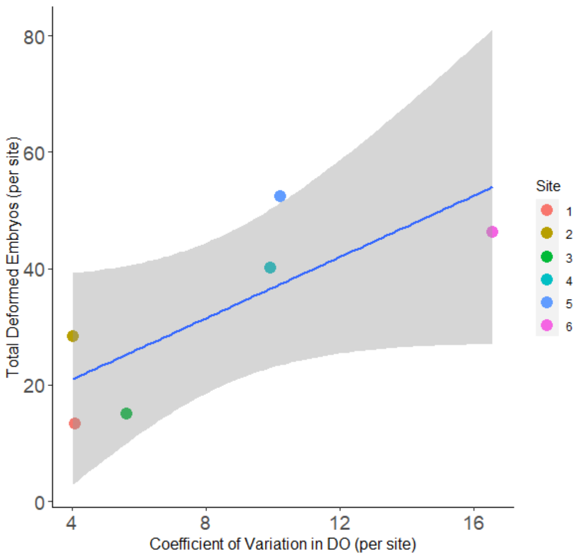

Figure 3.13: Total number of resampled deformed embryos and coefficient of variation in dissolved oxygen concentration per site. Total Deformed Embryos = 10.404 + (2.633*variation in DO per site).

DISCUSSION

I investigated site level environmental factors (i.e., cumulative degree days, average daily water temperatures, coefficient of variation in DO and water levels) and bundle-level incubation conditions (i.e., distribution of fine and coarse sand particles within incubators) that may be influencing Atlantic Salmon embryo mortalities and deformities while they incubate within Jordan/Scotty incubators of the Serpentine River. If an inadequate number of Atlantic Salmon die during the embryonic stages of development, or if many of them develop deformities and malformations, it can negatively impact their abundance at later life cycle stages and can reduce both their individual and the overall populations resilience (i.e., lower number of emigrating wild smolt with high fitness) within freshwater ecosystems.

The main findings highlighted that less than half of the embryos we incubated died (i.e., 8096/18000= 45%), and despite the large range in the proportion of embryo mortalities per incubation bundle between field, hatchery, and transport controls (i.e., 23.0% mortality at Site 2 vs 67.6% at MBF hatchery control site) these between site differences were not statistically significant (p>0.05). Additionally, the proportions of embryo mortalities we observed within the Serpentine River are typically what wild Atlantic Salmon embryos experience and align with other similar in-situ incubation experiments conducted within the Miramichi River (Lavery, 2017) and other tributaries of the Tobique River (Flanagan, 2003). Conversely, the main findings for embryo deformities highlighted that overall, a very small number of the remaining alive embryos (i.e., 18000 incubated – 8096morts=9904 alive) were deformed (i.e., 265/9904= 2.68%) and that the range in the proportion of embryo deformities per incubation bundle between field, hatchery, and transport controls were relatively small (0.3% in MBF transport control site vs 9.0% at Site 6). Like our findings for embryo mortality, the results from our one-way ANOVA confirmed that these between site differences in the proportion of embryo deformities per incubation bundle were also not statistically significant (p>0.05). Furthermore, the range in the proportion of field deformities at our sites along the Serpentine River (3.0-4.4%/site) were much lower than the larger range of site-level deformities (1-86%) observed in the previous study conducted in 2013 (A. Bartlett, unpub. data). Therefore, given we did not observe a similar deformity phenomenon as in 2013 and since there are no statistically significant differences between our field and control sites, there does not appear to be any additional embryo mortality or deformity within the Serpentine River beyond the baseline-levels experienced at Mactaquac Biodiversity Facility (MBF). Additionally, given the results from our two-sample *t*-test were above our established alpha level (0.05) we were also able investigate within control type (i.e., hatcher vs transport controls) differences in proportion of embryo mortalities and deformities and rule out any potential handling and transport effects. Moreover, after performing a Pearson's chi-square test on genetic cross type and percent survival the results were statistically significant (i.e., $p<0.01$), meaning there were some crosses with substantially greater proportions of mortalities. However, due to our embryo randomization process at MBF best efforts were made to ensure that no specific field or control incubators received a disproportionate number of these mortality prone embryos, which thereby spread the genetic effects across all sites equally. Furthermore, all these results seem to suggest that perhaps embryo mortalities are influenced by their genetic background and the semi-captive rearing conditions of Tobique brood stock fish within hatchery environment, rather than the in-situ field incubation conditions as we previously thought. These findings have important implications for any similar future in-situ incubation studies, however, exploring how hatchery rearing conditions may influence embryo survival and development (i.e., epigenetic expression) was beyond the current scope of this project.

The main aim of our research was to investigate what may be influencing Atlantic Salmon embryo mortalities and deformities during their early winter incubation period. However, given that the ANOVA results found no statistically significant difference between the proportion of mortalities or deformities within incubation bundles at field or control (i.e., hatchery and transport) sites it essentially answered my main research question, and suggested that perhaps between cross type differences are influencing embryo survival. These results are ultimately a good news story for incubated Atlantic Salmon embryos as it confirms that we did not observe the same deformity phenomena as in 2013 and that landscape composition and it subsequent influence on incubations conditions within Serpentine River substrates did not have a meaningfully impact. However, the secondary component of my research question was focused on explicitly testing the between field site and bundle level environmental factors within the Serpentine River that could potentially influence embryo mortality and deformities. Although the results of the bundle- and site-level statistical models (i.e., simple linear regressions) were not statistically significant there is still value in discussing them as it places the result of our experiment in the wider context of the literature and highlights potential limitations that future in-situ Atlantic Salmon incubation experiments should avoid while also discussing recommendations for future research.

Effect of bundle-level fine sediment on proportion of embryo mortalities and deformities

I initially predicted that as the proportion of fine sediments within Jordan/Scotty incubator bundles increased that the proportion of embryo mortalities and deformities would also increase. I believed that increasing proportions of fine sediments could have contributed to both mortalities and deformities because finer sand particles can coat the chorion and temporarily disrupt DO exchange (i.e., contribute to embryo deformity), or at the extreme end it could completely smother incubating embryos in sediment and disrupt DO exchange more permanently (i.e., contribute to embryo mortality). An alternative explanation for the potential influence of fine sediments on embryo deformity could be related to the indirect effect of coarser sand particles (0.250-2.0mm), which can infiltrate the interstitial spaces within Jordan/Scotty incubators, thereby decreasing hyporheic flows and displacing DO available to incubating embryos. When investigating field sites, and explicitly testing if bundle level proportions of fine and coarse sand particles (0.075-2mm) influenced the proportion of embryo deformities or mortalities (per bundle), neither of our simple linear regressions were statistically significant (p>0.05).

There have been numerous field and laboratory-based studies, which found that the accumulation of fine and coarse sand particles within redds contributed to greater embryonic mortality in salmonid species (Sear et al., 2016; Jensen et al., 2009; Louhi et al., 2008; Levasseur et al., 2006; Julien & Bergeron, 2006; Reiser & White, 1988; Turnpenny & Williams, 1980). Initial research conducted by Reiser & White (1988) found that the accumulation of fine sand (<0.84mm) can be detrimental to the survival of the very early embryonic stages of salmonid development (i.e., blastulation) given their increased oxygen demand and reliance on hyporheic flows to flush sediments. It is also important to consider the potential mechanical effects of sand particles on the abrasion of embryonic membranes as a contributing factor influencing the mortality of incubating embryos (Krise, 2001; Lapointe et al., 2004) or the additional sediment transport that may be co-occurring with ice formation and break-up events (Nafziger et al., 2017).

It is important to note that there may be a few limitations regarding our bundle installation techniques and sediment sampling regime that may have contributed to why we did not observe a similar influence of fine sediment on embryo survival. Firstly, the vertical installation of Jordan/Scotty egg incubators may have influenced sediment distribution within bundles as the orientation of alevin escape hole was slightly elevated to the right-hand side of each cell, and not oriented at the bottom of each cell. However, the bundles were still parallel to flow as per manufacture recommendations but given the variation in site substrate composition and the assumed relationship with hyporheic flows and variations in DO experienced at each site, we are unable to determine to what extent vertical vs horizontal installation of incubators truly impacted survival. Recent field experiments conducted in Newfoundland on sediment distribution within Jordan/Scotty bundles under four different installation techniques also discovered no statistical differences in sediment fraction sizes between incubators installed either perpendicular or parallel to river flows (Purchase et al., 2018). Secondly, the sediment sample we used to quantify bundle-level distribution of particle sizes only represented a proportional sample of sediment within incubators at a temporally discreet period (i.e., time of sampling) and does not accurately reflect the total temporal variation in sediment distribution during the entire incubation period. Thirdly, the total weights of the proportional sediment sample per incubation bundle were very small and ranged from 4.5g – 78.6g, which does not reflect the total amount of accumulated sediment or the total space available within Jordan/Scotty incubators for sediment to accumulate.

Although our results indicated that there were no between field and control site differences in the proportion of embryo mortalities and deformities, I proceeded with investigation of environmental factors at our field sites. The within site exploration of embryo mortalities discovered an influence of fine sand particles, but due to the limitations of our sediment sampling regime these findings should be interpreted with caution. Additionally, although our ANOVA models did not find any differences in the proportions in mortalities and deformities between field and control (i.e., hatchery and transport control) sites it does not mean that our control embryos were not influenced by similar fine sand particles, it just means that they had a similar effect on mortality despite differences in incubation conditions. Future studies should collect the total amount of sediment within each incubation bundle rather than a sub-sample, especially if risk of embryos freezing at extreme temperatures (i.e., average air temperature during sampling was -20°C) can be further minimized. Any future in-situ incubation experiments should consider having more robust sediment sampling regimes that use more continuous sediment monitoring equipment (i.e., Lindorm SediMeter) that track sedimentation over the entire year to better understand transport dynamics and preclude any potential incubation sites with relatively elevated levels of sedimentation.

Effect of site-level water quality conditions on total embryo mortality and deformity

When exploring between field site differences in the total number of embryo mortalities and deformities I hypothesized that changes in both water temperature and dissolved concentrations would influence survival and development. I predicted that as water temperatures approached the freezing point (i.e., 0°C) the number of mortalities would increase due to ice crystal formations and that similar increases in deformities would occur as dissolved oxygen concentrations decreased due to potential exposure to potential hypoxic incubation conditions. When analyzing field data, I noticed there was great variability in mortality, especially at site 2 with more embryos tending to die at upstream sites (Site 1-3), which also had the fewest cumulative degree days (Figure 3.7). Likewise, there was similar variability in field embryo deformities, especially at site 3, with slightly more deformed embryos at downstream sites (Site 4-6), which also had the greatest coefficient of variation in DO concentrations (Figure 3.11). Given the relationship between thermal units (i.e., CDD) and estimated stages of embryonic development (i.e., tau-somite calculations) I expected there to be a statistically significant influence of water temperature on embryo mortality. Likewise, given the relationship between the DO requirements for incubating embryos and embryonic development I anticipated any fluctuation or temporary disruptions in DO to have a statistically significant influence on embryo deformity. However, when explicitly testing for between field site differences in total number of mortalities and cumulative degree days the results from our simple linear regression were not statistically significant. I also tested other relevant site-level environmental factors, which included the average daily water temperature, coefficient of variation in dissolved oxygen and coefficient of variation in water levels, but none had a significant influence on the total number of mortalities we observed at each field site. Likewise, when explicitly testing for between field site differences in the total number of deformities and the coefficient of variation in dissolved oxygen concentration, the results from the simple linear regression were not statistically significant. I also tested other relevant site-level environmental factors, which included cumulative degree days, average daily water temperature, and coefficient of variation in water levels, but none had a significant influence on the total number of mortalities we observed at each field site.

There are numerous field and lab-based studies that have focused on how variable water temperatures (and related water temperature metrics) experienced during incubation can greatly influence embryo growth and survival (Peterson et al., 1977; Bisaillon et al., 2007; Hagedorn et al., 2004; Tang et al., 2020). Initial laboratory experiments conducted by Peterson et al. (1977) established the sensitivity of pre-eyed embryos to water temperatures below 4°C, as well as the adaptations and temperature tolerance different stocks of wild Atlantic Salmon can exhibit. Similar research conducted on by Bisaillon et al. (2007) in Quebec, Canada highlighted that perhaps embryo mortality is due to winter harshness, as exemplified by greater cumulative freezing degree days and lower mean discharge during winter months (i.e., November – February). Additionally, more locally relevant research conducted by Flanagan (2003), who conducted a similar in-situ incubation experiment of Atlantic Salmon embryos within tributaries of the Tobique River, found that variable flow regimes on regulated rivers tended to influence intergravel water temperatures, which had a greater influence on embryo mortality than accumulated fine sediments within unregulated rivers. Likewise, more recent research conducted by Lavery (2017) on the Miramichi River, New Brunswick demonstrated that a combination of abiotic incubation conditions (water temperature, water level, DO, ice conditions, substrate) were influencing embryo mortalities. When comparing the mean level of mortalities observed within the Serpentine River sites (42%), they were comparable to those of Flannagan (2003), who reported similar mean eyed embryo survival within similar regulated river environments (River Dee: 69% in 1999, 74% in 2000; River Don: 31% in 1999, 43% in 2000). Likewise, the embryo mortalities observed by Lavery et al. (2019), who also used Jordan/Scotty incubation bundles (vertical installation), observed high mean mortality within their Otter Brook site (44%). Unfortunately, relatively few studies have been performed on Atlantic Salmon embryonic deformities, especially in the context of in-situ field-based studies which test how naturally variable water quality condition influence pre-eyed development. However, there are some notable laboratory experiments being conducted by Wood et al. (2020) who suggested exposure to daily cyclical hypoxia (~100-25% DO saturation within 24h) can cause build-up of metabolic wastes and can slightly delay growth, with significant deformities and near total mortality observed under constant severe hypoxia (27% DO saturation for >24 hr). Regardless, our ANOVA and simple linear regression results indicated that there was no statistically significant difference in incubation conditions at our field sites within the Serpentine River that were influencing embryo mortalities or deformities.

It is important to acknowledge some key limitations within our study when we were investigating between site differences in incubation conditions. Firstly, two incubation bundles at Site 3 were lost and therefore I had to use resampled data for the total number of embryo mortalities and deformities per site. Secondly, I had a single upstream water temperature and DO measurement per site and the data loggers were installed at a depth of ~15cm rather than maximum depth of ~30cm that incubator bundles were installed at. Given that the hyporheic zone is a dynamic environment where mixing of surface water and groundwater occurs it is possible that low water temperature events (i.e., <0°C) may have occurred within the upper portions of incubation habitat (0-10cm) or relatively warmer water temperature events (i.e., ±1°C) may have occurred within the lower portions of incubation habitat (20-30cm). Given the importance of thermal influences on the embryonic metabolism and development of Atlantic Salmon embryos, very minor fluctuations in water temperatures (e.g., ±1°C) could drastically impact the total number and proportion of embryo mortalities (i.e., ice-formation events) and deformities. Furthermore, it is possible there are potential micro-scale interactions between hyporheic flow, fine sediments, and DO exchange across the membrane of incubating Atlantic Salmon within Jordan/Scotty incubators that may have influenced total number of mortalities and deformities, but without additional data loggers or available embryos this fine scale approach was not feasible or realistic. In future in-situ incubation experiments it is recommended that relatively low-cost water temperature loggers are installed with each incubation bundle, with at least one at the top of the incubator (i.e., 0-10cm) to help inform when potential ice formation events occur and another at the base of the incubator (i.e., 20-30cm) to help inform potential influence of warmer hyporheic flows on embryo development.

In summary, I found no between field or control site differences in the proportion of either embryo mortalities or deformities per incubation bundle, which suggests incubation within Serpentine River substrates did not have any meaningful impact on survival or development. Additionally, I did not observe a high proportion of embryo deformities as the previous experiment did in 2013 (A. Bartlett, unpub. data), which suggests that the fitness and overall freshwater resilience of these embryos are not being negatively impacted by deformities. Although I did observe a high overall proportion of embryo mortalities (i.e., 8096/18000= 45%), they are comparable to other studies conducted within New Brunswick, which used similar incubation equipment (Lavery, 2017) or embryos from wild caught brood stock (Flanagan, 2003). Furthermore, I was able to rule out any potential handling or transport induced effects on embryo mortality or deformity, which confirmed our experimental controls were adequate. However, I was not able to rule out the potential genetic effects, despite randomization procedures since certain random crosses we used for this experiment had significantly different rates of survival. Therefore, any future efforts at improving the survival and overall freshwater resilience of Atlantic Salmon embryos incubated within the Serpentine River should explore how the semi-captive rearing conditions at Mactaquac Biodiversity Facility hatchery may influence the fitness of embryos and affect overall population abundance.

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CHAPTER 4: GENERAL DISCUSSION

The aim of this research was to investigate and quantify environmental factors that may influence the growth and development of incubating Atlantic Salmon (*Salmo salar*) embryos within the Serpentine River, New Brunswick. Specifically, this research sought to identify whether certain landscape characteristics, water quality conditions, or substrate compositions may be contributing to pre-eyed embryonic mortalities and deformities within Jordan/Scotty incubators. In Chapter 2, I discussed how the spatial and temporal variation in landscape characteristics helped shape the instream water chemistry and substrate composition at our six incubation sites. Our results indicate that natural landscape characteristics such as catchment-scale topography, drainage area and percent forest cover are factors influencing the variation in water quality and substrate composition. In Chapter 3, I explored how these variations in water quality conditions and distribution of fine and coarse sand particles may have influenced the total number and proportion of embryonic mortalities and deformities within each incubation bundle and within each incubation site. The results indicate that there are no between field or control site differences in either mortality or deformity, and that there was also no transport or handling related impacts. Additionally, there was no evidence of the embryonic deformity phenomena previously observed in the 2013 incubation study (A. Bartlett, unpub. data). However, given there was a statistically significant difference in embryo mortality between the 32 random crosses, it suggests potential genetic impacts from hatchery rearing environment may be influencing their survival, but this was beyond the scope of the current project.

By increasing our understanding of how landscape composition at multiple nested spatial scales can influence the incubation conditions within Jordan/Scotty incubators we can gain insight into how impacts experienced at this very early life cycle stage may reduce their freshwater resilience and the recruitment success of future generations. If we can better understand the limiting factors influencing embryo growth and development, we can ultimately work towards improving in-stream incubation techniques that more effectively contribute to the long-term abundance and stability of wild Atlantic Salmon populations.

Connecting conceptual pathways

The Driver-Pressure-State-Impact-Response (DPSIR) framework is used as an environmental policy decision-making tool because it outlines a complex pathway of links between drivers, pressures, states, and impacts that lead to legislative or management responses (Baird et al., 2016; Smeets et al., 1999). This framework is useful here for conceptualizing multiple factors that might be contributing to real observed phenomena (e.g., embryo deformity/mortality phenomena). Another benefit of the DPSIR framework is that linkages between various factors can be characterized by the directionality of their impact in response to other factors (e.g., ↑ landscape disturbance is linked to increase in (↑) instream sedimentation). I used a modified version of the DPSIR framework to describe the interactions among drivers, pressures/stressors, states, and impacts related to our research questions about the survival and development of Atlantic Salmon embryos within the Serpentine River. I did not consider any legislative or management responses within the context of this research, but the conceptual pathways described here can help contribute to future recommendations.

For my study, the overall drivers of landscape composition within the watershed represent both anthropogenic land use from activities such as silviculture or hydroelectric power generation, and natural variability in landscape characteristics, such as the relative percent forest cover or underlying bedrock geology (Figure 4.1). Pressures are defined as direct effects from drivers and here they represent landscape disturbances such as clearcutting of forests and forestry (i.e., unpaved) road construction and variability in natural processes, such as ice formation events and changes in flow regime. Stressors are defined as secondary effects from pressures, and here represent instream environmental factors, such as increased sedimentation and reduced water quality conditions within Serpentine River. Meanwhile, the state is defined as the current condition of the environment, which here represents the direct measure of ecosystem and fish health, such as the number of embryo mortalities and deformities observed within each incubation bundle at each site. The impacts within the DPSIR framework are the observed effects from environmental degradation, which here represent the current state of Atlantic Salmon habitat and population abundance, such as their reduced freshwater resilience and poor recruitment. Finally, responses are defined as the response by society to the environmental situation and relate here to the observed capacity and willingness to return the aquatic environment to its previous state (e.g., desire to reduce embryo mortalities and deformities). Using the DPSIR framework to conceptualize the functional links from drivers through to responses helps to identify potential habitat and population restoration activities (e.g., instream incubation) we can employ to improve the freshwater resilience of Atlantic Salmon populations. Responses are not included in the modified DPSIR diagram below and some of the boxes are transparent, because although they are relevant within the Serpentine River watershed their relationships were not explicitly explored here. Below I discuss the findings from each chapter within this modified DPSIR framework and highlight how these results could help guide future fish population management activities and instream incubation techniques within the Serpentine River.

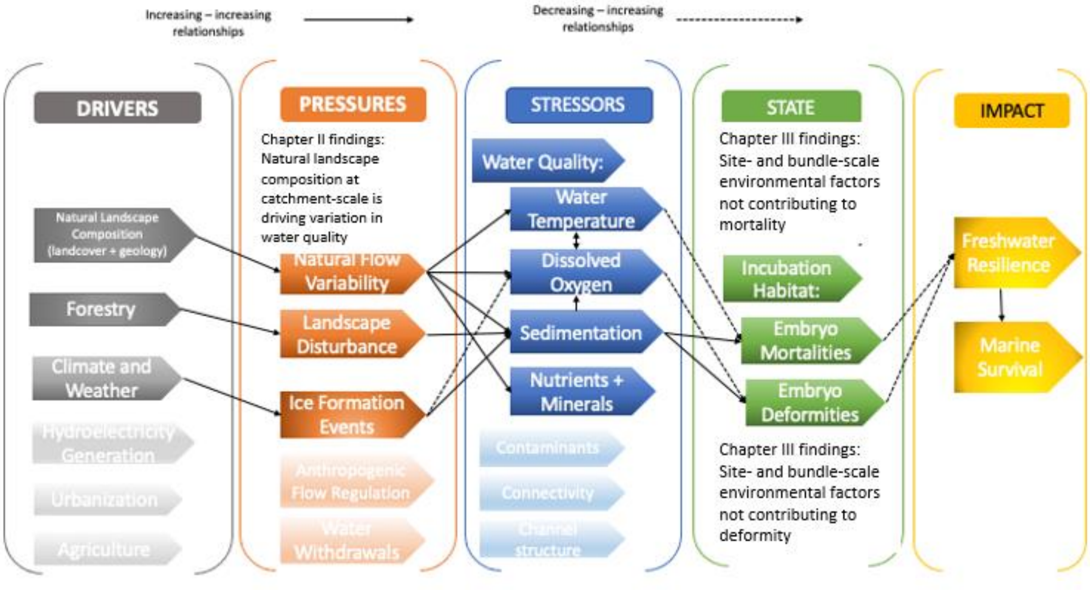

Figure 4.1: Proposed DPSIR framework for Atlantic Salmon within the Serpentine River watershed. Boxes represent relevant drivers, pressures, stressors, states and impacts with solid lines indicating increasing-increasing (↑-↑) relationships and dashed lines indicating decreasing-increasing (↓- ↑) relationships. Note that transparent boxes represent relevant factors, but relationships were not explicitly measured and described within this study.

Significance of spatial and temporal scales

By conceptualizing our findings within the adapted DPSIR framework the mechanistic linkages between environmental factors can be highlighted and how their directionality is influenced by different spatial and temporal scales can be explained. The findings suggest that natural landscape characteristics at the catchment-scale, such as topography, land cover and surficial bedrock geology, are influencing incubation habitat conditions within the Serpentine River. These natural drivers are influencing the physical and chemical composition of potential Atlantic Salmon incubation habitats and exerting pressure on the water flow regimes within the Serpentine River. Through the processes of erosion, runoff and chemical weathering the incubation habitat conditions at each site varied, which influenced the distribution of fine sediment particles within incubator and contributed to variation in water temperatures (i.e., represented by number of cumulative degree days) and dissolved oxygen concentrations, which are all major stressors for incubating embryos. However, the severity of stressors at each incubation site was influenced by their relative position along the longitudinal gradient within the Serpentine River, which reflected the unique impact of embryo deformity or mortality experienced at each specific spatial and temporal scale. While overall observed deformities within incubators were very low (~2.6%) and more prevalent at downstream sites, the overall observed mortalities were moderate (~45%) and more prevalent at upstream sites. The simple linear regressions indicated that neither the total number of field embryo deformities or mortalities were influenced by site-specific stressors, such as increasing variation in dissolved oxygen conditions at downstream sites or decreasing number of cumulative degree days at upstream sites. Likewise, simple linear regressions indicated that bundle-specific stressors, such increasing distribution of fine and coarse sediment particles within incubators did not significantly influence the proportion of embryo mortality or deformity as expected (Figure 4.1). Although, the spatial and temporal impacts of these multiple pressures and stressors did not have a direct impact on the state of incubation habitat within the Serpentine River, it may have a corresponding impact on the resilience of the freshwater life cycle stages of Atlantic Salmon in future incubation studies. Therefore, it is important to understand the complex interactions of drivers, pressures, stressors, states, and potential impacts on the very early embryonic life cycle stages of Atlantic Salmon in freshwater environments because they can ultimately alter the population structure and contribute to the reduced survival within the marine environments. With degraded freshwater habitat conditions, it is likely that fewer number of Atlantic salmon embryos will survive post-embryonic life cycle stages and be less able to recover from exposure to variable water quality conditions (i.e., less resilient) within the marine environment, thereby threatening future population viability of the wider OBoF population.

Chapter 2 findings indicate that upstream catchments (i.e., Sites 1 - 2) were characterized by greater amounts of natural land cover types, such as wetlands and more homogenous bedrock types. Conversely, downstream catchments (i.e., Sites 5-6) were characterized by greater amounts of anthropogenic disturbances, such as clearcutting and more heterogeneous bedrock types. It is also important to note that this temporal variation in anthropogenic activities, such as the lack of recent clearcutting within upstream and mid-stream (i.e., Sites 3-4) catchments is likely contributing to this longitudinal pattern of incubation sites conditions, as described by the river continuum concept (Vannote et al., 1980), which can further influence the downstream interaction of drivers, pressures and stressors.

Sites along the Serpentine River also followed a longitudinal gradient in substrate composition, with upstream sites generally characterized by coarse substrate sizes (i.e., greater cobble and fewer pebbles) and downstream sites characterized by relatively finer substrate sizes, except for minor deviations in proportion cobble substrate at Site 5. This longitudinal gradient in substrate composition supports the spatial hierarchy theory of freshwater ecosystems, which highlights the mechanistic pathway flow regimes play in shaping downstream habitats (Church, 2015). Additionally, our sites along the Serpentine River also followed a longitudinal gradient in water quality conditions. Headwater sites were characterized by slightly greater heavy metal concentrations such as iron and manganese, which reflect the chemical properties of the underlying felsic and granitic bedrock geology rather than any potential deviation from environmental water quality guidelines. However, these local-scale influences on water quality conditions were attenuated at downstream sites, which were characterized by greater concentrations in magnesium and reflect the underlying mafic bedrock geology.

Chapter 2 results did not support my hypothesis where I expected anthropogenic land uses at the catchment-scale to have a greater impact on site-scale incubation habitats. The relatively finer substrates at the most downstream site (i.e., greater pebbles and fewer cobble) are most likely associated with the natural erodibility characteristics of bedrock geology rather than direct impacts from clearcutting or forestry road networks. Thus, greater variability in the flow regime, which is facilitated by natural catchment-scale landscape characteristics, was the main mechanism influencing the spatial and temporal variation of incubation habitats throughout the watershed. Future decisions with respect to anthropogenic land use within the Serpentine River should take the wider spatial and temporal context of watershed-based management into consideration to avoid exacerbating cumulative impacts in downstream environments (e.g., clearcutting in headwater regions).

Chapter 3 findings suggest that the driver-pressure-stressor interactions from natural landscape characteristics on the water flow regime and incubation habitat conditions within the Serpentine River are not significantly contributing embryonic stressors. Site-level variations in water temperatures and DO concentrations, and bundle-level variations in distribution of sand particles within incubators were expected to impact embryo mortalities and deformities, but no significant difference was found between field embryos or control embryos. These findings suggest that the spatial and temporal dominance of natural landscape characteristics within the Serpentine River are helping preserve existing incubation habitat, and perhaps the high mortality rates observed in this experiment were due to the genetic influences of rearing brood stock fish within a hatchery environment. It is evident that the abundance and recruitment success of wild Atlantic Salmon populations within the Serpentine River is severely depressed, which makes the substitution of embryos from hatchery reared brood stock with completely wild embryos difficult. Regardless, in-stream incubation of Atlantic Salmon embryos using Jordan/Scotty incubators appears to be a viable option for bolstering wild populations, especially if epigenetic influences during embryogenesis can be minimized and alternative installation techniques can help decrease mortality. The findings from this in-situ incubation experiment were able to rule out any potential deformity phenomena from further impacting the post-embryonic fitness and overall freshwater resilience of Atlantic Salmon embryo incubated within the Serpentine River. However, there are still additional stressors within the wider Tobique River watershed and Saint John River drainage basin that need to be addressed if Serpentine River population is to recover.

Most notable stressor is habitat fragmentation from hydroelectric facilities, which span very large spatial scales and can significantly impede upstream and downstream migrations. Finally, it is also uncertain how the all-encompassing stressor of climate change will influence Atlantic Salmon populations, especially given the rapidly decreasing temporal scales at which climatic changes are occurring, which only further strengthen the need to foster greater freshwater resilience.

There are obvious limitations of our current in-stream incubation experiment, namely the vertical bundle installation technique, limited data logger deployment, and failure to quantify total amount and temporal variation in accumulated sediments within incubation bundles. However, the spatial and temporal scales of environmental factors discussed above, and the conceptualization of individual drivers-pressures-stressors-impacts interactions provide a useful framework for adaptive management approaches to any future in-stream incubation experiments. By continuing to refine in-stream incubation methods and techniques can we hope to alter some of the more influential DPSIR interactions currently limiting Atlantic Salmon embryonic growth and development and foster greater freshwater resilience.

Lessons learned and future research

This research highlights the care needed when selecting potential in-stream incubation sites, with special emphasis on locating areas with a relatively low level of sedimentation and stable water temperatures and DO concentrations. It is also important that a more comprehensive catchment-scale analysis of landscape characteristics be considered when selecting potential incubations sites within river systems. Currently, natural landscape characteristics are driving changes in site water quality and substrate compositions within the Serpentine River, but if future anthropogenic land use activities become spatially concentrated in one sub-catchment (e.g., clearcutting in headwaters) it could alter the suitability of downstream incubation habitats.

This research also highlights the benefit of using Jordan/Scotty incubation boxes as a conservation tool in protecting embryos from mechanical shock, fungal infection, and predation while exposing them to naturally variable flows. Ideally, habitat restoration and improvement of upstream/downstream fish passage structures within the Tobique River and the larger Wolastoq-Saint John River drainage basin would help wild populations recover. Since the survival and fitness of fish are improved when they are exposed to naturally variable flows, a greater understanding of the mechanisms of epigenetic expression may be achieved if embryos harvested from wild Atlantic Salmon are incubated within Jordan/Scotty incubators, especially as can help complement current efforts at preserving their genetic diversity (Burgerhout et al., 2017; Christie et al., 2012; Gavery et al., 2018). Research on comparing the survival of embryos from semi-captive (i.e., hatchery-reared) brood stock versus wild Atlantic Salmon is currently occurring within the Miramichi River by Roth et al. (unpub) which may be able to provide insight into how to reduce potentially detrimental epigenetic influences. If the survival and fitness of Atlantic Salmon embryos reared in Jordan/Scotty incubators increases, it will have a cascading effect on later stages of development and contribute to greater freshwater resilience. If greater freshwater resilience within the Outer Bay of Fundy (OBoF) population of Atlantic Salmon can be achieved, it will help buffer against effects of high marine mortality and contribute to greater recruitment success and population stability.

Future research should adopt adaptive management principles and explore modified incubation techniques that are suitable for local conditions, such as installing incubators above the river substrate as has previously been done by the Friends of the Kouchibouguacis in eastern New Brunswick. This watershed group has observed fewer Atlantic Salmon embryonic mortalities over multiple years when installing incubators above the substrate, as it has had the greatest potential at reducing sediment accumulation (M. Rushton, pers. comm.). There are, however, risks associated with installing incubators above the substrate since ice formation events (i.e., anchor ice, frazil ice, or ice scour) can negatively impact embryo survival by freezing the embryos or dislodging incubators. The selection of instream incubation sites used by the Friends of the Kouchibouguacis has evolved over multiple years and almost exclusively installs incubators within smaller tributary rivers to avoid potential issues posed by ice scour events more common in larger mainstem rivers. If other restoration groups are interested in replicating similar instream incubation projects, local ecological knowledge is required to ensure success as unseasonal discharge regimes from upstream hydroelectric reservoir lakes can cause large debris to dislodge incubators (as seen within our 2019 installation). Future incubation studies should also be paired with a detailed genetic analysis program for emigrating smolts and returning adults to investigate the effectiveness of instream incubation on survival and fitness. Jordan/Scotty egg incubation boxes are a viable restoration tool for fisheries managers aiming to recover or replenish depleted stocks and can contribute to greater embryo survival and fitness of Atlantic Salmon with exposure to naturally variable flows, thereby limiting hatchery rearing effects. Overall, this research established that deformities of Atlantic Salmon embryos incubated within the Serpentine River, New Brunswick were very low compared with the earlier 2013 study (A. Bartlett, unpub. data) and thereby are not a contributing factor limiting their fitness and overall freshwater resilience. With continued experimentation in instream incubation techniques, improved incubation site selection and further exploration of epigenetic influences from hatcher rearing environment it is anticipated that Atlantic Salmon populations within the Serpentine River can achieve greater stability for future generations.

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Appendix A. Supplementary Material

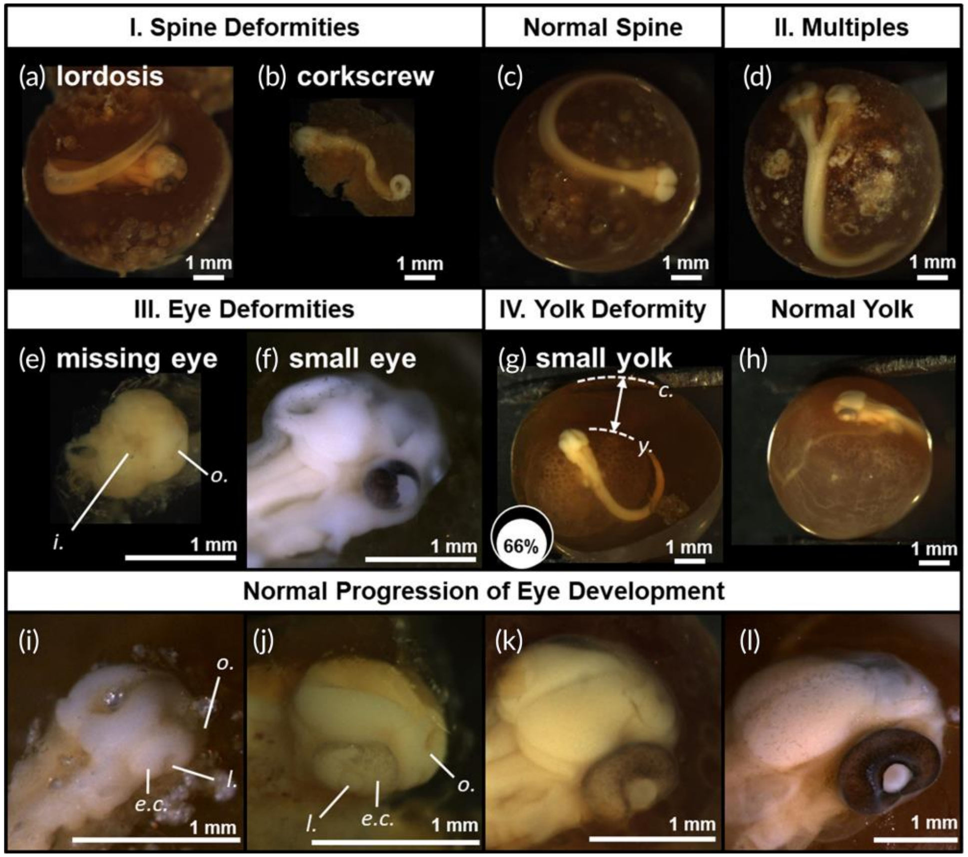

Figure A.1: Deformity assessment index showing the different deformity types observed in Atlantic Salmon embryos in Tobique River (Bartlett et al., 2022)

Appendix B: Raw PCA Input Data

Table B.1: Catchment Scale Variables

Site	Drain_Cat (km2)	For_Cat (%)	Wet_Cat (%)	Bare_Cat (%)	CC_Cat (%)	Spray_Cat (%)	Dist_CC (km)	Yr_CC	Dist_Spray (km)	Yr_Spray	Rd_Dens_Cat	Mafic_Cat (%)	DWC_Cat (%)	FI_Cat (%)	FV_Cat (%)
1	85.86	72.61	4.40	0.02	10.99	0	0.10	3	3.53	1	1.65	0	34.14	60.19	0
2	157.12	76.30	3.73	0.06	11.98	0.07	0.04	0	0.26	1	1.74	0	30.13	66.76	0
3	190.01	78.10	3.27	0.09	11.63	0.07	0.81	7	2.58	1	1.78	0	29.14	68.30	0
4	288.57	79.30	2.54	0.06	13.11	0.08	0.05	5	5.38	0	2.21	0.01	39.15	59.15	0
5	328.81	77.93	2.36	0.05	15.07	0.04	0.10	1	8.6	0	2.16	1.17	42.94	51.91	2.49
6	379.77	76.87	2.28	0.04	16.64	0.30	1.21	5	2.29	0	2.17	1.01	46.61	44.94	6.14

Table B.2: Reach Scale Variables

Site	MSlope_Rch (m)	RipWet_Rch (%)	Flood_Rch (%)	MCA_Rch (%)	Rbuff_Rch (%)	PLPM5_Rch (%)	PLPM9_Rch (%)	PLPM10_Rch (%)	PLPM11_Rch (%)	PLPM12_Rch (%)
1	3.96	1.06	61.98	0	11.29	13.38	75.35	10.64	0	0
2	2.56	0.09	71.72	1.49	7.30	0	50.91	0	0	46.89
3	2.32	0.39	82.87	0	0.34	56.27	0	0	0	42.37
4	6.79	0	11.44	72.55	6.71	97.89	0	0	0	0
5	4.39	0	45.65	10.38	17.39	83.37	0	0	13.28	0
6	0.30	1.36	7.96	75.70	2.81	15.87	0	0	81.55	0

Table B.3: Site Scale Variables

Site	Site_Elev (m)	Dist_2_Rd (km)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	ALKY	Conduct	Al (mg/L)	Fe (mg/L)	Mn (mg/L)	WetWidth (m)	Propn_Gravel (%)	Propn_SPeb (%)	Propn_LPeb (%)	Propn_SCob (%)	Propn_LCob (%)	Pct_Bould (%)	Pct_BedR (%)	wD50	CV_Temp	CDD	CV_DO	CV_Lvl
1	362.19	0.10	0.255	2.16	0.365	6	22	67	110	15.5	9.41	1.0	10.0	43.0	32.0	11.0	3.0	0	7.5	145.13	81.82	4.09	14.41
2	343.74	0.04	0.275	2.88	0.47	8	27	72	80	6	11.31	3.0	14.0	43.0	38.0	1.0	0	0	50.5	141.66	78.07	4.04	28.01
3	335.26	0.81	0.315	3.295	0.525	10	28	72	85	7	27.26	1.0	13.0	50.0	21.0	2.0	4.0	0	50.5	155.13	72.84	5.63	39.41
4	279.41	0.05	0.295	2.64	0.5	7.5	26.5	57	75	5	30.71	1.0	13.0	47.0	34.0	2.0	0	2.0	50.5	139.80	81.31	9.93	21.41
5	252.81	0.10	0.29	2.775	0.525	8.5	27.5	55.5	70	4.5	34.66	0	13.0	36.0	44.0	6.0	1.0	0	6.4	135.44	82.38	10.21	28.28
6	237.78	1.21	0.295	3.24	0.575	10	31	50.5	60	3	46.48	2.0	21.0	49.0	21.0	4.0	3.0	0	5.75	131.77	90.54	16.57	23.03

Appendix C: Correlation Matrix

Table C.1: Raw correlation matrix of PCA input variables (R>0.8 highlighted).

Variables	Site	Drain_Cat	Site_Elev	MSlope_Rch	For_Cat	Wet_Cat	Bare_Cat	CC_Cat	Dist_CC	Yr_CC	Spray_Cat	Dist_Spray	Yr_Spray	Rd_Dens_Cat	Dist_2_Rd	RipWet_Rch	Flood_Rch	MCA_Rch	Rbuff_Rch	PLPM5_Rch	PLPM9_Rch	PLPM10_Rch	PLPM11_Rch	PLPM12_Rch	Mafic_Cat	DWC_Cat	Fl_Cat	FV_Cat	K	Ca	Mg	ALKY	Conduct	Al	Fe	Mn	WetWidth	Propn_Grav	Propn_SPeb	Propn_LPeb	Propn_SCob	Propn_LCob	Propn_Bould	Propn_BedR	wD50	CV_Temp	CV_DO	CV_Lvl
Site	1																																															
Drain_Cat	0.99	1																																														
Site_Elev	-0.98	-0.99	1																																												
MSlope_Rch	-0.20	-0.12	0.06	1																																										
For_Cat	0.63	0.66	-0.57	0.24	1																																								
Wet_Cat	-0.97	-0.98	0.95	-0.01	-0.79	1																																						
Bare_Cat	0.14	0.11	0.02	-0.08	0.73	-0.26	1																																					
CC_Cat	0.95	0.94	-0.96	-0.32	0.39	-0.86	-0.12	1																																			
Dist_CC	0.53	0.44	-0.37	-0.78	0.14	-0.37	0.23	0.49	1																																		
Yr_CC	0.22	0.17	-0.10	-0.11	0.30	-0.22	0.37	0.01	0.64	1																																	
Spray_Cat	0.73	0.71	-0.66	-0.63	0.26	-0.60	0.03	0.77	0.81	0.35	1																																
Dist_Spray	0.40	0.42	-0.50	0.59	0.31	-0.48	-0.16	0.33	-0.33	-0.09	-0.28	1																															
Yr_Spray	-0.88	-0.92	0.95	-0.22	-0.56	0.90	0.14	-0.85	-0.15	-0.07	-0.50	-0.62	1																														
Rd_Dens_Cat	0.90	0.94	-0.95	0.21	0.69	-0.95	0.04	0.83	0.18	0.14	0.52	0.57	-0.98	1																													
Dist_2_Rd	0.57	0.53	-0.52	-0.71	-0.12	-0.36	-0.30	0.69	0.79	0.25	0.92	-0.30	-0.37	0.32	1																												
RipWet_Rch	0.08	0.01	-0.04	-0.63	-0.59	0.16	-0.52	0.23	0.65	0.33	0.54	-0.36	0.06	-0.15	0.80	1																											
Flood_Rch	-0.72	-0.77	0.80	-0.15	-0.33	0.70	0.33	-0.74	-0.22	-0.18	-0.64	-0.31	0.88	-0.85	-0.56	-0.22	1																										
MCA_Rch	0.69	0.73	-0.72	0.05	0.44	-0.69	-0.11	0.65	0.37	0.39	0.74	0.09	-0.78	0.79	0.59	0.24	-0.95	1																									
Rbuff_Rch	-0.05	-0.01	-0.12	0.47	-0.23	0.02	-0.53	0.08	-0.69	-0.73	-0.52	0.70	-0.24	0.12	-0.37	-0.31	-0.01	-0.29	1																								
PLPM5_Rch	0.40	0.44	-0.44	0.70	0.73	-0.58	0.34	0.17	-0.23	0.28	-0.21	0.79	-0.57	0.63	-0.43	-0.59	-0.31	0.27	0.23	1																							
PLPM9_Rch	-0.84	-0.83	0.77	0.00	-0.89	0.91	-0.58	-0.63	-0.46	-0.48	-0.49	-0.41	0.69	-0.79	-0.20	0.24	0.46	-0.53	0.25	-0.68	1																						
PLPM10_Rch	-0.65	-0.67	0.57	0.13	-0.89	0.75	-0.73	-0.50	-0.28	-0.09	-0.44	-0.04	0.45	-0.59	-0.08	0.48	0.24	-0.35	0.29	-0.37	0.79	1																					
PLPM11_Rch	0.73	0.70	-0.69	-0.66	0.04	-0.55	-0.23	0.84	0.78	0.20	0.93	-0.12	-0.53	0.50	0.97	0.68	-0.62	0.63	-0.27	-0.27	-0.36	-0.24	1																				
PLPM12_Rch	-0.42	-0.45	0.56	-0.33	0.10	0.38	0.70	-0.50	0.03	-0.05	-0.18	-0.64	0.71	-0.58	-0.32	-0.33	0.74	-0.54	-0.46	-0.34	0.13	-0.32	-0.37	1																			
Mafic_Cat	0.81	0.79	-0.84	-0.31	0.20	-0.71	-0.21	0.90	0.35	-0.18	0.49	0.51	-0.71	0.65	0.52	0.19	-0.47	0.30	0.38	0.15	-0.49	-0.32	0.69	-0.50	1																							
DWC_Cat	0.84	0.85	-0.91	-0.05	0.22	-0.76	-0.42	0.91	0.30	0.01	0.61	0.51	-0.91	0.84	0.62	0.30	-0.86	0.71	0.27	0.26	-0.48	-0.20	0.75	-0.81	0.84	1																						
Fl_Cat	-0.78	-0.78	0.84	0.22	-0.05	0.65	0.50	-0.90	-0.39	0.01	-0.65	-0.40	0.81	-0.72	-0.73	-0.47	0.79	-0.63	-0.25	-0.08	0.36	0.09	-0.83	0.78	-0.86	-0.98	1																					
FV_Cat	0.81	0.78	-0.79	-0.60	0.10	-0.64	-0.24	0.92	0.69	0.09	0.86	0.08	-0.63	0.58	0.89	0.57	-0.62	0.56	-0.07	-0.15	-0.43	-0.28	0.97	-0.44	0.84	0.83	-0.90	1																				
K	0.59	0.55	-0.44	-0.15	0.84	-0.66	0.83	0.33	0.55	0.63	0.39	0.11	-0.31	0.46	0.08	-0.24	-0.12	0.30	-0.54	0.52	-0.90	-0.78	0.19	0.25	0.18	0.09	0.00	0.20	1																			
Ca	0.57	0.51	-0.39	-0.63	0.59	-0.53	0.73	0.44	0.75	0.40	0.64	-0.29	-0.14	0.27	0.41	0.03	-0.02	0.21	-0.64	0.00	-0.69	-0.79	0.48	0.45	0.29	0.08	-0.08	0.45	0.83	1																		
Mg	0.89	0.86	-0.78	-0.36	0.78	-0.88	0.54	0.76	0.63	0.32	0.72	0.11	-0.61	0.71	0.46	-0.07	-0.43	0.52	-0.36	0.32	-0.90	-0.88	0.60	0.03	0.59	0.50	-0.44	0.64	0.83	0.87	1																	
ALKY	0.66	0.59	-0.49	-0.63	0.58	-0.61	0.66	0.54	0.80	0.44	0.66	-0.15	-0.24	0.34	0.46	0.09	-0.08	0.24	-0.56	0.07	-0.75	-0.74	0.55	0.31	0.43	0.20	-0.21	0.55	0.84	0.98	0.90	1																
Conduct	0.83	0.80	-0.71	-0.54	0.65	-0.78	0.49	0.76	0.71	0.26	0.82	-0.10	-0.50	0.59	0.59	0.06	-0.39	0.50	-0.44	0.08	-0.77	-0.84	0.70	0.13	0.56	0.45	-0.44	0.71	0.73	0.91	0.97	0.91	1															
Al	-0.85	-0.88	0.93	-0.04	-0.32	0.80	0.35	-0.89	-0.28	-0.09	-0.61	-0.53	0.95	-0.90	-0.58	-0.24	0.91	-0.79	-0.22	-0.36	0.55	0.25	-0.70	0.81	-0.77	-0.99	0.94	-0.78	-0.16	-0.08	-0.53	-0.20	-0.46	1														
Fe	-0.91	-0.92	0.87	0.25	-0.73	0.91	-0.33	-0.86	-0.43	-0.04	-0.73	-0.16	0.75	-0.82	-0.47	0.13	0.60	-0.63	0.15	-0.28	0.79	0.86	-0.63	0.11	-0.67	-0.64	0.57	-0.69	-0.60	-0.69	-0.93	-0.71	-0.92	0.66	1													
Mn	-0.83	-0.84	0.77	0.18	-0.83	0.88	-0.51	-0.73	-0.35	-0.06	-0.62	-0.12	0.66	-0.76	-0.31	0.30	0.48	-0.54	0.21	-0.34	0.82	0.95	-0.47	-0.06	-0.53	-0.48	0.38	-0.52	-0.69	-0.74	-0.93	-0.73	-0.90	0.51	0.97	1												
WetWidth	0.98	0.95	-0.93	-0.24	0.61	-0.93	0.18	0.89	0.65	0.42	0.76	0.36	-0.82	0.85	0.60	0.18	-0.69	0.71	-0.19	0.41	-0.87	-0.59	0.74	-0.42	0.74	0.79	-0.74	0.80	0.67	0.61	0.88	0.71	0.81	-0.82	-0.84	-0.75	1											
Propn_Grav	-0.21	-0.19	0.26	-0.50	-0.18	0.27	0.07	-0.08	0.16	-0.22	0.38	-0.89	0.35	-0.31	0.34	0.19	0.07	0.08	-0.48	-0.74	0.35	-0.16	0.22	0.54	-0.31	-0.28	0.21	0.06	-0.19	0.26	0.01	0.10	0.23	0.30	-0.11	-0.14	-0.26	1										
Propn_SPeb	0.75	0.73	-0.68	-0.67	0.29	-0.62	0.07	0.81	0.77	0.20	0.98	-0.27	-0.50	0.52	0.89	0.45	-0.57	0.66	-0.44	-0.24	-0.49	-0.53	0.93	-0.10	0.57	0.60	-0.64	0.87	0.38	0.69	0.77	0.71	0.87	-0.58	-0.80	-0.70	0.75	0.42	1									
Propn_LPeb	0.06	0.03	0.08	-0.34	0.18	-0.03	0.38	-0.07	0.67	0.83	0.52	-0.60	0.14	-0.05	0.40	0.40	-0.14	0.42	-0.97	-0.14	-0.24	-0.16	0.28	0.25	-0.39	-0.17	0.16	0.08	0.47	0.49	0.28	0.43	0.34	0.10	-0.08	-0.11	0.23	0.36	0.40	1								
Propn_SCob	-0.14	-0.06	-0.02	0.59	0.01	0.02	-0.25	-0.05	-0.86	-0.84	-0.58	0.52	-0.16	0.11	-0.58	-0.66	0.07	-0.29	0.88	0.26	0.22	0.02	-0.47	-0.15	0.14	0.05	0.03	-0.30	-0.44	-0.54	-0.30	-0.54	-0.37	-0.03	0.04	0.02	-0.32	-0.22	-0.47	-0.91	1							
Propn_LCob	-0.29	-0.31	0.18	0.09	-0.77	0.41	-0.82	-0.12	-0.18	-0.16	-0.32	0.33	0.10	-0.27	0.05	0.49	0.05	-0.26	0.55	-0.18	0.53	0.88	-0.01	-0.59	0.15	0.18	-0.28	0.05	-0.67	-0.71	-0.63	-0.58	-0.64	-0.11	0.60	0.75	-0.26	-0.45	-0.38	-0.41	0.14	1						
Propn_Bould	-0.03	-0.14	0.16	-0.57	-0.33	0.19	0.03	-0.06	0.70	0.63	0.21	-0.21	0.32	-0.34	0.38	0.69	0.26	-0.18	-0.44	-0.27	-0.02	0.33	0.30	0.03	0.04	-0.09	-0.05	0.24	0.21	0.28	0.03	0.36	0.06	0.13	0.27	0.33	0.15	-0.19	0.13	0.46	-0.77	0.35	1					
Propn_BedR	0.13	0.22	-0.21	0.76	0.52	-0.32	0.09	-0.03	-0.33	0.28	-0.04	0.27	-0.45	0.50	-0.26	-0.40	-0.55	0.61	-0.07	0.64	-0.31	-0.20	-0.24	-0.32	-0.30	0.15	0.03	-0.28	0.18	-0.22	0.05	-0.27	-0.08	-0.28	-0.14	-0.20	0.14	-0.16	-0.13	0.22	0.12	-0.31	-0.52	1				
wD50	-0.31	-0.28	0.39	0.26	0.48	0.13	0.75	-0.52	-0.20	0.20	-0.22	-0.39	0.35	-0.19	-0.51	-0.60	0.31	-0.07	-0.51	0.18	-0.12	-0.43	-0.55	0.71	-0.72	-0.67	0.78	-0.65	0.39	0.26	0.05	0.10	0.04	0.57	0.02	-0.18	-0.29	0.35	-0.22	0.42	-0.07	-0.77	-0.32	0.45	1			
CV_Temp	-0.66	-0.71	0.77	0.06	-0.10	0.59	0.50	-0.83	-0.05	0.40	-0.54	-0.30	0.78	-0.70	-0.55	-0.17	0.78	-0.61	-0.39	0.00	0.21	0.22	-0.66	0.62	-0.74	-0.87	0.86	-0.73	0.18	0.03	-0.35	-0.03	-0.38	0.84	0.63	0.48	-0.53	-0.08	-0.59	0.35	-0.31	-0.07	0.43	-0.10	0.55	1		
CV_DO	0.94	0.93	-0.94	-0.26	0.41	-0.86	-0.11	0.95	0.60	0.27	0.85	0.27	-0.86	0.85	0.76	0.35	-0.85	0.83	-0.12	0.22	-0.67	-0.44	0.87	-0.58	0.76	0.91	-0.89	0.90	0.39	0.43	0.75	0.52	0.73	-0.92	-0.80	-0.67	0.94	-0.10	0.83	0.18	-0.27	-0.12	0.05	0.15	-0.44	-0.74	1	
CV_Lvl	0.16	0.11	0.01	-0.29	0.57	-0.23	0.93	-0.04	0.32	0.26	0.02	-0.10	0.20	-0.05	-0.22	-0.41	0.48	-0.32	-0.39	0.21	-0.53	-0.66	-0.14	0.71	0.00	-0.37	0.40	-0.10	0.78	0.78	0.55	0.74	0.51	0.36	-0.31	-0.46	0.20	-0.02	0.09	0.20	-0.22	-0.64	0.21	-0.25	0.49	0.48	-0.11	1

Appendix D: Site-Scale Variables

Table D.1 : Site-scale variables for six Serpentine River incubation sites.

Site	Average water temperature (°C)	Coefficient of variation in water temperature (%)	Cumulative degree days (#)	Average dissolved oxygen concentrations (mg/L)	Coefficient of variation in dissolved oxygen (%)
1	1.87	145.14	81.82	13.07	4.09
2	1.74	141.66	78.07	13.42	4.04
3	1.62	155.13	72.84	13.14	5.63
4	1.69	139.80	81.31	12.48	9.93
5	1.74	135.45	82.38	12.19	10.21
6	1.83	131.78	90.54	11.76	16.57

Appendix E: Additional Water Temperature and Dissolved Oxygen Plots (Bundle and Site Level Summaries)

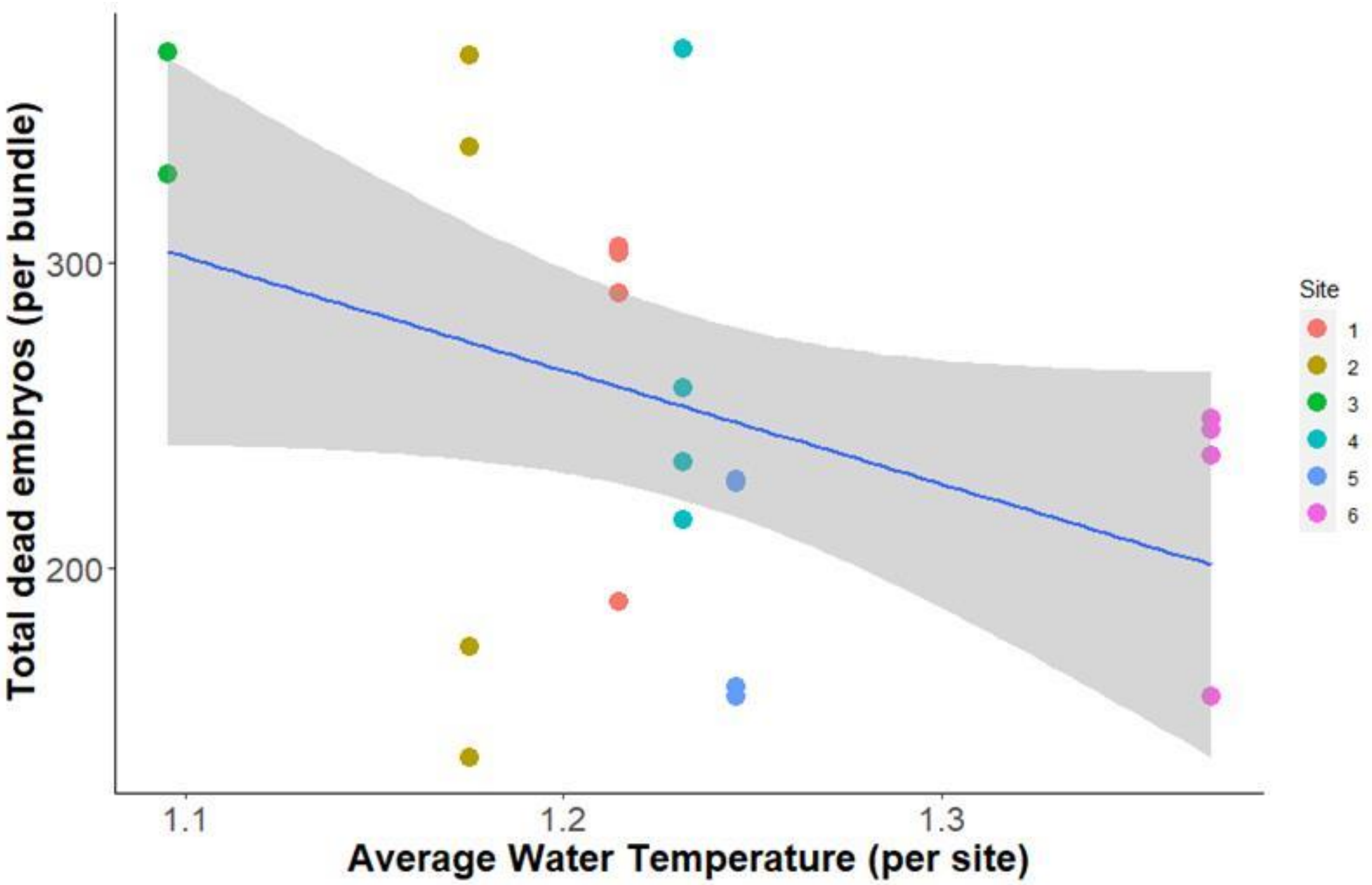

Figure E.1: Scatterplot of total dead embryos (per bundle) and average water temperature (per site).

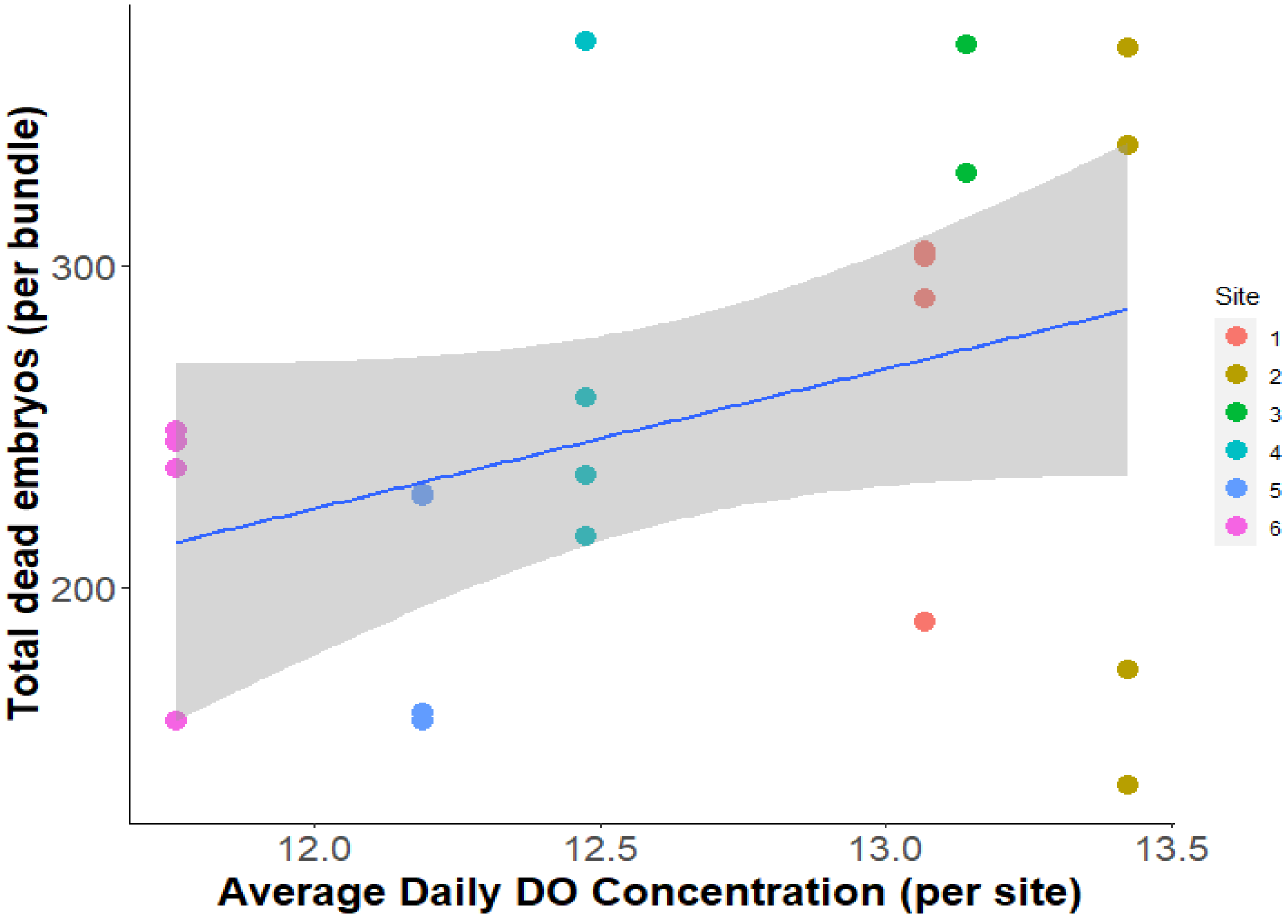

Figure E.2: Scatterplot of total dead embryos (per bundle) and average daily dissolved oxygen concentration (per site).

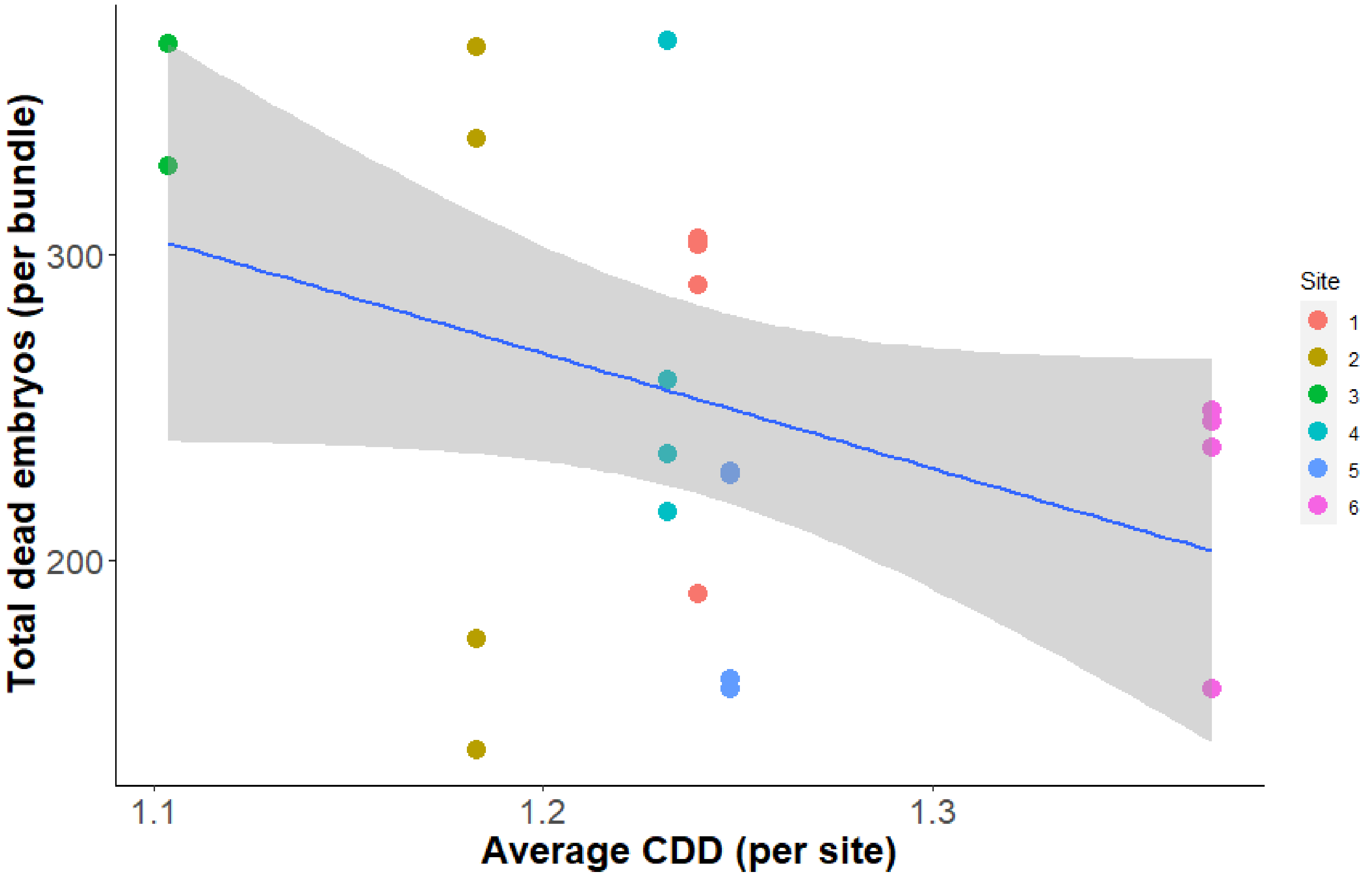

Figure E.3: Scatterplot of total dead embryos (per bundle) and average cumulative degree days (per site).

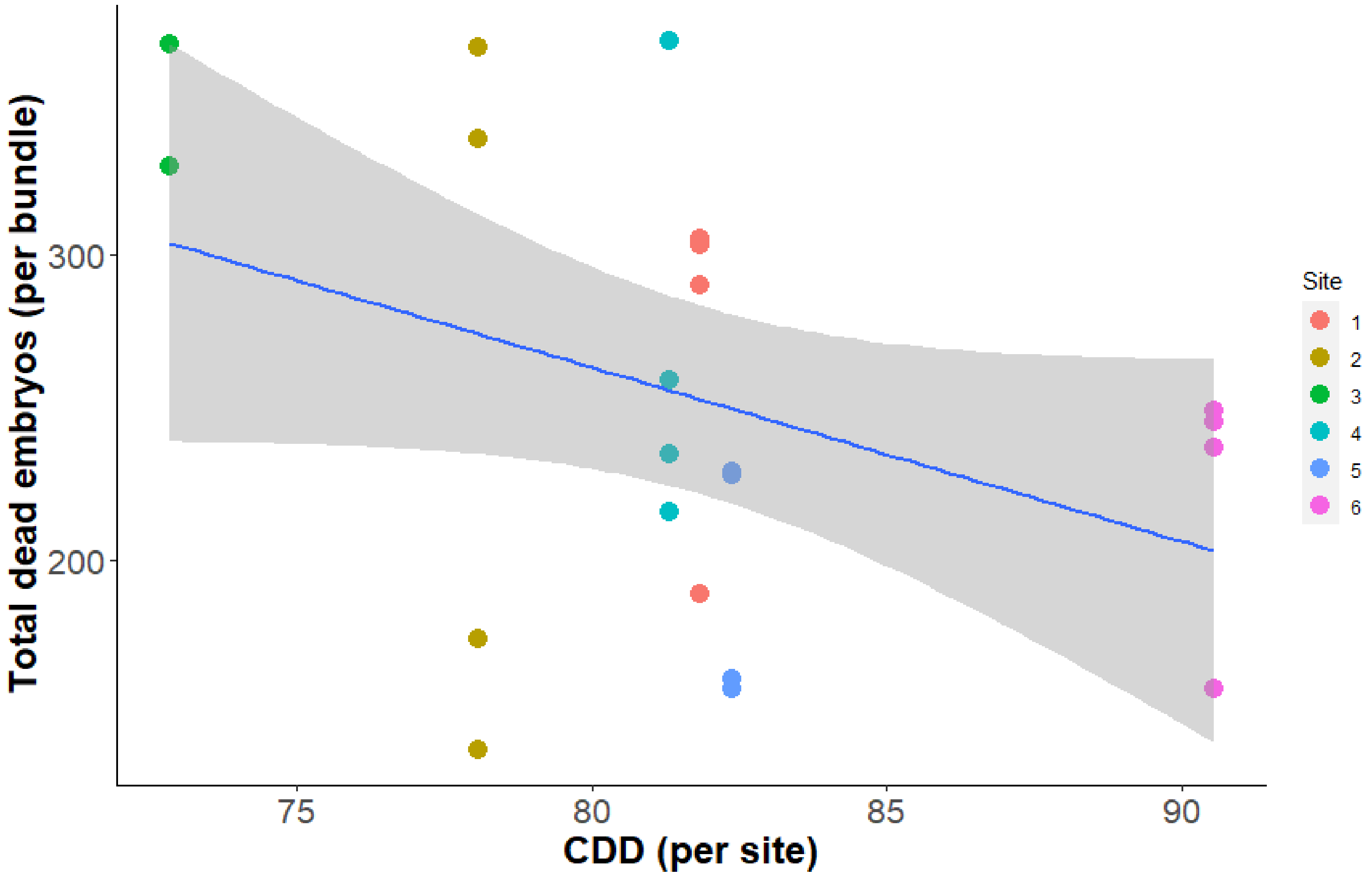

Figure E.4: Scatterplot of total dead embryos (per bundle) and cumulative degree days (per site).

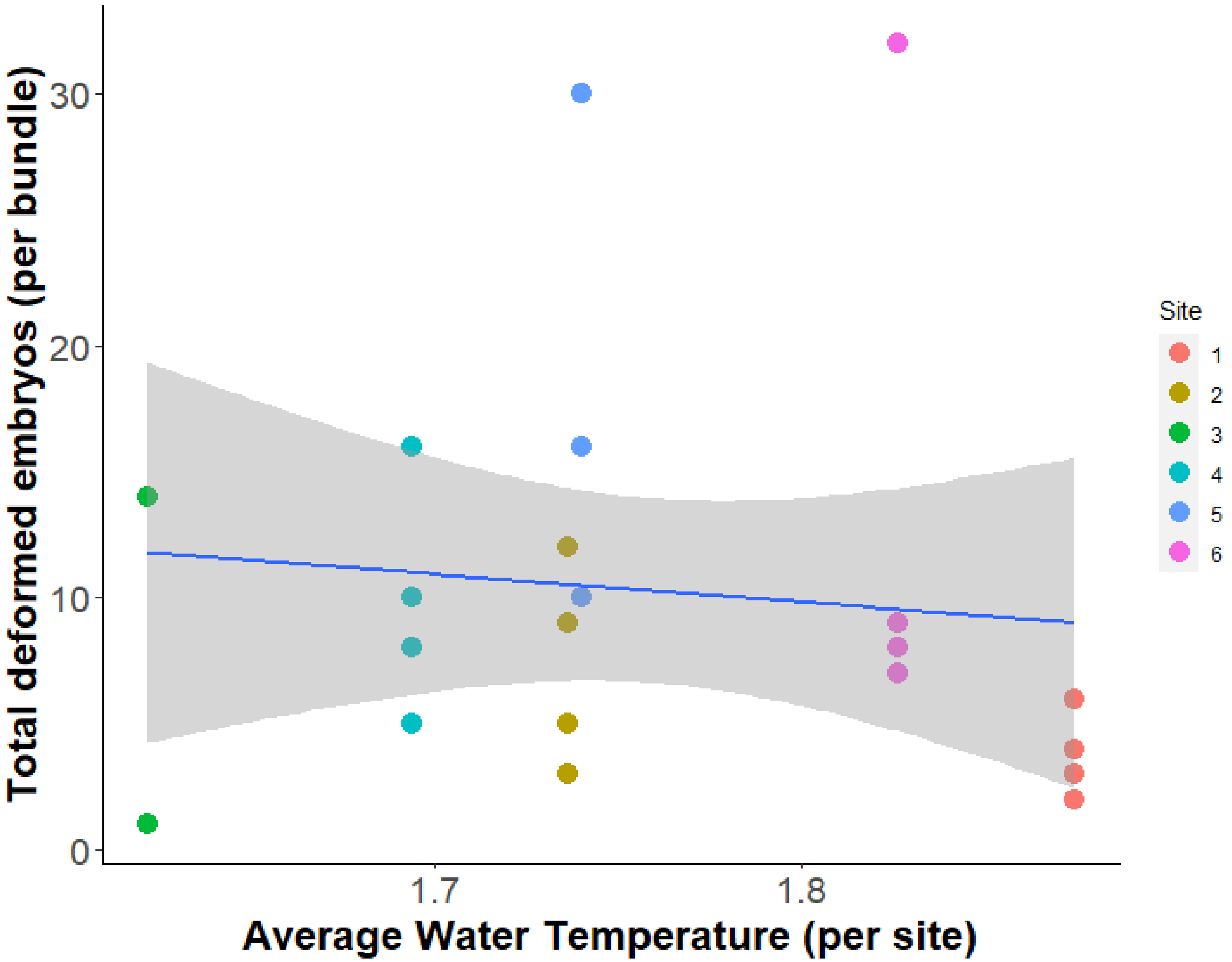

Figure E.5: Scatterplot of total deformed embryos (per bundle) and average water temperature (per site).

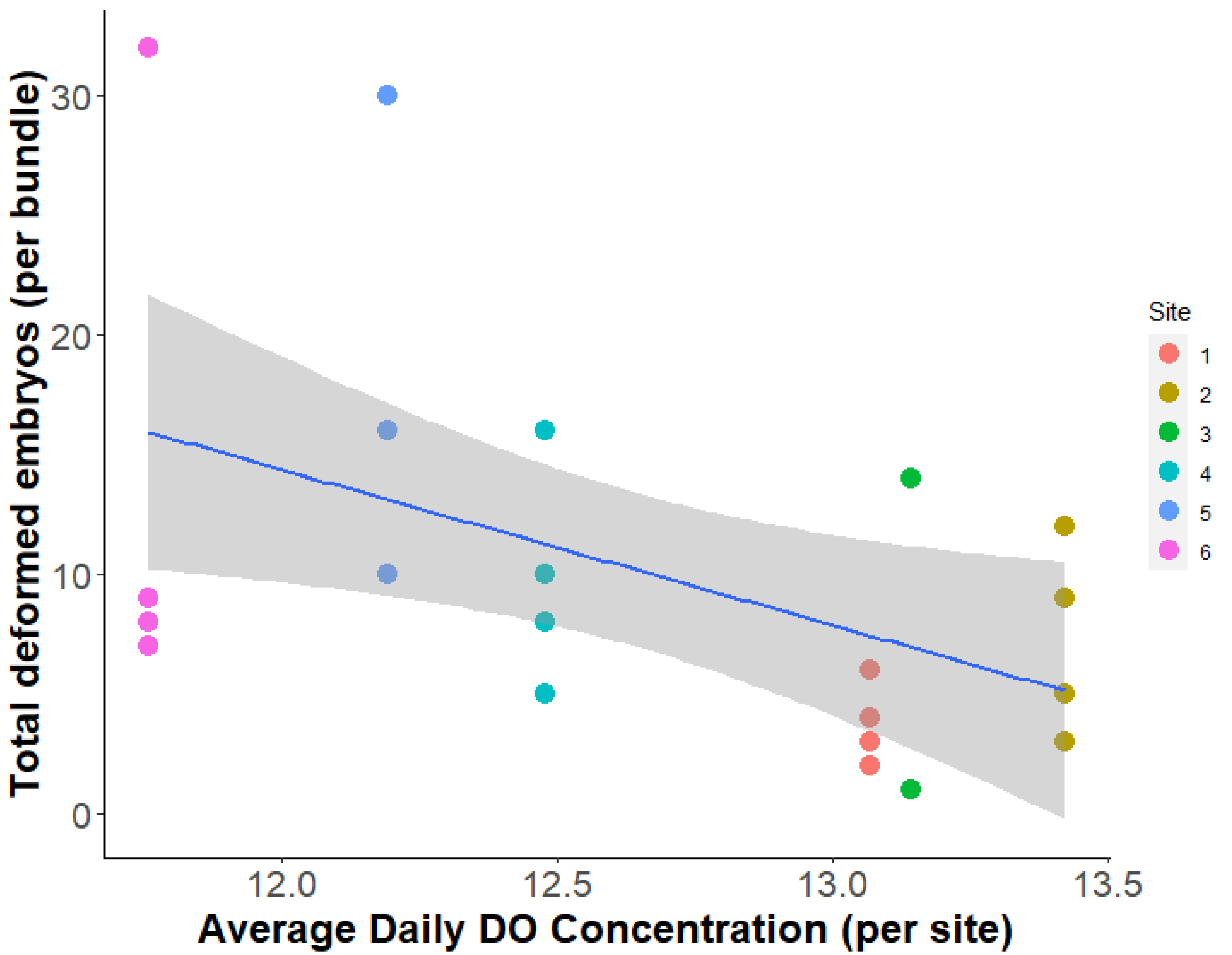

Figure E.6: Scatterplot of total deformed embryos (per bundle) and average daily dissolved oxygen concentration (per site).

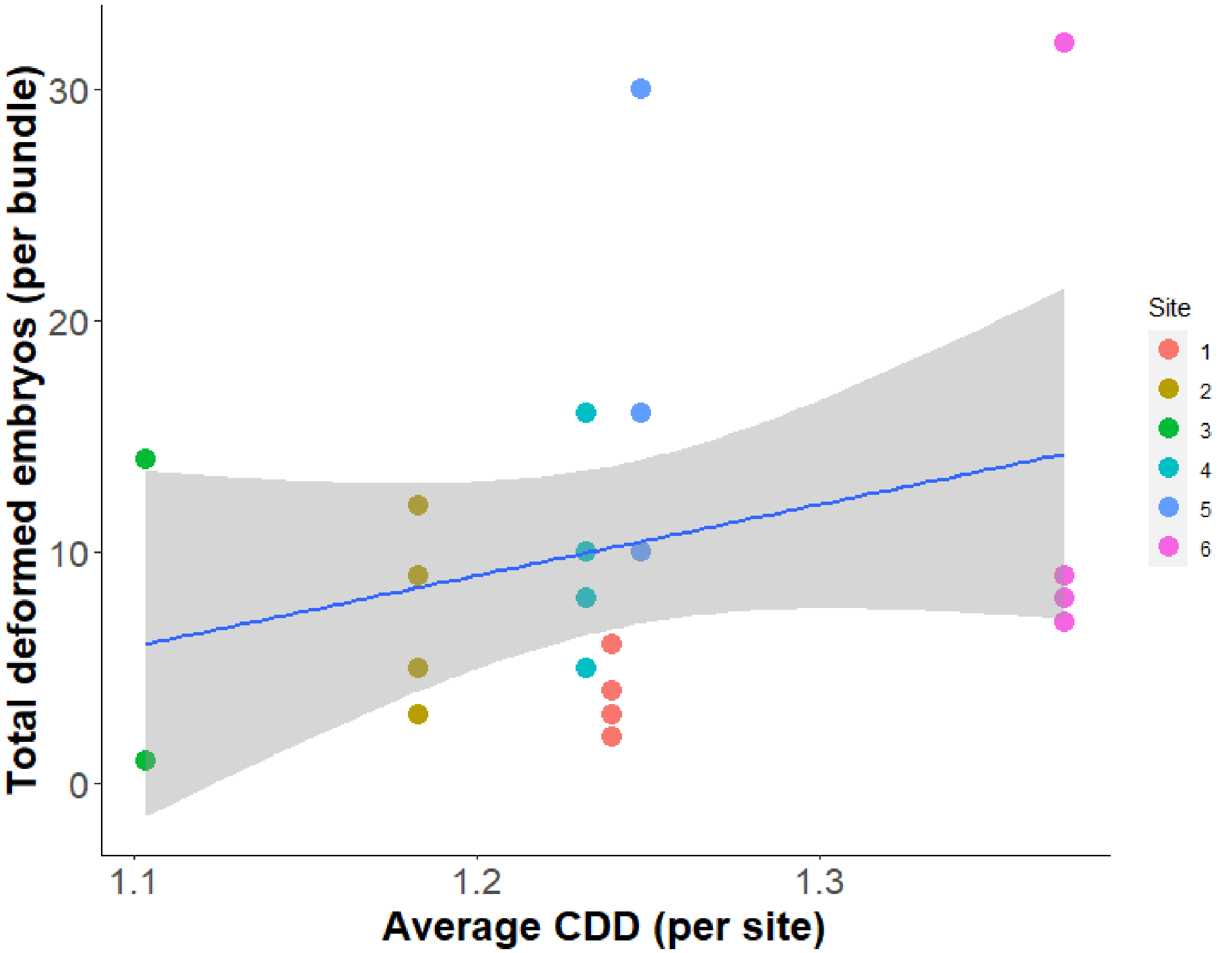

Figure E.7: Scatterplot of total deformed embryos (per bundle) and average cumulative degree days (per site).

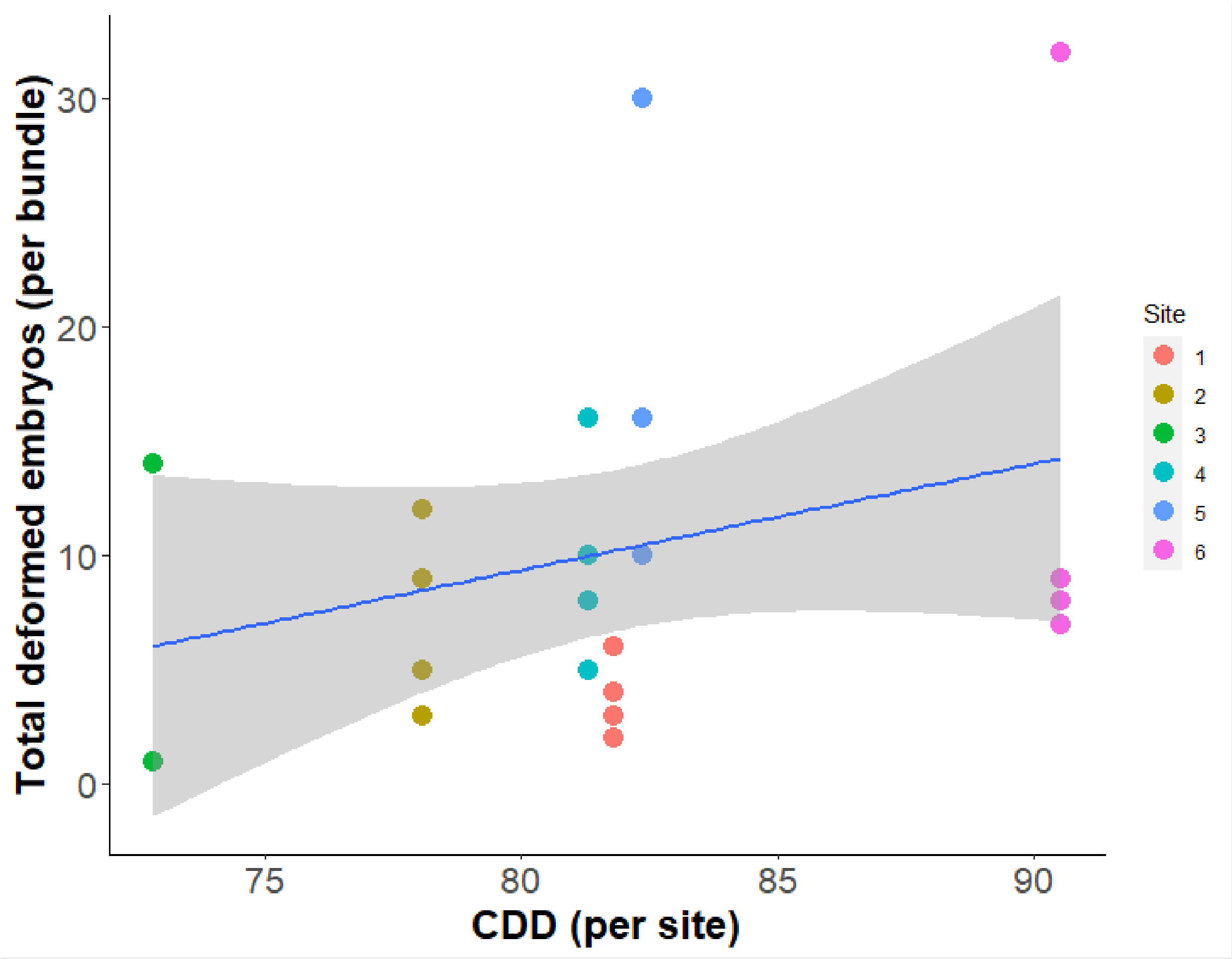

Figure E.8: Scatterplot of total deformed embryos (per bundle) and cumulative degree days (per site).

Curriculum Vitae

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Universities attended (with dates and degrees obtained):

Saint Francis Xaiver University - Bachelor of Arts in Aquatic Resources (May 2013)

Conference Presentations:

Millar, W (2021, May 6th-7th). Variation of landscape characteristics and water quality conditions within Serpentine River Watershed [Conference Poster Presentation]. 2021 University of New Brunswick Graduate Research Conference, Fredericton, New Brunswick, Canada