TEMPORAL AND SPATIAL PATTERNS OF OUTMIGRATION OF JUVENILE SOCKEYE

SALMON IN RIVERS INLET

by

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B.Sc., Thompson Rivers University, 2012

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

The Faculty of Graduate and Postdoctoral Studies

(Oceanography)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

October 2016

ABSTRACT

The sockeye salmon, *Oncorhynchus nerka*, is economically, ecologically and culturally important for British Columbia and Canada. Currently, ~75% of sockeye populations are declining with a precipitous decline reported in Rivers Inlet (RI). The purpose of this study was to investigate the variability of juvenile sockeye residence and growth in RI during their outmigration to the Pacific Ocean. The point of the marine entry was estimated by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (*LA-ICP-MS*) using otolith elemental composition (Sr:Ca ratio). A significant correlation was found between the day of entry and the duration of the migration. Average transit time through the inlet was ~ 2 weeks in 2011 and 2009, compared to ~ 4 weeks in 2008 and 2010. In terms of BL/s, the fish travel through RI with an average speed of 0.2– 0.5 BL/s. The size of the smolts at the entry to the inlet ranged between 54 and 83 mm. Detailed analysis of the full outmigration period in 2009 identified two possible migration behaviors, dependent on the day of entry and potentially the size of the smolts. Smaller fish that entered the inlet prior to mid-June exhibited a "holding" pattern at the head of the inlet, with an average residence time of 2-3 weeks. Smolts that entered the inlet later in the season swam continuously throughout the inlet. Using GAM models, it was determined that 78% of the weight at catch and 64% increase in weight were explained by the combination of water temperature, freshwater discharge, zooplankton densities and inlet entry size of smolts. The duration of the outmigration was dependent on river discharge and temperature, with 62% of the variation in outmigration duration explained by the model. Results suggest that there is a set of optimal environmental conditions for smolt growth during their outmigration. Outmigration timing was found to be the key determinant of conditions experienced and appeared to act as an equilibration mechanism between small and large smolts, resulting in similar size being attained by both by the inlet mouth.

Temporal and Spatial Patterns of Outmigration of Juvenile Sockeye Salmon in Rivers Inlet, is original, unpublished, independent work written by Y. Egorova. This work forms part of the collaborative effort of Rivers Inlet Ecosystem Study (RIES), funded by the Tula Foundation, with the primary objective to "understand the dynamics of spring productivity and how it affects the growth of juvenile sockeye salmon." It has been written to fulfill the graduation requirements of the MSc in the Faculty of Graduate Studies at the University of British Columbia (UBC). My research question was formulated together with my supervisor, Evgeny Pakhomov and the other members of the research committee Brian Hunt and Scot Hinch. The research was interesting and challenging and provides further insight into the early marine stage of the juvenile sockeye salmon.

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ACKNOWLEDGEMENTS

DEDICATION

This work is dedicated to my grandfather, Vladimir Egorov. Thank you for your love, support, and for teaching me the value of knowledge. I wish I had half of the wisdom you have.

1.1. Sockeye Salmon of BC

Sockeye salmon (*Oncorhynchus nerka*; Walbaum 1792) is one of the most abundant salmon species in the Pacific Ocean (Figure 1). They are an important part of the food webs in terrestrial, freshwater, and marine environments. Salmon fisheries as a whole contribute significantly to the economic wealth of individual provinces as well as the whole country (www.env.gov.bc.ca/omfd/fishstats; Hinch et al. 2012). Sockeye are also the most commercially important salmon species in Canada, and they are central to the culture of the First Nations in the Pacific region (Cooke et al. 2011). Thus, the state of the sockeye populations in the North Pacific is of great significance, and much research has been dedicated to understanding the factors that determine their abundance variability.
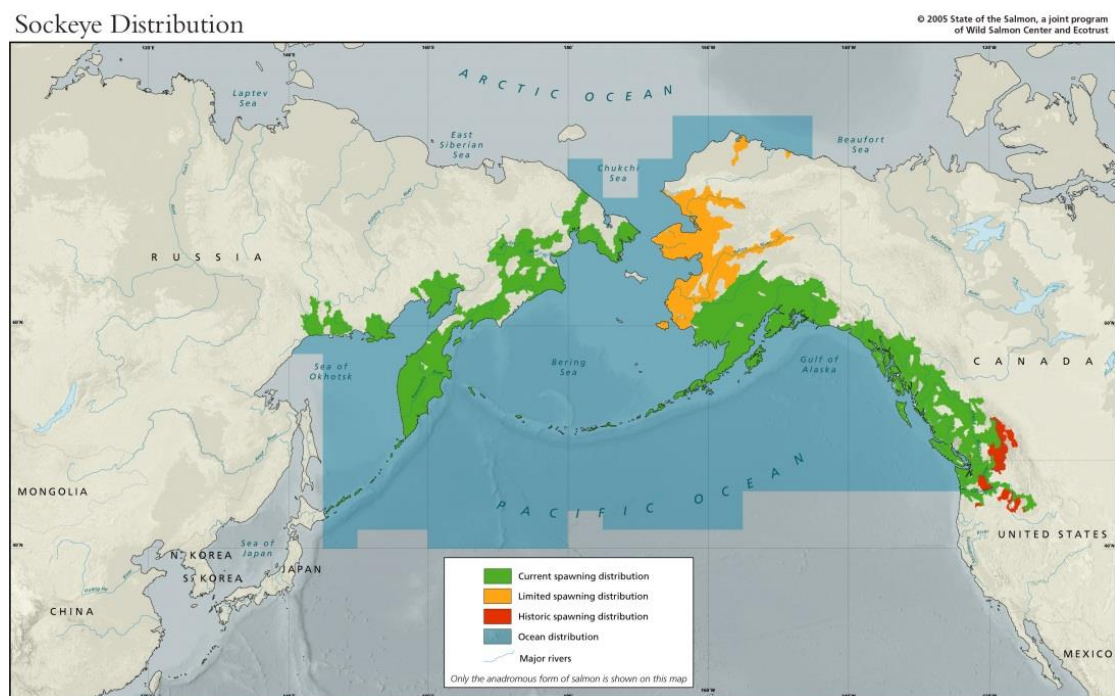
Figure 1: Oceanic distribution and spawning locations of sockeye salmon in North Pacific region (Hermansen 2006).

Sockeye salmon are anadromous fish, utilizing both marine and freshwater environments during their life cycle. Adults of sockeye salmon migrate to freshwaters from the ocean to mate and spawn. Fry rear

for a certain amount of time in freshwater before migrating to the sea where they grow and mature. Therefore, during their life cycle, sockeye salmon experience three different phases: oceanic, fresh-water and early-marine stages. Mortality happens at each of these lifecycle stages, and this is discussed in more detail in section 1.2.

1.2. Seaward Migration

Many different hypotheses have been proposed to address the question of what determines salmon survival success. These can broadly be divided into two categories: (a) anthropogenic forcing, e.g., overfishing, forestry, and hatchery enhancement programs, (Raymond 1988, Nehlsen et al. 1991, Thorstad et al. 2008, Kostow 2009), and (b) environmental forcing, e.g., climate cycles and long-term climate change effects on ocean conditions (Hare and Francis 1995, Friedland et al. 2000, Pyper et al. 2001, Mantua et al. 2010).

Hypotheses pertaining to the environmental drivers of recruitment can be divided into three life stage specific categories: (1) Open ocean conditions, focusing on post-juvenile stage salmon, (2) freshwater conditions, focusing on the eggs, fry and smolts, and (3) the early marine phase, focusing on juveniles during their first months at sea. The early marine phase includes both the transition from freshwater to the marine environment when juveniles undergo considerable changes in their biology (to adapt to saline water and life in the ocean as well as to a new prey community), as well as growth in the first weeks at sea. Survival during this is highly variable and is considered to be one of the primary drivers of recruitment (Peterman 1981, Mathews 1984, Sinclair et al. 1988). Currently, we know little about the sockeye oceanic phase (Peterman et al. 1998, Mueter et al. 2002b).

Recently, significant attention has been drawn to the earlier marine phase in the salmon life cycle between freshwater and high seas (Johannessen and Ross 2002, Cooke et al. 2011, Hinch et al. 2012). The timing of smolt migration may be a major factor determining juvenile marine survival rate

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(McCormick et al. 1998). McCormick et al. (1998) introduced the term 'smolt window' and proposed two mechanisms that may affect smolt survival. The first is a physiological window, reflecting the physiological and behavioral readiness of smolts to start the seaward migration. This window is controlled by photoperiod, water temperature, river flow and other factors (McCormick et al. 1998). The physiological condition of the smolts is broadly correlated with the adult returns (Virtanen et al. 1991; Staurnes et al. 1993). The second window is an environmental window that describes the optimum conditions for smolt survival. It is controlled by seasonal changes in rivers, estuaries, and coastal environments to create appropriate conditions for fish survival. When both windows match/coincide, high adult returns follow. When the windows are out of phase by two weeks, adult returns are poor (McCormick et al. 1998). This implies that even if the conditions between the years are the same, the actual timing/duration of migration may ultimately determine marine survival. Conditions for growth are crucial during the first weeks/months at sea because larger individuals tend to have higher survival rates (McKinnell et al. 2001).

The other evidence supporting the importance of the early marine stage in determining the survival success of the smolts is the difference between the southern and northern sockeye stocks. Climate and ocean conditions can have opposite effects on southern and northern salmon stocks in the Northeast Pacific Ocean (Mueter et al. 2002a) while the survival of the adjacent stocks strongly correlates with coastal environmental variables (upwelling index, sea surface temperature, and salinity) at local spatial scales (<500 km intervals; Pyper et al. 2001, Mueter et al. 2002a, 2002b). For instance, drastic decline in the 1960s in Rivers and Smith Inlets were not observed for other sockeye stocks such as Fraser stocks over the same period, although juvenile Fraser River sockeye pass by the mouth of both Rivers and Smith Inlets and spend one-two winters rearing at the same area (Tucker et al. 2009). Such observation indicates that local conditions are the very important in determining the survival success of the stocks.

Recent studies have identified three different behavior patterns of seaward migration. Fish can 1) use the estuary as a migration corridor (McKinnell et al. 2001), 2) interchangeably move between freshwater and adjacent marine zone at an early stage (Simmons et al. 2013), or 3) may spend time in the estuary foraging. The choice among the above behaviors is determined by the unique properties of the environment.

1.3. Sockeye Salmon in Rivers Inlet

Rivers Inlet is a fjord-type estuary surrounded by temperate rainforest and the territory of the Wuikinuxv (Oweekeno) First Nation situated on the central coast of British Columbia, Canada (Figure 2). The Rivers Inlet system is about 45 km long and 3 km wide (Buchanan 2006). Physically, the inlet is similar to the many other inlets along the BC coast (Wood 1970). Nowadays, it is a popular destination for recreational fisheries, forestry and tourism (McKinnell et al. 2001, Levy 2006). Historically, Rivers Inlet supported the second largest sockeye salmon run in BC (Foskett 1958, Wood 1970, McKinnell et al. 2001).

Figure 2: Map showing the major features of Rivers Inlet system.

The majority of Rivers Inlet sockeye rear in Owikeno Lake. Spawning areas are located in various streams and rivers that flow into the lake. The Wannock River (6.5 km long, 100m wide) connects Owikeno Lake with Rivers Inlet and is also used by sockeye as a spawning ground (DFO 1996). It was estimated that the maximum annual carrying capacity of salmon fry in the Owikeno Lake is around 41,000 kg (McKinnell et al. 2001).

The sockeye salmon population of Rivers Inlet has attracted much attention due to a drastic decline in total abundance that led to a complete closure of the commercial fishery in 1996 (McKinnell et al. 2001). Prior to the 1990's, Rivers Inlet had an annual average sockeye return of more than one million fish (Rutherford and Wood 2000). Starting from the late 1960s, the population of sockeye salmon began to experience pronounced fluctuations. There were two peaks of more than 2.6 million sockeye salmon returns in 1968 and 1973 with a precipitous drop in abundance in between (Figure 3).
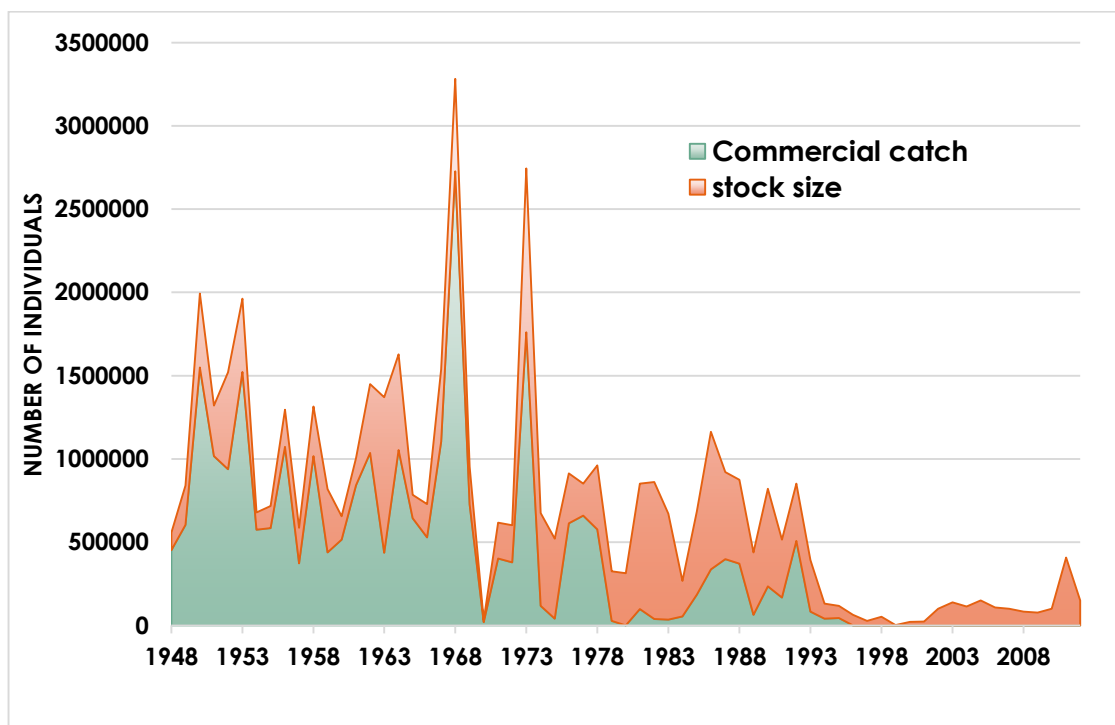
Figure 3: Commercial catch and escapement data of sockeye salmon in Rivers Inlet between 1948 and 2012 (graph built based on the data from DFO, 2013).

After these events, the sockeye population underperformed compared to its historical returns. Despite the application of adaptive management procedures (Walters et al. 1993) and later complete closure of commercial sockeye salmon fishery in the region, the returns did not increase (Rutherford and Wood 2000). The population gradually decreased to the lowest return of ~3,600 fish in 1999. Since then returns have increased to 100,000–150,000 fish, below the DFO set limit (over 200,000 fish) for the commercial fishery re-opening (DFO 1996). The cause of this dramatic population collapse is to date unclear. Notably, similar stock declines were observed in other systems along the BC coast, but it was the most dramatic in Rivers Inlet (Rutherford and Wood 2000, McKinnell et al. 2001, Riddell 2004). In 2011, the sockeye return was estimated to be 400,000 fish but have been at lower levels since 2011. Returns for 2014 that came from low brood year escapements and was significantly impacted by severe flooding in 2010 (DFO 2014).

One of the potential factors determining juvenile marine survival rate is the timing and duration of the outmigration from Rivers Inlet (McCormick et al. 1998).This information is crutial, as during this time smolt mean weight may double (Buchanan 2006). Larger smolts have a smaller predator window and may be more physically fit compared to the smaller smolts.

1.4. Research Objectives and Predictions

The purpose of this research study was to determine the rate and dynamics of juvenile sockeye salmon outmigration through Rivers Inlet, based on juvenile salmon surveys conducted annually between 2008 and 2011. This overarching goal was divided into four sub-components, each testing specific predictions:

Chapter 2. Determine the inter-annual variability in the duration of the outmigration of sockeye juveniles smolts between 2008 and 2011, and the role of fish size in the outmigration rate.

Prediction 1.1: Migration rate differs significantly between years – based on preliminary results and data analysis, sockeye salmon smolts spent more time in Rivers Inlet in 2010 and less in 2008/2011.

Prediction 1.2: Increase in smolt size is related to the timing of the migration and initial size at an entry to the inlet.

Chapter 3.1. Investigate temporal patterns of the sockeye smolt migration over the entire Rivers Inlet length in 2009.

Prediction 2.1: Sockeye smolt migration rates vary spatially and temporally due to fish dynamically using "holding points".

Chapter 3.2. Determine whether outmigration dynamics are controlled by freshwater discharge or zooplankton availability.

Prediction 2.2: Freshwater discharge is strongly correlated with the timing and rate of outmigration of sockeye salmon in Rivers Inlet. Fish spent more time in the areas of high zooplankton densities.

Chapter 4. Explore the effect of the combination of the environmental factors (river discharge, water temperature and zooplankton densities) on physiological state of the smolts (weight at catch and increase in weight)

Prediction 3: Environmental conditions in the inlet determine the weight at catch and the increase in weight at the end of the outmigration through Rivers Inlet.

CHAPTER 2: DETERMINATION OF SPECIFIC LENGTH OF MIGRATION OF SOCKEYE SALMON SMOLTS

2.1. Introduction

The estuarine residence is a critical period for the salmon life history. First, the estuary is the transition phase between freshwater and open ocean where smolts spend time adjusting to the new environment (Fried et al. 1978, LaBar et al. 1978, Thorstad et al. 2007, 2012, Kocik et al. 2009). Second, the estuary provides smolts with necessary habitat and shelter from predators (Healey 1980, Levy and Northcote 1982, Shreffler et al. 1992), creating an opportunity for rapid growth.

The speed and the length of outmigration may vary dramatically across the systems depending on migration behavior and/or environmental conditions. Welch et al. (2011) tracked the outmigration of smolts from the Fraser River and through the Strait of Georgia using telemetry. The smolts covered 400 km in 22 days with an average speed of 18 km/day or 1 body length per second (BL/s) for 13-25 cm smolts. Theoretically determined optimal speeds of smolt active migration have been estimated at 0.80-2.00 BL/s (Welch et al. 2011, Martins et al. 2012). These estimates were based on larger (> 120 mm) smolts and, as a result, may not apply to smaller or weaker smolts. Wood et al. (1993) studied the outmigration of sockeye salmon smolts (standard length 70-78 mm) down the Stamp and Somass Rivers, through Alberni Inlet and Barkley Sound (Vancouver Island, BC, Canada). The average ground speed through Alberni Inlet was 1.9 km/day (0.3 BL/s) and 1.6 km/day in Barkley Sound (0.2 BL/s).

In Rivers Inlet, if smolts maintained the average speed of 1 BL/s, then they would swim through the inlet within 6– 7 days, with a mean speed of 6.3– 7.5 km/day. However, previous estimates in Rivers Inlet have been of an average residence time of 1– 3 weeks with an average migration speed of 1.9– 2.9 km/day (Buchanan 2006, Ajmani 2011). More recently, Stocks et al. (2014) estimated that the residence time was 3 to 7 weeks, yielding an average migration speed of 0.7– 4.2 km/day. The latter estimate was

obtained from a relatively small data set of 19 samples, focused on the larger fish, and as a consequence likely did not capture the full range of migration rates for the system.

2.1.1. Chemical Tracers

Current aging techniques use various registering structures. For example, tree rings are broadly used to assess the age of trees (Kuniholm et al. 1996) and layering of ice used for determination of the approximate age and temporal dynamics of glaciers (Baumgartner et al. 1989). In the animal kingdom numerous registering structures such as shells and coral skeletons are found (Dodge and Thompson 1974, Lutz and Rhoads 1980, Goren et al. 1987, Zuk 1987). Fish are not an exception, and their age can be determined from, e.g. otoliths, scales, vertebra, and opercula (Brown and Gruber 1988, Baker and Timmons 1991, Robillard and Marsden 1996, Campana 1999). Among them, fish's scales and otoliths are most commonly used to assess the age of teleost fish (Casselman 1987).

Otoliths, also known as "ear stones", are fragile structures located in the inner ears of the fish helping with balance and sound perception. They exist in three pairs: lapilli, sagittae and asterisci and are located symmetrically in vestibules that are part of a fish's ear (Figure 4). Sagittae are most commonly used in otolith studies (Secor et al. 1992, Popper et al. 2005).
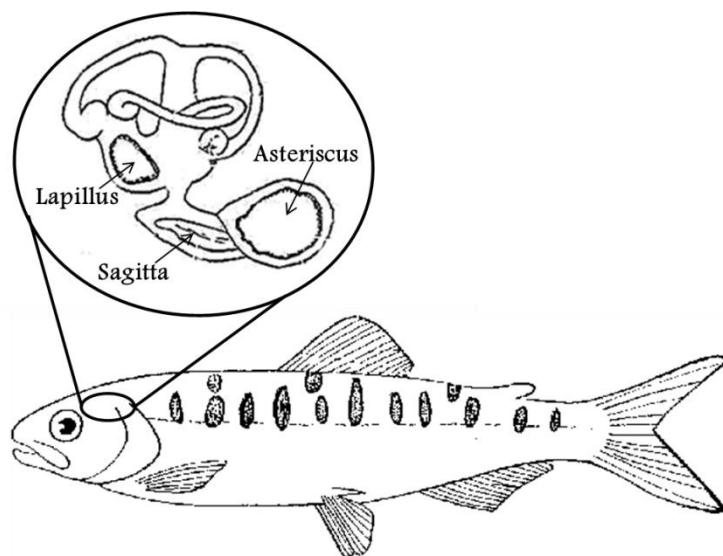
Figure 4: Structure of fish's otolith (modified from Calliet (1986) and www.fpc.org).

Considering their chemical structure, otoliths are primarily compromised of aragonite (97%), a crystalline form of calcium carbonate ($CaCO_3$), with the remaining ~3% compromising minor (Na, K, S, N, Cl, P, Ba and Sr) and some trace elements (Campana 1992). Since otoliths are metabolically inert, meaning any elements incorporated into the structure are permanently retained there. Therefore, they provide a high-resolution record (daily growth increments or rings) of conditions experienced by the fish (Campana and Nelson 1985). Incorporation of elements into the otolith is a complicated, multi-stage process (Olsson et al. 1998). Although it has been hypothesized that the majority of elements deposited in otoliths originate in the water that fish reside in, it has been found that some fraction can also be incorporated from dietary items (Limburg 1995).

Aragonite is deposited on the surface of the otolith on a daily basis (Asano and Mugiya 1993). Divalent elements with similar ion size to Ca^{2+}, such as Mg^{2+} and Sr^{2+}, tend to incorporate directly into the otolith structure as calcium substitutes or associated with the protein matrix system (the otolith consist of aragonite crystals and a protein-made organic matrix). Larger divalent ions (Ba^{2+} or Pb^{2+}) can substitute calcium, so they tend to co-precipitate into the otolith matrix (Morse and Mackenzie 1990).

The concentration of calcium is the most influential property of water controlling elemental uptake, and as a consequence, the element to calcium ratio is often more indicative of environmental change than the elemental concentration itself (Mayer et al. 1994). A good marker of the movement of diadromous fish such as sockeye salmon between fresh and salt water environments is a change in Sr:Ca ratio in the otolith because strontium is usually 1 or 2 orders of magnitude higher in seawater compared to freshwater (Campana 1999, Secor and Rooker 2000).

Another indicator of the transition between freshwater and marine environments is the Ba:Ca ratio. Similar to the Sr:Ca ratio, ambient Ba:Ca concentration are positively correlated with the element concentration in a fish's otolith (Bath et al. 2000, Elsdon and Gillanders 2003, Wells et al. 2003). Ba

concentrations tend to be higher in freshwater estuaries due to inputs of Ba-rich sediments (Li and Han 1979). Therefore, Ba concentrations are typically higher in fresh water than in salt water (Turner et al. 1981), and hence, the otolith chemistry will reflect this characteristic when a fish moves between habitats.

Hale & Swearer (2008) found that Sr:Ca ratio was more statistically consistent in determining marine entry than Ba:Ca; and although it may be species specific the Ba:Ca signal may change faster than the Sr:Ca signal. Another approach used in the recent work of Stocks et al. (2014) was to apply the Sr:Ba ratio. The authors found a significant (a few orders of magnitude) change in the Sr:Ba signal in fish that moved from fresh to salt water, allowing for the determination of the marine entry point into the Inlet. This "breakpoint" is a valuable tool for determination of the migration history of the fish.

2.1.2. Otolith Analysis

The development of microanalytical techniques that can detect trace elements has always been a major concern for fish biologists. Trace elements are 'fingerprints' in the otolith structure and are a valuable source of information for determining fish movements, discriminating fish populations, and monitoring the aquatic environment (Thorrold et al. 1998, 2001).

A wide range of techniques has been used since the discovery of daily growth increments in otoliths (Edmonds et al. 1991, 1995, Hoff and Fuman 1995, Jones and Chen 2003). Perhaps the most powerful tool for accessing chronological information is the laser beam technique. This technique has been used for otolith analysis since 1995 (Fowler et al. 1995) and provides greater sensitivity and ability to analyze trace metals. This technique is called laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS; Gray 1985, Denoyer et al. 1991, Jones and Chen 2003). Following this method, a high-powered laser beam is used to vaporize the otolith in fine scale increments. The gaseous sample is carried with a carrier gas (Ar) into plasma where it atomized and ionized. The ionized parts are then separated and detected by a mass spectrometer (Denoyer et al. 1991).

2.1.3. Goals for Chapter 2

Stocks et al. (2014) used laser ablation coupled with daily growth rings to do a preliminary analysis of the migration duration of Rivers Inlet sockeye smolt in 2008, 2009 and 2010. They found that fish spent 12 – 31 days in the inlet in 2008, 18 – 40 days in 2009, and 22 – 55 days in 2010. This analysis was based on a small sample size and focused on the largest fish, and likely did not capture the full range of migration durations, and did not examine seasonal variability. So the first aim of this study was to expand the work of Stocks et al. (2014) and test Prediction 1.1 (Chapter 1) that the duration of the seaward migration of sockeye smolts differed significantly between 2008 and 2011. Secondly, the size of the fish was investigated to determine the interannual variability in smolt size and test Prediction 1.2, that an increase in smolt size is related to the timing of the migration and initial size at an entry to the inlet.

2.2. Methods

2.2.1. Otolith Collection, Extraction, and Storage

Juvenile salmon were caught at 14 different locations along the length of Rivers Inlet between May and early July of 2008, 2009, 2010 and 2011 (Figure 5). All samples were collected during the RIES (Rivers Inlet Ecosystem Study) surveys on board the MV Western Bounty, a fishing vessel operated by the Wuikinuxv First Nations. Sampling was conducted fortnightly, between late April and early July to overlap with the expected period of migration based on previous studies (Buchanan 2006). Five surveys were completed in 2008 and 2009, six in 2010, and three in 2011 (exact dates are shown in Table 1). A herring seine (364 m long by 29 m deep) was provided by the Fisheries and Oceans Canada (Pacific Biological Station, Nanaimo, BC). The mesh size was 2.5 to 3.75 cm with a 0.6 cm knotless bunt. A subset of captured fish was frozen for later processing. In the lab, fish were defrosted, weighed and measured (standard fork and total length) and a pair of sagittal otoliths (left and right) was removed from each fish.

Table 1: Survey number and its corresponding date for each sampling year.

Survey #	1	2	3	4	5	6
2008	29-Apr	12-May	28, 29–May	10,11,12,13 –Jun	25, 26–Jun	-
2009	1-May	18-May	31-May; 1,2-Jun	15, 16 –Jun	29, 30–Jun	-
2010	6-May	19-May	4-Jun	19-Jun	27, 28-Jun	5, 6–Jul
2011	25–May	8- Jun	23–Jul	-	-	-

Samples were stored at room temperature prior to the analysis. Samples from 2011 originally were stored in eppendorf tubes sealed with air and then were temporarily stored in a -20ºC freezer before being kept at the room temperature for consistency with other years.

Two locations, Bosquet Shore and Bosquet Open (sites 1 and 2 on the map), were chosen to answer the questions of the first part of this research work (101 samples were analyzed). These two sites were chosen for three main reasons. First, these locations were close to the mouth of the inlet, and thus, fish caught would have the longest residence time in the inlet. Second, catch data indicated that Darby Channel was the primary migration pathway for Owikeno sockeye smolts. Third, the other sites located near the mouth of the inlet (Dimsey Shore, Dimsey Open and Mouth) were excluded from this part of the analysis because of potential "contamination" of Owikeno Lake smolts by Drainey and Smith Inlet stocks. Thus, overall, the Bosquet Shore and Bosquet Open fish were likely to be representative of Owikeno smolts only.
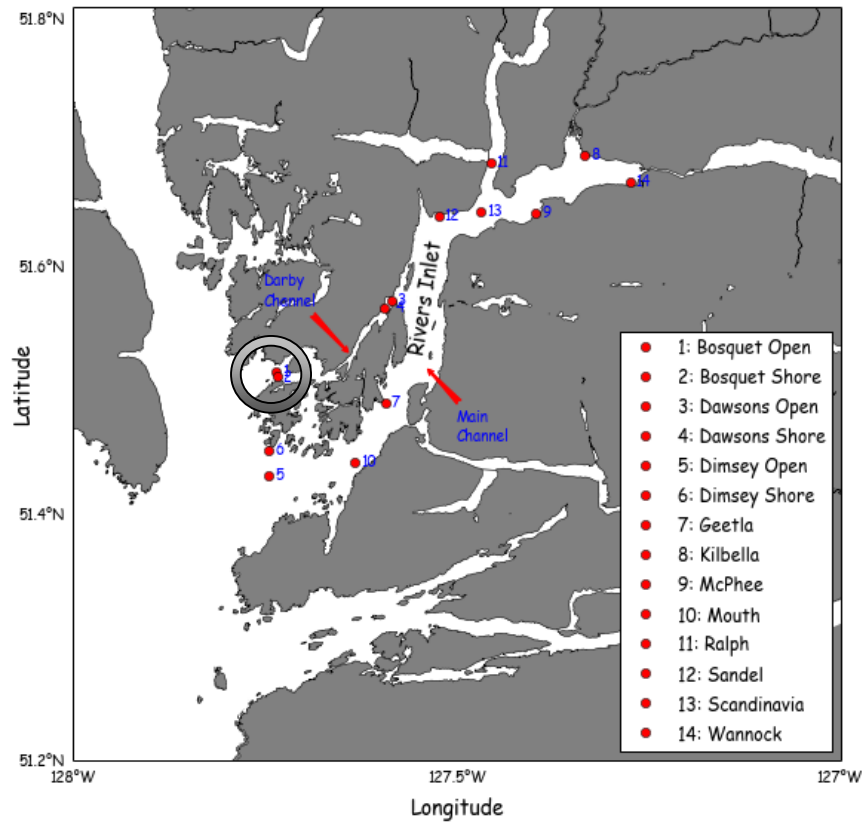
Figure 5: Map of Rivers Inlet showing the location of 14 seining stations (red dots). Arrows indicate two proposed migration routes from the Inlet. Grey circle shows the sites used for analysis in this chapter.

2.2.2. Otolith Sample Preparation

The otolith samples require special preparation for both laser ablation and ring count procedures. For ring counts and laser ablation, otoliths were polished on their ventral side making the daily rings more apparent under the microscope (Figure 6), while also allowing the laser to focus on the targeted area. This is crucial for laser ablation because if the laser is run on a rounded surface, the laser path may go through several rings which could result in a mixed signal. Therefore, polishing of the otolith to a flat surface was performed. Due to the small size of the otoliths, the samples were first fixed on the surface and then polished to the desired state.

Otolith Mounting

Prior to polishing, all the equipment was washed with distilled water. Gloves were worn during all procedures. Glass slides were cleaned with distilled water and then dried with a Kim Wipe. A piece

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of Scotch[R] double-sided tape was placed on the glass slide. A plastic ring was then placed on top of the tape, so that the sticky area was inside the ring. Then the otolith was removed from its vial with forceps and was placed arbitrarily on the glass slide (sulcus side up) and pressed into the scotch tape to ensure that the samples did not move prior to adding the resin. Usually, about ten otoliths were placed on the glass in a recorded pattern. Their position and identification were recorded in the log book. Broken otoliths were discarded.

The adhesive was made by mixing EpoxiCure[TM] Epoxy Resin and EpoxiCure[TM] Epoxy Hardener in a 4:1 ratio (by volume). The mixture was stirred carefully with a wooden rod to avoid bubbles until a clear colour was achieved. Then the adhesive was added to the samples, filling the ring up to its rim. Samples were left overnight to allow the resin to harden.

Sample Polishing

After the resin was hardened and the otoliths were securely fixed, the plastic ring was removed from the glass slide with a razor blade. The sample was then progressively polished with 240, 400, and 600 grit Buehler CARBIMET® abrasive paper rolls on a Buehler HANDIMET® II Roll Grinder instrument. The degree of polishing was checked regularly on a Fisher-Lite MI-150 microscope with a High-Intensity Illuminator and C-W10xA/22 Nikon lenses (Japan) and to ensure that the otoliths protruded above the adhesive resin. However, the resolution of this microscope was not sufficient to display the rings. Therefore, the otoliths were also investigated under an inverted light microscope (Model Eclipse E600, Nikon, Japan) to check for potential over-polishing / clarity of rings. It is important to note that the positioning of the otolith on the glass slide is critical. Improper positioning resulted in the over-polishing of one side of the otolith and loss of information from the rings. In addition, over-polishing of the sample can lead to the destruction of the rim and thus loss of the rings at the edge of the otolith.

The final polishing was done on a Minimet 100 Grinder Polisher using Metadi® fluid and Metadi®

diamond polishing powder of 6 µm (2 times), 3 µm (1 time), and 1 µm (1 time). Each step was done for

4 min. The state of the sample was also regularly checked under the microscope. A photograph of each

otolith was taken, which was also used to determine the actual size of the otolith. The length (distance

between the rostrum and postrostrum) and the width (distance between dorsal and ventral edges) of each

otolith was carefully measured (Figure 7).

2.2.3. Analytical Instrumentation

The ablation of otoliths was performed using a Resolution M50-LR Ablation System (ASI,

Australian Scientific Instruments, Australia) equipped with a Compex Coherent ArF laser and coupled

to an Agilent 7700 x Quadrupole-ICPMS system (Agilent Technologies, Japan). The instrument was

tuned for maximum sensitivity and stability and ThO/Th < 0.5% in order to enhance counting statistics

and analytical performance and to reduce potential interferences and matrix effects. To remove surface

contamination, samples were pre-ablated first (50% spot overlap, 5µm spot size, 5 Hz laser repetition

rate, 1 pass per cycle). Livestream viewing of the samples was available through a video monitor

controlled by the software. All instrument parameters are listed in Table 2.

Table 2: LA-ICPMS settings, operating conditions and data acquisition parameters for multi-element analysis of otolith samples.

Laser Ablation Parameters:	
Instrument Model	ASI Resolution M50-LR
Wavelength, nm	193
Ablation Gas	He
He Flow Rate, mL/min	800
Ablation Mode	Scan line with rotating slit
Slit size, µm	2 x 142
Repetition Rate, Hz	5
Scan Rate, µm/s	2
Fluence, J/cm^2	1.8

ICP-MS Parameters:

Instrument Model	Agilent Technologies 7700x Series
RF Power, W	1350
Carrier Gas	Ar
Ar Flow Rate, L/min	0.53
Additional Gas	N_2
N_2 Flow Rate, mL/min	2.00
Isotopes Monitored:	^{25}Mg, ^{43}Ca, ^{44}Ca, ^{88}Sr, ^{137}Ba, ^{138}Ba,
Standards	SRM NIST 612 and NIST 610
Internal Standard Element	Ca

One of the disadvantages of the LA-ICPMS system was the inability to view the daily rings of the otolith: the transmitted light source was too far from the sample, and/or the thickness of the puck did not allow light to pass through the embedded sample (Figure 6). To ensure accurate positioning of the ablation path, otoliths were photographed under the inverted light microscope and then compared with the view under the ablation camera.

Figure 6: View of otolith via the LA camera.

According to previous studies and test ablations, the transition zone is located at the edge of the otolith. Hence, the area of the core of the otolith was not further investigated to reduce analysis time and costs. The ablation path was set to start from ~150-200 µm from the edge (depending on the size of the

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otolith) and was run towards the edge of the otolith. The path did not end at the edge of the otolith but continued into the resin to ensure a complete coverage of the edge (see an example of the path in Figure 8). Otoliths were run in blocks of 30 samples with standards bracketing every 3– 4 samples. Samples and standards were analyzed in time-resolved mode (with an acquisition time of 0.331 s).

2.2.4. Data Reduction and Analysis

Six isotopes were monitored in this study: ^{25}Mg, ^{43}Ca, ^{44}Ca, ^{88}Sr, ^{137}Ba, ^{138}Ba. Raw data obtained from the instrument were reduced using Iolite, a self-contained package for Igor Pro$^®$ (Wavemetrics Inc. of Lake Oswego, Oregon, USA) (Paton et al., 2011). To check the accuracy of integration, the silicate glass standard SRM NIST 612 was used for external calibration while a Ca concentration of 40% (m/m) was used as internal standard for the aragonitic samples. SRM NIST 610 was cross-checked for quality control purposes.

Using natural abundance values, the measured counts for each isotope were transformed into elemental counts and element:Ca ratios were calculated. In addition, the Sr:Ba ratios were calculated. The external precisions (relative standard deviation, RSD) were only available for spot analyses but not calculated for line scans. However, as the repetition rate was 5 Hz with a 2 µm/s scan speed, so that 5 values of the signal were obtained at the same position of the slit, a standard deviation for the average of the 5 values was calculated.

To determine the point when the fish changes its environment, segmented regression analysis (SRA) was performed. The SRA was performed using the R statistical software (R Core Team 2014). The package *segmented* was used to estimate a linear model with one or multiple breakpoints (Vito, 2003, 2008). This package also computes the summary of estimates of the slopes and the location of a breakpoint. The point of transition from freshwater to saltwater was determined based on the change of the Sr:Ba elemental ratio that was obtained from Sr:Ca and Ba:Ca ratios (Stocks 2012). However, the breakpoint was also determined based on Sr:Ca, Ba:Ca and Mg:Ca independently to confirm the validity

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of the method established by Stocks et al. (2014). The duration of the outmigration then was calculated using the position of the transition point that is discussed further in this chapter.

The right sagittal otolith was primarily used in the microchemistry analysis, with the left otolith being used when the right was broken or missing. The difference between the otoliths in a pair has been found to be insignificant (Campana and Fowler, 1994). To confirm this pairs of otolith (left and right) were analyzed for 12 fish (see Appendix B).

2.2.5. Ring Counting

The greatest length, width and otolith radius (i.e. distance from otolith core to its margin) of the otoliths were measured under the inverted light microscope (measured under 4x magnification) prior to the ablation. Counting of daily rings was performed after the ablation so that the counting could be done along the ablation path. It was not possible to detect rings under direct light, so an inverted light microscope was used for the analysis. The resolution of the camera lenses was important. The photos were still blurry with the resolution of 2560 x 1920 (~4.9MP).
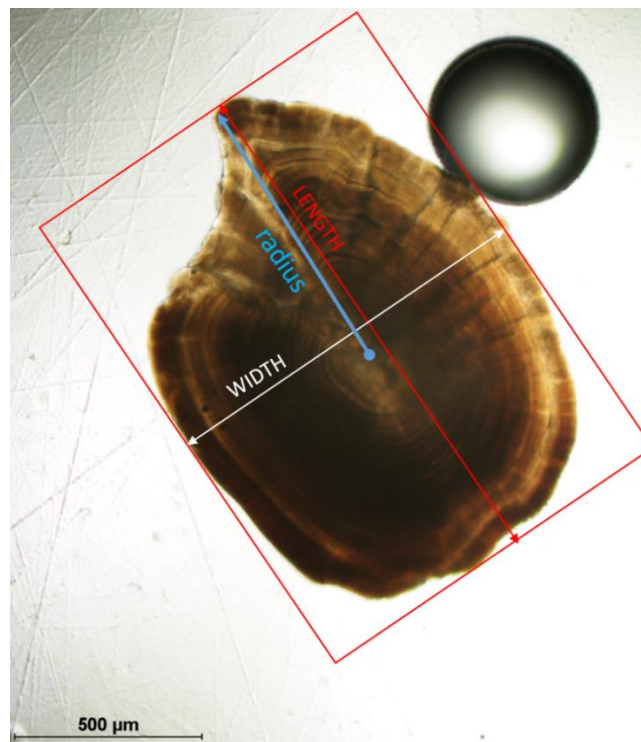
Figure 7: Left otolith view under the inverted light microscope with the illustration of radius, width and the length of the otolith. Otolith is shown ventral side up (sulcus down).

The number of daily increments was estimated by dividing the width of the marine section determined by the ablation path by the average otolith ring width that was measured for each fish. The accuracy of the average ring width approach was then tested against the actual counts from each of the 10 otoliths with clearly evident rings (see Appendix D). The otoliths were photographed under a microscope at 20x and 40x magnification (depending on the width of the rings), and analyzed using ImageJ software in combination with the ObjectJ plugin (www.sils.fnwi.uva.nl/bcb/objectj/7a-Examples.html).
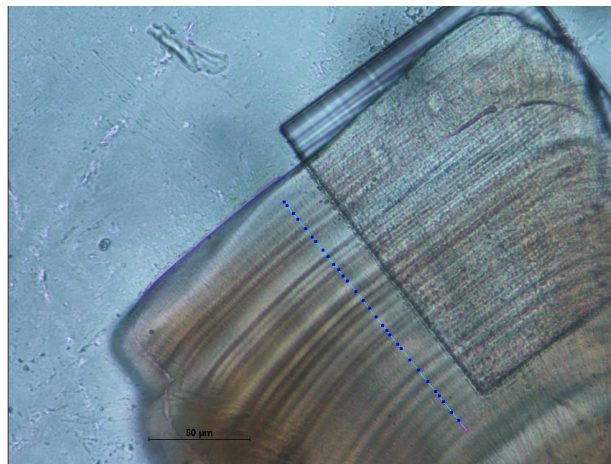
Figure 8: Example of the ImageJ software window with ObjectJ automatic ring detection option (light blue line with dark blue dots). On the right, the ablation path is shown.

The width of otoliths' ring is not constant and may vary daily depending on, for example, feeding conditions. The average otolith ring width was used in this study. The following assumption may be applicable only over a small length as for the longer distances this might introduce error into the analysis. However, as marine entry happens almost at the end of the otolith (~100µm from the edge), our assumptions were considered reasonable to apply in our analysis. Refer to Appendix D for method verification details.

Due to the fact that the ablation path was longer than the actual distance to the edge from the start of the ablation point (Figure 8), the distance between the start of the ablation path and the edge of the otolith was found based on the two approaches. The first approach was visually measuring the ablation path distance under the inverted microscope:

$$visual\ distance\ to\ the\ edge\ [\mu m] = \text{ablation path length } [\mu m] - 1\mu m$$

1 µm was subtracted from the visual measurement because the visual path is 1µm greater from each side (as the width of the slit is 2 µm). However, in several cases, the otolith was not transparent enough (too dark) to determine the start of the ablation path. Therefore, a second method was used to calculate the length of the laser path along the otolith. The length of the path was determined as the difference between total ablation path length (that was obtained from the log file of Iolite) and visual length of the laser path along the resin section (difference between the visual ablation path length across the resin section and distance to the edge):

$$distance\ to\ the\ edge\ [\mu m] = \text{ablation path length } [\mu m] -$$
$$(visual\ ablation\ path\ length\ [\mu m] - visual\ length\ to\ the\ edge\ [\mu m])$$

Both methods were used to determine the distance to the edge of the otolith for all samples. Then the average distance to the edge of these two methods was taken. On average, the difference between two methods was within 2-3 µm.

After the breakpoint had been determined, the number of days spent in the Inlet was estimated by dividing the distance from the breakpoint to the otolith edge (estuarine marine radius) by the average width of the otolith rings. Knowing the number of days that each smolt spent in Rivers Inlet as well as the date when the smolts were caught, the entry date into the inlet was back-calculated for each fish (as the difference between the catch day and the number of days spent in the Inlet). Using the regression

equation for each year (left panel of Figure 13), the approximate date of the end of the migration was determined (note that as dates were used as an x-axis, the regression model transformed the dates into Julian days, with a default date of origin at 1st Jan 1970). Travelling speed may differ for fish of different sizes. To take into account the effect of size, swimming speed was also calculated in terms of body length per second (BL/s).

2.2.6. Estimation of the Initial Size at Entry to the Inlet

The size of the smolts when they first enter the inlet was back-calculated from the measured size of fish after it was caught (further will be referred as size at catch). Two otolith features can be used to calculate the size: otolith radius and otolith length. The former is the most commonly used method for displaying the relation between the size of the fish and the otolith (Campana 1992, Campana and Casselman 1993, Freshwater et al. 2015).

In this study, ablation path doesn't always coincide with otolith radius (Figure 9) either due to the curvature of the otolith tip or inability to determine the width of the daily rings along the otolith radius. The number of days that fish spent in the estuary was determined from ablation data. So by measuring the width of the rings along the otolith radius and multiplying it by the duration of the marine residence, the width of the marine zone along the otolith radius can be determined (Figure 9).
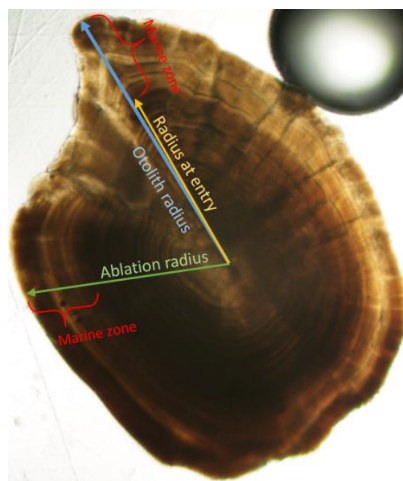
Figure 9: Difference between the ablation radius (radius of the ablation path) and the otolith radius (distance from the center of otolith along the of maximum otolith growth axis). Radius at entry represents the radius of the otolith when smolt enters into the Inlet. Marine zone represents the time when fish spends in the Inlet.

It was possible to measure the rings along the otolith radius for only 92 samples, so the size at entry was determined for only 92 smolts. Then, the size of the otolith radius at the entry into the Inlet was calculated as a difference between the measured otolith radius and the width of the marine zone (Figure 9).

There was a significant linear relationship ($p \ll 0.05$) between the size of the fish and otolith radius. Subsequently, size at the entry was estimated using this (Figure 10; $R^2 = 0.50$):

Method #1: Size at entry [mm] = 12.8 + 0.0658 x otolith radius at entry [µm]
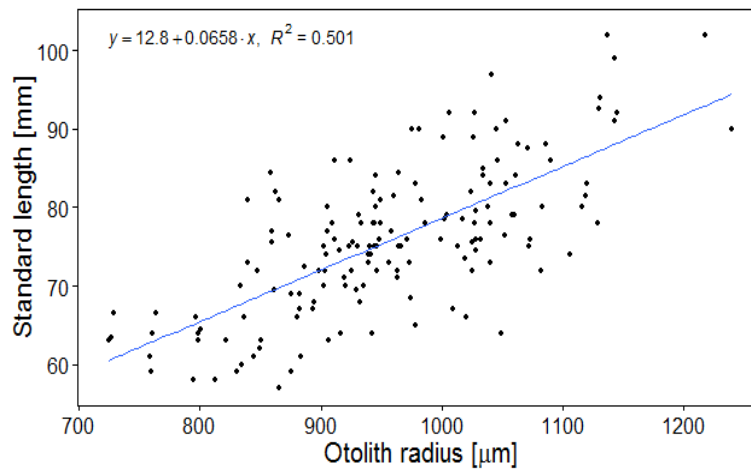
Figure 10: The relationship between the otolith radius (in µm) and the standard length of the smolt (in mm). The relationship is built based on all measured samples. The blue line is the fitted linear regression.

However, the relationship between size and otolith radius was not as strong as expected (see Discussion section). In addition, measuring the otolith radius was complicated as some otoliths have two primordia centers or the otolith preparation method did not allow for sufficient light penetration to see the center of the otolith. Therefore, a second method was applied to estimate the size at entry using the second feature of the otolith (otolith length). This method was based on two relationships: 1) otolith

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length vs smolt size (left panel, Figure 11; $R^2 = 0.60$), and 2) otolith length vs otolith radius (right panel, Figure 11; $R^2 = 0.84$). Both relationships were statistically significant ($p \ll 0.01$).
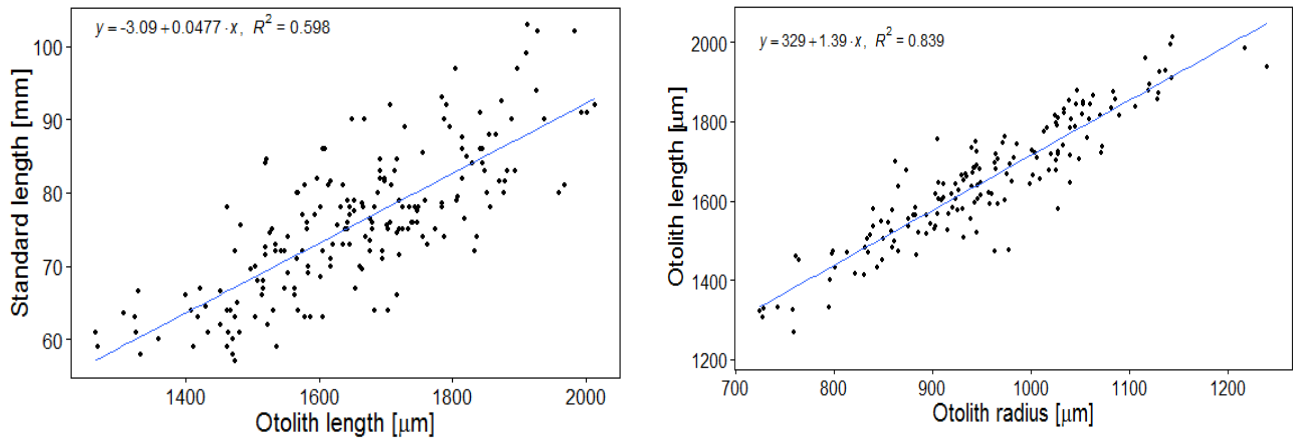
Figure 11: Relationships between the standard length of sockeye salmon smolts (mm) and total otolith length (μm; left panel) and between otolith length (μm) and otolith radius (μm; right panel). The graph was built based on data from all sites and years. The blue line is the fitted linear regression.

Combining two equations from the regression lines (Figure 11), the otolith length at entry was estimated, and then the size of entry was calculated using the following equation:

Method #2: Size at entry [mm] = 12.60 + 0.0663 x Otolith radius at entry [μm]

Then the results from the both methods for determining the initial size of the fish were compared. Both methods produced similar results of the estimate of the smolt's size at entry with no significant difference in calculated sizes (ANOVA, $p \gg 0.05$). As a result, the calculations from Method #1 were used in the analysis.

2.2.7.   Interannual Variability in Smolts' Sizes.

In the previous section, the duration of the outmigration through Rivers Inlet was determined for four years (2008 – 2011). It was of interest to see the interannual variability in size of the smolts in the inlet. To determine whether there was an interannual difference in smolt sizes in Rivers Inlet, the size of smolts

was compared from two locations from the available records (n = 791): the head (n = 45; Wannock site) and the mouth (n = 128; Bosquet Open and Shore) of the inlet during 2008 - 2011.

To validate whether the back-calculated sizes at entry that were calculated in this research work were a valid approximation of the actual fish size, it was important to obtain the reference estimate of the size of smolts at entry for this area. To do this, only smolts from the Wannock site (n = 45) were chosen from the available data set. From the existing records of smolt sizes, fish at the head of the inlet (Wannock site) were the smallest in 2009 (mean size 61.9 ± 4.0 mm), and the largest in 2011 with average size of 73.4 ± 3.4 mm (Table 3).

Table 3: Interannual smolts' mean size (mm), weight (g) and corresponding ranges for 2008– 2011 period from the original dataset collected from the field data. Means are listed with ± 1 standard deviation (SD).

Site	Year	Sample size (n)	Size, mm		Weight, g	
			Range	Mean (± 1 SD)	Range	Mean (± 1 SD)
Wannock	2008	4	62.5 – 74.0	69.3 ± 5.0	2.76– 4.82	3.77 ± 0.86
	2009	20	51.5 – 68.0	61.9 ± 4.0	1.98– 3.74	2.69 ± 0.42
	2010	9	58.5 – 69.5	66.4 ± 3.3	1.92– 3.70	3.24 ± 0.53
	2011	12	69.0 – 80.0	73.4 ± 3.4	3.49– 6.05	4.68 ± 0.69
Bosquet Open	2008	14	62.0 – 87.5	76.3 ± 8.5	3.01– 7.11	5.21 ± 1.44
	2009	22	67.0 – 113.0	76.3 ± 9.9	3.19– 7.41	5.36 ± 2.90
	2010	24	77.0 – 105.0	87.3 ± 8.2	3.47– 4.71	8.91 ± 2.98
	2011	5	64.0 – 88.0	76.9 ± 8.6	2.75– 7.39	5.36 ± 1.71
Bosquet Shore	2008	16	58.0 – 104.0	76.7 ± 10.2	2.39– 4.31	5.35 ± 2.72
	2009	14	68.5 – 100.0	77.9 ± 9.4	3.21– 1.49	5.75 ± 2.49
	2010	20	72.0 – 93.0	81.3 ± 6.1	5.36– 1.53	7.62 ± 1.74
	2011	13	73.0 – 97.0	82.3 ± 6.5	4.11– 10.37	6.06 ± 1.56

However, the fish that were caught at the head of the inlet may have already spent some time at that location and as a result gained some weight and size during that period. So the obtained reference numbers might be an overestimate of the original size. Then, these results were compared with the back-calculated size at entry in this study (n = 63).

Size gained was calculated as the difference between the sizes at the head and the mouth of the inlet for each fish where this calculation was possible. To eliminate the influence of the duration of the

stay in the inlet size gained per day was calculated as a ratio between size gained and a duration of the stay in the inlet. Then, using the size – weight relationships (see Appendix F) for four years (2008 - 2011), the increase in size was correlated with the weight gained during the outmigration period to test the statement listed in McKinnell et al. 2001 and Buchanan 2006 that smolts double in weight during the seaward migration through Rivers Inlet.

Fulton condition factor (CF; Ricker (1975), Anderson and Gutreuter (1983)) which estimates condition under the assumption of isometric growth was used as an indicator of overall health or condition of the fish and was calculated the following way for the analyzed dataset (n = 103). The factor was slightly modified by Barnham and Baxter (1998) to describe condition in salmonid fishes.

$$CF = \frac{W}{SL^3} \text{ x } 10^5,$$

where W is weight of the smolt (in g) and SL is the standard length (in mm).

2.2.8.   Statistical Analysis of Data

Simple regression is used to examine the relationship between the following pairs: 1) fish size and otolith length or radius, 2) otolith radius and otolith length, and 3) gained size and the size of the smolts at entry. One-way ANOVA was used to determine the significance of the difference in mean duration of outmigration between the years; effect of year on traveling speed (both in km/day and BL/s), size density distribution. ANOVA was used to check the difference in smolt sizes at the mouth (available and back-calculated) and head of the inlet between the years as well as to explore the difference between gained size and weight across four years (2008– 2011). One-way ANOVA was used to test for significance of the difference in the condition factor between the years. Data were analyzed using two-factor univariate ANOVA to assess the effects of year and size class on the duration of the outmigration. Assumptions of ANOVA were tested using Levene's test for homogeneity of error variances and normal probability plots. Post hoc comparisons of group means were conducted using Tukey's HSD tests.

ANCOVA was used to investigate the duration smolts spent in the RI with the respect of day of entry and year.

Fewer fish were sampled in 2011 because there were only three sampling events (25 May, 8 June and 23 July) compared to ≥ 8 seining surveys between beginning of May to early July in 2008-2010. Smaller data sets tend to produce more variability in the data. T-test can be used to assess if there are differences in the means as this method takes into account the standard error of the estimates of the means for each group.

To estimate the error of the Element:Ca ratios, the relative error was calculated as the ratio between the standard deviation and a mean. The uncertainty for Sr: Ba ratio was determined using the propagation of errors and was calculated as a square root of sum of squares of relative errors of Sr:Ca and Ba:Ca ratios:

$$\frac{SD(Me:Ca)}{\overline{Me:Ca}} = \sqrt{\left(\frac{SD(Me)}{\overline{Me}}\right)^2 + \left(\frac{SD(Ca)}{\overline{Ca}}\right)^2}$$, where *Me*, and *Ca* are measured metal and calcium counts

from an experiment, respectively; and SDs are the standard deviations of those variables.

2.3. Results

2.3.1. Method of Validation for Determination of a Break Point

Stocks et al. (2013) established a new technique to determine the marine entry of sockeye salmon smolts based on the Sr:Ba elemental ratio. In this study, the break point determined by this ratio was compared to the break points obtained by Sr:Ca or Ba:Ca ratios, across 71 samples. Based on the obtained results, Sr:Ca ratio was chosen for determining the break point in this study as the data were more consistent and the change in the signal was more evident for this ratio compared to the Sr:Ba and Ba:Ca (see Appendix A for more information on method validation).

2.3.2. Average Time Spent in Rivers Inlet

The calculated mean residence time in Rivers Inlet for each of the four survey years are shown in Figure 12. In addition, the data were divided into five size classes of 10 mm increments to explore the effect of fish size on the duration of residence in the inlet (Figure 12).
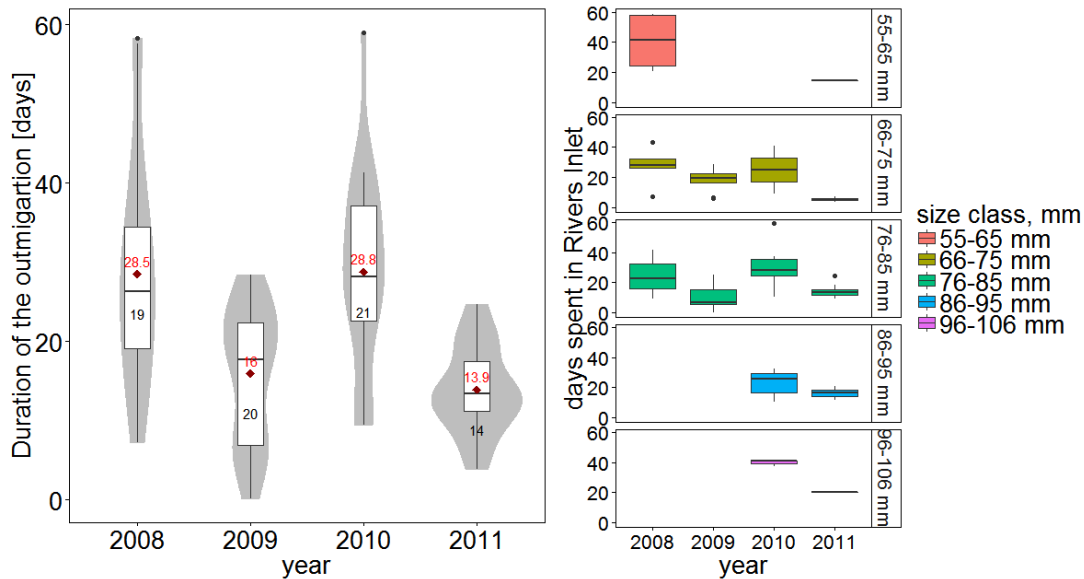
Figure 12: Time spent in Rivers Inlet in 2008 to 2011. Left plot presents box plots of residence time for each year; the right graph shows box plots of residence time by size class for each year color coded based on different size classes. Median values (black solid horizontal line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Red dots and numbers are the sample means; black numbers are the sample sizes and the gray area around the boxplot is kernel density.

On average, juvenile sockeye salmon spent ~4 weeks in the inlet in 2008 and 2010, and ~2 weeks in 2009 and 2011 (Figure 12; Table 4). Both 2009 and 2011 were significantly different from 2008 (ANOVA; $p \ll 0.05$), but were not different from each other. No significant difference in residence time was found between size classes within years, or same size classes across four years, except for fish size of 76– 85 mm in 2010 which spent significantly more time traveling through the Inlet than the fish with similar size in 2009 (ANOVA; $p < 0.05$). In addition, fish of size 96– 106 mm in 2010 were significantly longer in the Inlet than the 76– 85 mm fish in 2009 (ANOVA; $p < 0.05$).

Table 4: Average duration of the seaward migration of sockeye smolts between 2008 and 2011 years.

Year	Mean, days	Standard deviation, days	# otoliths
2008	28.52	14.27	19
2009	15.96	8.51	20
2010	28.79	12.35	21
2011	13.90	5.66	14

A significant correlation ($p \ll 0.05$; $R^2 = 0.55$) was found between the day of entry and the time spent in Rivers Inlet (left panel in Figure 13), indicating that the later fish entered the inlet, the less time they spent in it. The range of the entry days was April 17 – June 26 (difference of 69 days or ~10 weeks). No significant contribution of year to this relationship was found (ANCOVA, $p > 0.05$).
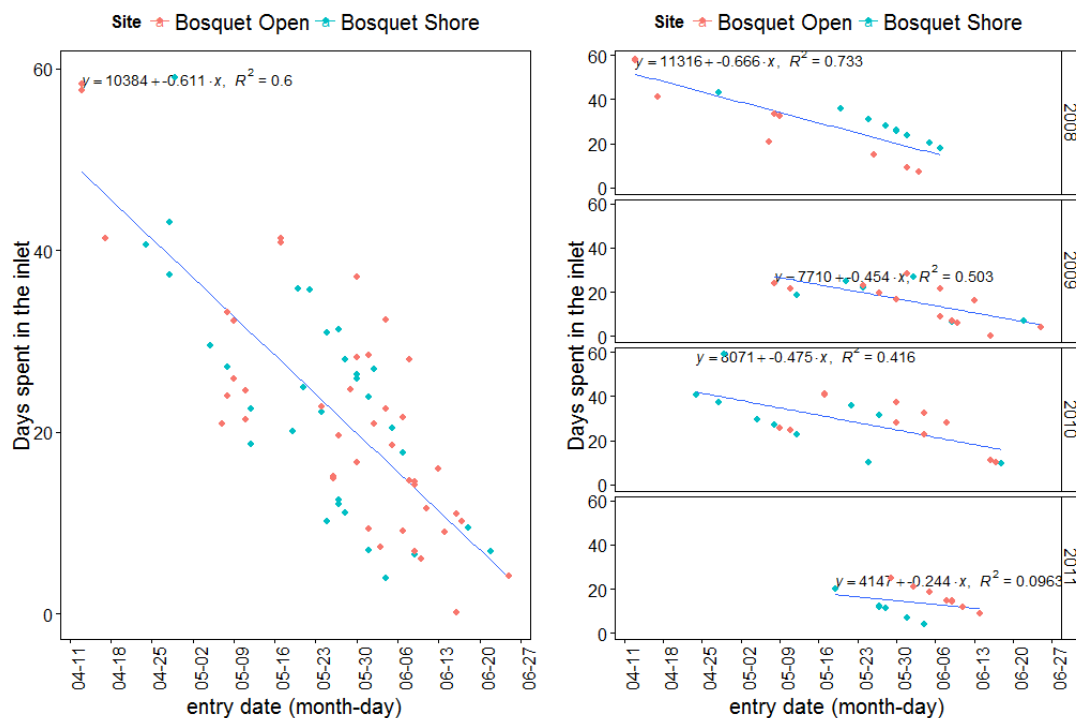
Figure 13: Relationships between the day of entry into the inlet and duration of the migration through the Inlet for combined 2008-2011 years (left panel) and for each year (right panel). The blue line is the fitted linear regression line.

Based on the available samples, migration from the Wannock River into Rivers Inlet happens over a 6-8 week period, with the exception of 2011 which was likely affected by the limited number of

samples available for that year (Table 5). A difference was observed between the two sites (Bosquet

Shore and Bosquet Open), though this did not follow a regular pattern. In 2011, Bosquet Open fish spent

a significantly longer time in the inlet than Bosquet Shore fish (ANOVA, $p < 0.05$). In 2008 the opposite

was observed, though the difference was not significant.

Table 5: Estimated period of migration from the Wannock River and estimated last day of the migration
through Inlet.

Year	First fish	Last fish	Duration of outmigration period from the Wannock River (weeks)	Estimated date of end of the migration
2008	Apr 17	Jun 8	7.4 weeks	Jul-18
2009	May 12	Jun 25	6.2 weeks	Jun-25
2010	Apr 24	Jun 18	7.9 weeks	Jul-11
2011	May 19	Jun 13	3.6 weeks	Aug-13

Assuming that fish constantly travel through the inlet, the average traveling speeds were

calculated as the ratio of length of Rivers Inlet (~45 km) and the number of days spent in the Inlet (Figure

14).
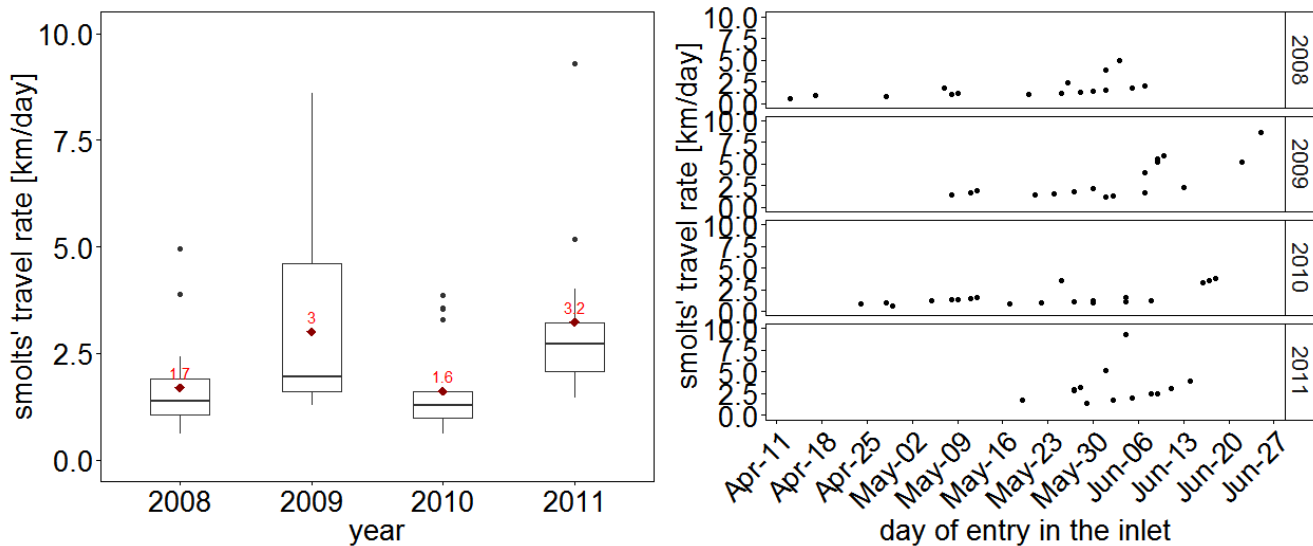
Figure 14: Box-plots of migration rate of smolt through Rivers Inlet in each year (left); and the
relationship between day of entry into inlet and travel speed (right). Median values (black solid horizontal
line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Red dots and
numbers are the sample means.

Fish traveled through Rivers Inlet at an average speed of 1.7, 3.0, 1.6, and 3.2 km/day in 2008, 2009, 2010, and 2011 respectively (ANOVA, $p \gg 0.05$). The rate of travel increased with the day of entry. In terms of body length per second, the traveling rates of the smolts were 0.3, 0.5, 0.2 and 0.5 BL/s in 2008, 2009, 2010 and 2011 respectively with no significant difference between the years (ANOVA, $p > 0.05$; Figure 15).
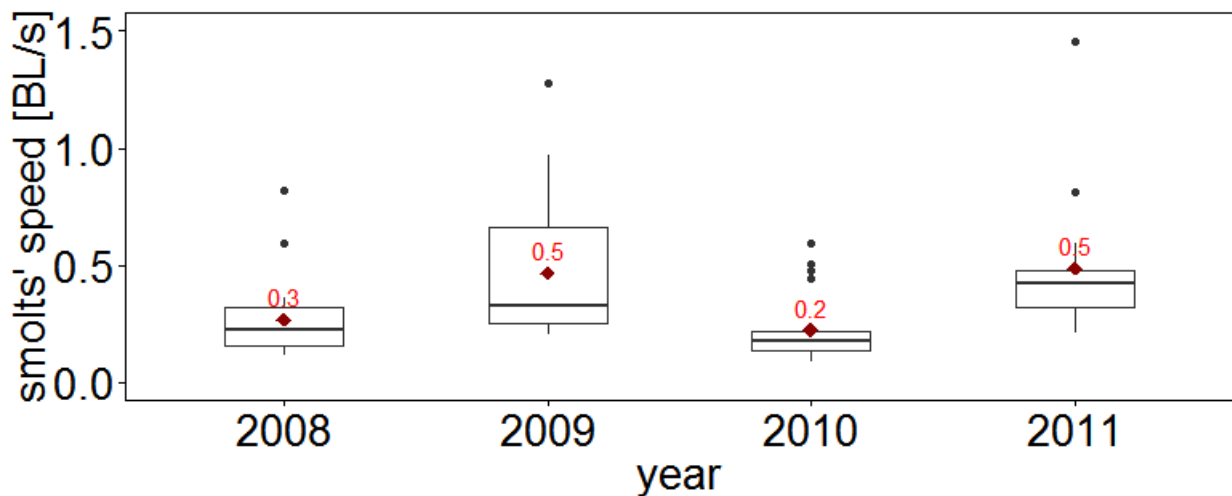
Figure 15: Outmigration rates of travel of juvenile sockeye smolts through Rivers Inlet in BL/s for four chosen years. Median values (black solid horizontal line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Numbers in red are the median values for each year. According to the obtained data, the traveling speeds were fastest in 2009 and 2011, and a bit slower in 2008 and 2010.

2.3.3. Interannual Variability

Based on the available data, there was an interannual difference in size density distribution between the head and the mouth of the inlet (Figure 16). Year 2010 was significantly different from 2008 and 2009 (ANOVA, $p \ll 0.01$), and 2011 differed from 2009 ($p \ll 0.01$). At the head of the inlet, smolts were larger in 2011 and 2008 (73.4 and 69.3 mm respectively) compared to 2009 and 2010 (61.9 and 66.4 mm respectively), with 2009 having the smallest fish (Table 3).

There was also a significant difference in smolt sizes at the mouth of the inlet between some years. 2010 was significantly different from both 2009 and 2008 (ANOVA, $p \ll 0.01$). The distribution of sizes were ~10 mm larger in 2008 and 2009 than in 2010 and 2011 (Table 3). Mean size was the largest

in 2010 (85 ± 8 mm) and the smallest in 2008 and 2009 (76 ± 9 mm), the average size of the smolts at

the mouth of the inlet in 2011 was 80 ± 7 mm.
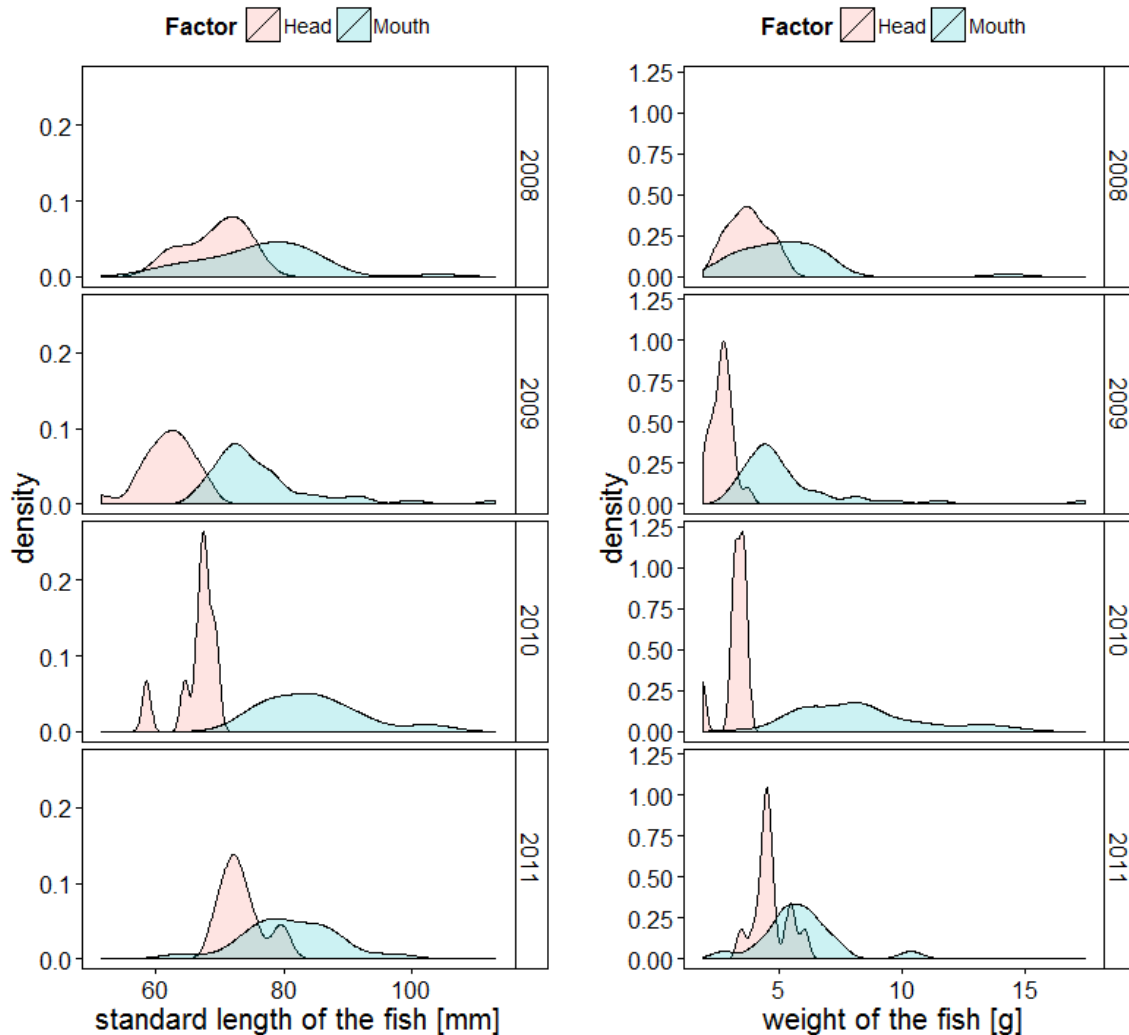
Figure 16: Comparison of size and weight density distributions at the head (Wannock) and mouth (Bosquet Open and Shore) of Rivers Inlet in 2008-2011. The graph was built based on all the available records from these sites (n=173).

Based on the back-calculated size at entry to the inlet, standard length of the smolts varied

between the years, but the only significant difference (ANOVA; $p < 0.05$) was found between 2010 and

2008, and between 2011 and 2008 years (Figure 17; top panel). On average, fish that were entering the

inlet in 2008 were the smallest with a mean size of 67.3 mm, and the largest smolts were in 2010 and

2011 (75.0 and 76.4 mm, respectively). Mean smolt size in 2009 was 71.9 mm (median and mean sizes did not differ much).

Mean size at the mouth of the inlet was significantly different (ANOVA; $p < 0.05$) across the years except for 2008-2009 and 2008-2011 pairs (Figure 17). Smolts were the largest in 2010 with a median size of 85.5 mm. Median size was similar in 2008 and 2011 (77.5 and 78 mm respectively). The smallest median smolt size was observed in 2009 (73 mm).
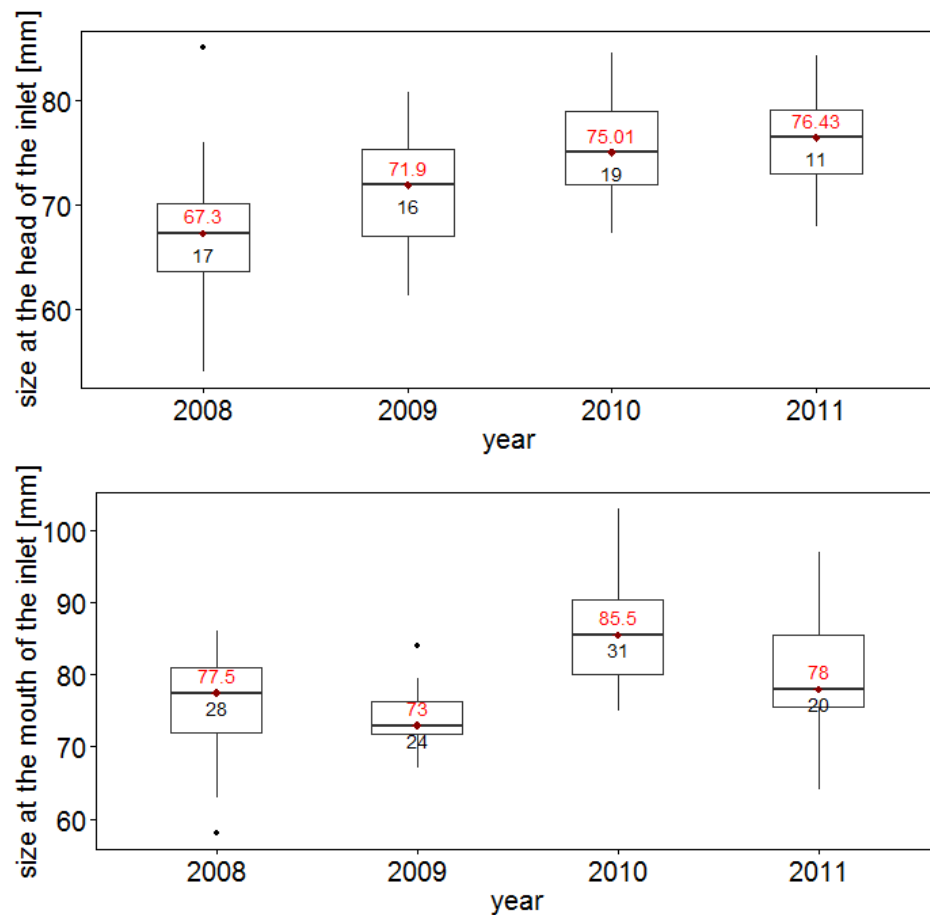
Figure 17: Standard lengths of the sockeye salmon smolts (in mm) at the entrance (left panel) and mouth (right panel) to Rivers Inlet. Median values (black solid horizontal line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Red dots and numbers represent the median values, and black numbers are the sample sizes. Size at the mouth of the inlet was back-calculated.

Size gained was calculated as the difference between the sizes at the head and the mouth of the inlet for each fish where this calculation was possible (Figure 18). Fish had the largest gain in size (8.8

mm) in 2010, followed by 2008, with an average gain of 6.2 mm. In 2009 fish only gained 3.8 mm and fish in 2011 gained only 2.1 mm.
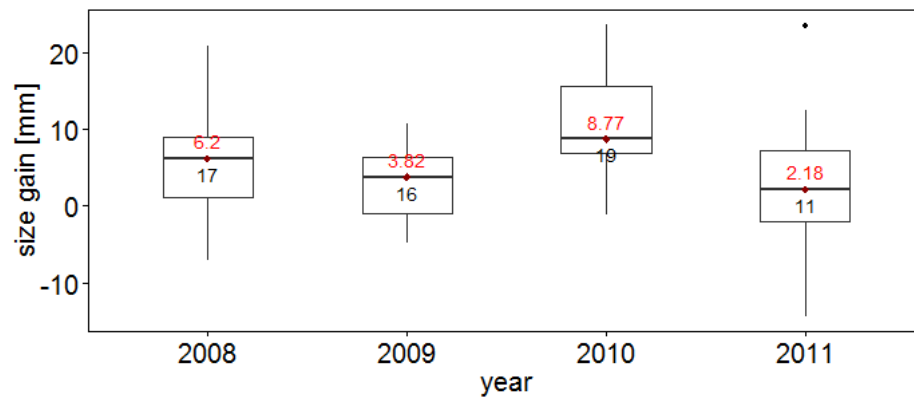
Figure 18: Annual difference of size gained between the 2008-2011 years (in mm). Median values (black solid horizontal line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Red dots and number represent the median values, and black numbers are the sample sizes.

To eliminate the effect of the duration of the stay in the inlet, the size gained per day was compared across four chosen years (Figure 19). Fish grew the fastest in 2011 with 0.4 mm gain per day. However, there was very high variability compared to all other years. The slowest growth per day was in 2008 (0.17 mm/day). Size gained per day in 2009 and 2010 were 0.22 and 0.33 mm/day respectively. The only significant difference in mean values was between 2010-2009 and 2010-2011 pairs (ANOVA; $p < 0.05$).
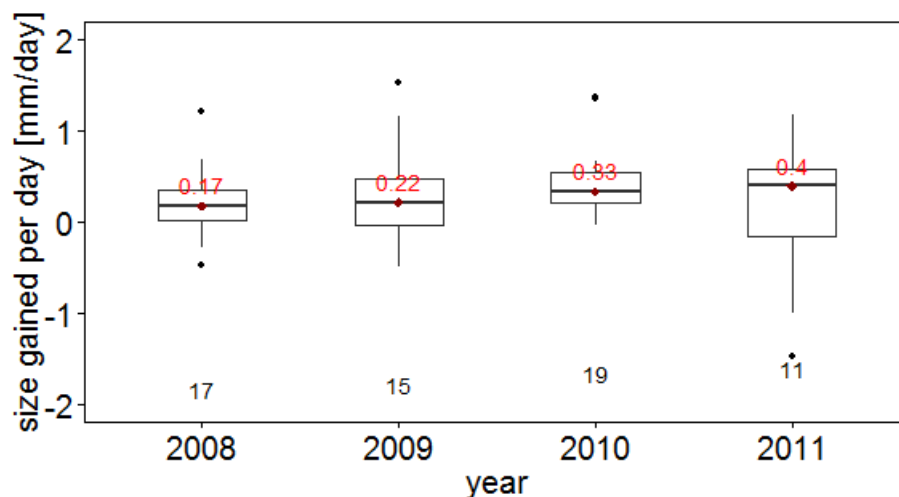
Figure 19: Size gained per day during the outmigration period. Median values (black solid horizontal line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Red dots and number represent the median values, and black numbers are the sample sizes.
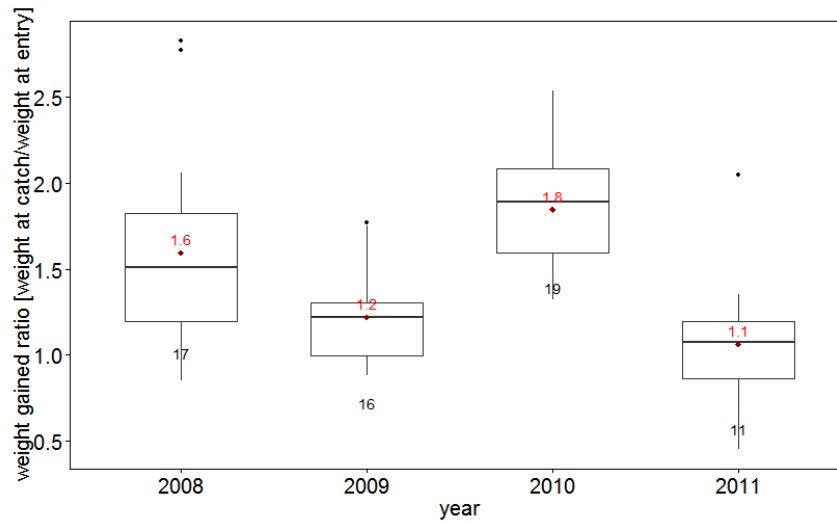
Figure 20: Increase in weight for smolts across four years (2008-2011). The increase in weight is represented as a ratio between the final weight and the initial weight at entry to the inlet. Median values (black solid horizontal line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Red dots and number represent the median values, and black numbers are the sample sizes.

Mean increase in weight, calculated as the ratio of weight at capture over the initial weight of the fish at entry to the inlet, was the largest in 2010 with smolts almost doubling their weight (~1.8 increase). The size gain in 2010 was significantly higher than in all other years (ANOVA, $p \ll 0.01$). In 2011 and 2009 smolt gained a little weight (~1.1 and 1.2 increase, respectively). The increase in size measured in 2008 was large compared to 2009 and 2011 (~1.6).
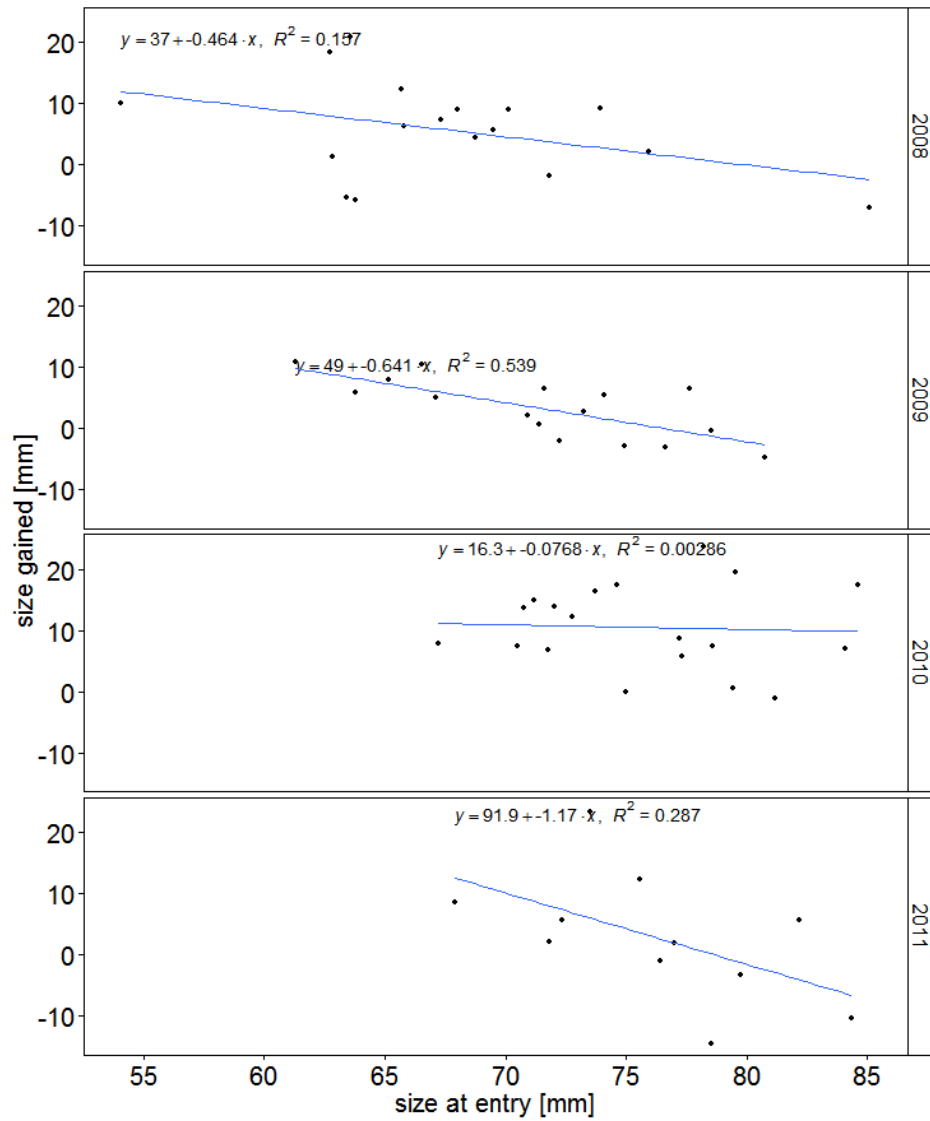
Figure 21: The relationship between size at entry and overall size gained during the outmigration through Rivers Inlet. The blue line is the fitted linear regression line.

During 2008, 2009 and 2011 smaller fish increased in size more compared to the larger individuals. However, there was no change in size gained between larger and smaller smolts in 2010. There was no statistical difference between size gained and size at entry between the years (ANCOVA, $p > 0.05$).
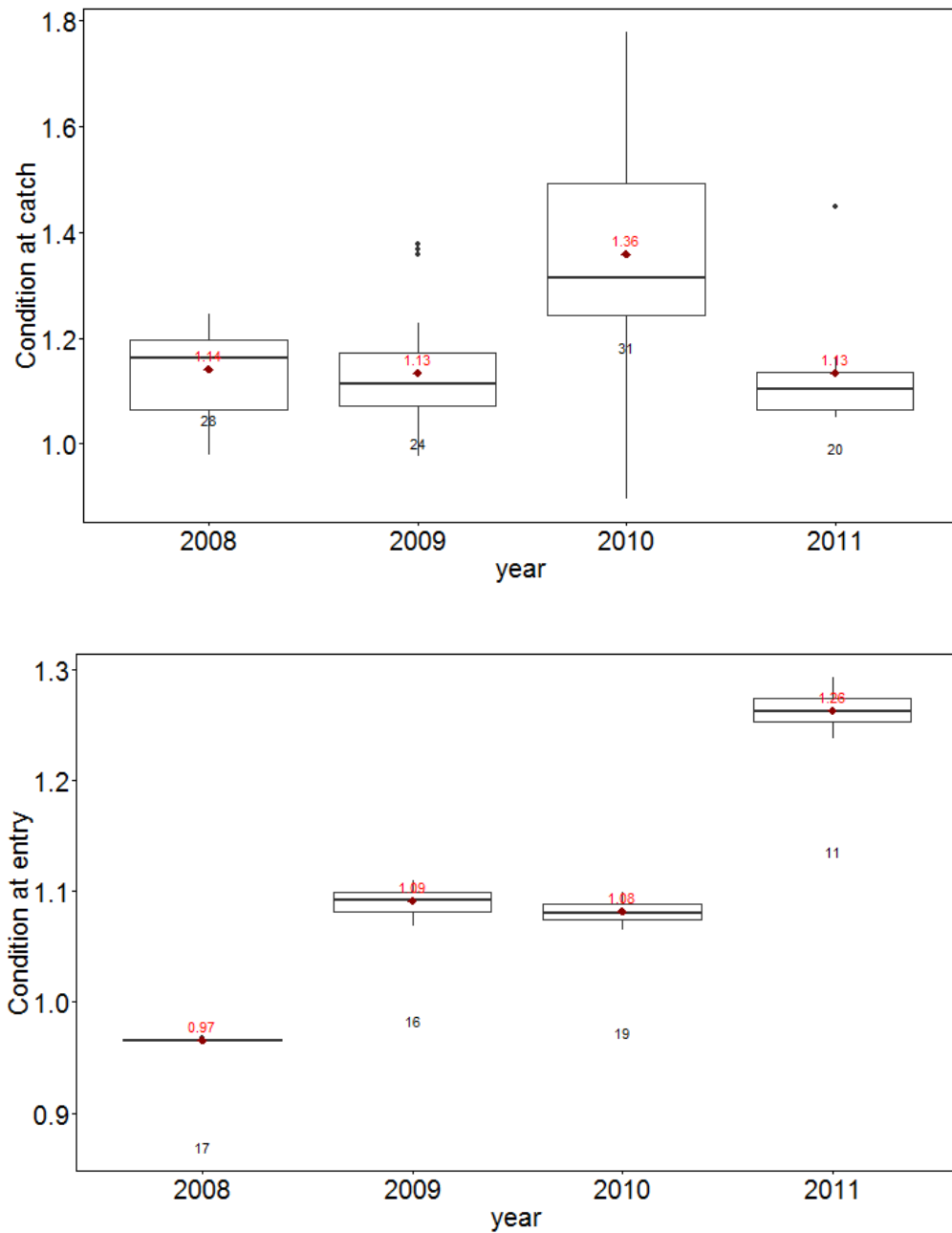
Figure 22: Condition of juvenile sockeye salmon smolts at catch (top panel) and at entry (bottom panel) from Rivers Inlet measured for four years (2008-2011). Median values (black solid horizontal line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Red dots and number represent the mean values, and black numbers are the sample sizes.

In 2010, the smolt condition at catch was the highest of four years (1.36) and was significantly different from the rest (ANOVA; $p \ll 0.01$). The mean condition factor in 2008, 2009 and 2011 was similar (1.13– 1.14) for three years. Condition factor was independent of size within the size range of

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sockeye smolts used in this study (58 – 103 mm). No significant relationships (ANCOVA; $p > 0.05$) were observed between the condition factor, and length except for 2009 and 2010 where the condition factor was dependent on smolt size (ANCOVA; $p < 0.05$). However, very low slopes (0.00125 and– 0.000996) along with low R^2 values (0.20 and 0.18) were observed in 2009 and 2010 respectively. In addition, the slope of the regression lines between log of body weight and log of standard length are all between 2.8 and 3.1, satisfying the assumption of an isometric growth associated with the use of Fulton's condition factor.

The condition of the smolts at entry to the inlet was the highest in 2011 (1.26) and the lowest in 2008 (0.97). Conditions in 2009 and 2010 were similar and were 1.08-1.09. Smolt conditions from 2008 and 2011 were statistically different from the other years (ANOVA; $p \ll 0.01$). Condition at entry may provide information on the overall physical condition of the smolts at the begging of outmigration. Stronger, healthier fish have a better chance for survival than smaller weaker counterparts.

2.4. Discussion

Our results showed that the Sr:Ca signal is a valuable marker of the migration history of juvenile salmon and clearly identified the transition between freshwater and marine environments. A disadvantage of this marker is its heavy dependence on the relationship between Sr:Ca in both otolith and ambient water. Although Sr seawater concentrations tend to stay relatively stable ($\backsim$90 µM), concentrations in freshwater can vary dramatically depending on geological and weathering conditions (Limburg 1995). This relationship can be altered by water temperature, large gradients in salinity within estuaries (Kraus and Secor 2004), fish growth rate, and physiology (Martin et al. 2004).

2.4.1. Duration of Outmigration Through Rivers Inlet

The duration of the outmigration from Owikeno Lake was determined assuming constant deposition of daily rings on the otolith. When attempting to estimate residence time using otoliths, it is important to determine whether there is a lag between the deposition of Sr into the aragonite structure and the change in the Sr concentration of ambient water. A lag may also be introduced during the ablation process as after the ablation, ionized material travels through the instrument before reaching the detector. However, the latter issue is not expected to influence the results of the study due to the high travel speeds (μs) of the analyzed material to the detector. Ruggerone and Volk (2003) performed a similar study on Chinook salmon. They compared the position of thermal and strontium mark methods with the assumption that the otolith thermal mark was incorporated on the same day as a temperature change. They found that the difference between the thermal and strontium mark methods was 0.1μm. Thus, for the purpose of this study, the difference between the incorporation rate of the element and environmental change was considered insignificant.

The first goal of this study was to determine the duration of the outmigration of juvenile sockeye salmon in Rivers Inlet. We determined that fish spent on average ~2 weeks in Rivers Inlet in 2009 and 2011, while in 2008 and 2010 the average duration was ~4 weeks. These average durations were lower than those measured by Stocks et al. (2014); ~3 weeks in 2008, 4 weeks in 2009 and 6 weeks in 2010. The lower average rates recorded in this study may have been attributed to a combination of larger sample size, which included a full-size spectrum of fish as opposed to only the largest fish in Stocks et al. (2014), and fish collected from throughout the outmigration period.

Closer examination found that the duration of stay in the inlet was not related to the size of the fish, but rather the day of entry into the inlet. An inverse relationship was found between the day of entry and the duration of outmigration (Figure 13). This relationship was evident for all four years. The later migration initiation date in 2009 and 2011 may, therefore, have been an important factor in the shorter inlet residence time in these years.

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Assuming that the catch data were representative of the population, the migration from the Wannock River started as early as 17 April in 2008 and lasted for 7.4 weeks. In 2010, migration from the river started one week later than in 2008 but lasted for a similar time period (~8 weeks). Migration in 2009 was initiated on 5 May and lasted for ~6 weeks. The start of migration was the latest in 2011, on 19 May, and lasted only for 3.6 weeks. A factor contributing to the short estimated outmigration period in 2011 would have been the relatively early date of the last capture of Bosquet fish (June).

From the collected data, the last fish exited Wannock River on 8 June in 2008, on 25 June in 2009, 18 June in 2010 and 13 June in 2011. However, extrapolation of the regression line (left panel Figure 13) gave the estimate of theoretical termination day of the migration as mid-July in 2008 and 2010, late June in 2009, and mid-August in 2011. When considering all years together, the mean end date of the migration from the Wannock was 13 July.

These results also partially support the connection between the lunar calendar and the seaward migration of Rivers Inlet juvenile sockeye salmon proposed by Buchanan (2006). Buchanan (2006) demonstrated that the migration begins at the time of the last full moon in May with peak achieved at a new moon in mid-June. The migration ends by the next full moon in late July. The largest juveniles were observed during mid-migration while the smaller members migrate either earlier or later in time (Buchanan 2006). In this study, the estimated outmigration dates from the Wannock River were indeed correlated with the lunar cycle when the first fish appeared in the full moon in mid-April to mid-May (depending on the year) and the migration ended in the 4th full moon after the start of the migration (Table 6).

Table 6: Comparison of first and last day fish entered the inlet with the lunar cycle for 2008-2011.

Year	First fish	First full moon	Estimated date of end of the migration	Last (4th) full moon	Estimated duration of the migration, days
2008	Apr 17	Apr 20	Jul-18	Jul 18	59
2009	May 12	May 9	Jun-25	Aug 6	58
2010	Apr 24	Apr 28	Jul-11	Jul 26	59
2011	May 19	May 17	Aug-13	Aug 13	57

Estimated duration of outmigration from the Wannock River was consistent between years, with an average of 58 days (2 months), coinciding with the duration of the three moon cycles. Moreover, the graphs show the change in the duration of outmigration through the Inlet with the day of entry in the Inlet.

Based on these residence times, average travel rates were found to be 1.7, 3.0 (median is 2.2), 1.6 and 3.2 km/day in 2008, 2009, 2010 and 2011, respectively. Estimated mean average speed in 2008 was slower in this study than the one calculated by Stocks et al., while in 2009 the calculated speed was faster than the one determined by Stocks et al. This could be due to two possible reasons: 1) only largest fish were used in the Stocks et al. study which may travel faster; 2) the majority of the fish in the Stocks et al. study came from a different location (Dimsey Site). It is possible that fish traveling through the main channel may have had different migration behavior and hence swimming speed. The mean speed of the migration found in this study for 2010 is consistent with the results of Stock et al. (2012). Again, as the Stocks data included sites inside the inlet, obtained reference speeds might not be totally comparable. The obtained speeds are travel rates and not necessarily represent the actual moving speed of the fish travelling through Rivers Inlet. As the fish might move faster or slower in certain areas, the average speed calculated for sites different from Bosquet may result in faster average speed. This will be further investigated in Chapter 3.

In terms of BL/s, the fish travel through Rivers Inlet at a speed of 0.2-0.5 BL/s. The difference between years was found to be insignificant (ANOVA; $p > 0.05$). Travel speed in Rivers Inlet was lower than theoretically determined optimal speed of active smolt migration, e.g., the 0.80-2 BL/s (Welch et al. 2011, Martins et al. 2012).

There was no consistent change in the smolt size with the period of outmigration within each year as there was no correlation between fish size and the day of entry. This finding concurs with an observation from other systems (e.g., Ibbotson et al. 2011). There are two potential explanations for this:

1) the timing of the outmigration of smolts is size-independent and driven by the lunar cycle, or 2) due to the environmental conditions in the lake, smolts enter the inlet at different sizes. The latter statement was investigated in the 3rd chapter. Potential variations in smolt length among the years appeared to be independent of the duration of the seaward migration and may be attributed to a number of environmental factors, including prey availability (quality and quantity), freshwater discharge, and etc. (see Buchanan 2006; Ajmani 2011; Hodal 2011). The environmental factors will be briefly discussed in Chapter 3 (Section 3.4).

2.4.2. Decoupling Between the Size of the Otolith and the Smolt Length

The initial back-calculated size of the fish was calculated by two methods based on 1) the otolith radius and 2) the otolith length. The first method used the direct measurement of otolith radius and size of the smolts (Figure 10) using the measuring unit. However, the relationship between these two values was not as strong with R^2 value being lower (0.50) than in similar relationships (Ruggerone and Volk 2003, Xu 2014) where R^2 values were close to 0.8-0.9. The weakened correlation may have been due to:

1) Errors in the measured radius– light penetration in some cases was not good enough to clearly see the center of the otolith, and so the center of the otolith was estimated.

2) Some otoliths have two primordia centers– to be consistent the center that was further away from the tip of the otolith was chosen as a starting point when measuring the otolith radius. However, other portions of the otolith have only one primordia center.

To investigate the first source of potential error (i.e the error in measured otolith radius) the relationship between the otolith length and otolith radius was inspected. However, the correlation between the otolith radius and length was quite strong with R^2 value of 0.84, meaning that errors might be minimum at this step. However, similar to the relationship between otolith radius and smolt size, the relationship between otolith length and smolt size also had a relatively low R^2 value (0.60). A possible explanation for this is "decoupling" between the growth of the smolt's otolith and the fish itself. Such

decoupling might be due to fish starvation/shortage of food resources or physiological processes that could alter the aragonite growth rates (Wright et al. 1990, Metcalfe et al. 1992); although Meekan et al.(n.d.) disproving this theory.

2.4.3.   Interannual Differences in Size Distribution

In 2008 and 2011, fish that were caught at the Wannock site were as small as the fish caught at the mouth of the inlet for the corresponding years. In fact, in 2011 there were fish caught at Bosquet that were smaller than those caught at the Wannock. The following might be the evidence that the period of the migration from the Owikeno Lake might take several weeks. Fish in all four years showed a noticeable shift in the mean size distribution between the mouth and the head of the inlet.

Analysis of the catch data enabled validation of whether the back-calculated entry sizes of the smolts were appropriate, and the obtained numbers were comparable with the ones in Table 3. The back-calculated size of smolts in 2008 was 67.3 mm compare to the 69.3 mm from the fish that were caught at the Wannock site during that year. Similarly, in 2011 the back-calculated size at entry was just 3 mm larger than the reference number (73.4 mm). However, back-calculated sizes at entry in 2009 and 2010 were higher (71.9 and 75.0 mm respectively) compared to the measured mean sizes of fish caught at the Wannock Site during those years (61.9 and 66.4 mm respectively). In 2009, the back-calculated size of the smolts were higher than the fish caught at the Wannock area because the fish that were caught at that location was only from two surveys in June; thus, the larger smolt might appeared earlier or later in the season (May or July). In 2010, fish was caught at the Wannock only during one day on May 19, so can't represent the actual mean size of the fish during the whole migration period. Therefore, the back-calculated sizes during 2009 and 2010 can be considered reasonable. Overall, back-calculated sizes seemed to be a reasonable representation of smolt sizes, and hence support the methods and techniques used in this study.

The size of the smolts varied significantly among years even prior to the start of the migration (54.1-85.1 mm). During 2008, 2009 and 2011 there was a correlation between the size at entry and overall size gained during migration through the inlet (Figure 21), with the smaller smolts gaining more than the larger individuals. Although there was no change in size gained between larger and smaller smolts in 2010, the data for the other years do indicate that the freshwater growth rate may have a strong influence on subsequent growth rates in the marine waters and may have a lingering effect on salmon beyond the influence of the initial size at entering marine environment (Ruggerone and Volk 2003).

Considering the size gained by fish during the outmigration through the Inlet, fish had the largest gain in size (8.8 mm) in 2010, followed by 2008 with an average gain of 6.2 mm. In 2009, fish only gained 3.8 mm and fish in 2011 gained only 2 mm. (Figure 18). However, fish spent different amounts of time in the Inlet, so when the effect of the duration of the outmigration was eliminated, smolts grew the fastest in 2011 with 0.4mm gain per day. However there was high variability in 2011 compared to all other years. The slowest growth per day was in 2008 (0.17 mm/day). Size gained per day in 2009 and 2010 were 0.22 and 0.33 mm/day respectively. Growth rates were comparable to salmonoids growth rates in other areas (Fisher and Pearcy 2005, Beakes et al. 2010, Volk et al. 2010)

Smolts weight seems to increase slightly from the past years. In 1914-1916, average fork lengths of the Owikeno smolts were 58.6 – 59.7 mm, with the size decreases as the season progresses, amounting to about 10 mm (Gilbert 1915, 1916, 1918). In 1956, smolts averaged at 61 mm in fork length (2.0 g in weight; Foskett 1958). Over the 26-year period (1950s– 1990s), the presmolt were 1.42 g (McKinnell et al. 2001, Rutherford et al. 2005). However, the trawl used in the surveys (1-m diameter conical trawl) to collect the juveniles was increasingly size-selective for the fish larger 40 mm (K. Hyatt in Shortreed and Morton 2003). After adjustment, average smolt weight in Owikeno Lake was 2.33 g and ranged between 1.0 and 5.5 g. In 2002, smolts averaged 80 mm in length and 4.3 g in weight (Shortreed and Morton 2003). In 2008-2011, the size of smolts at entry to the inlet varied significantly and ranged between 54

and 83 mm (mean 78.9 mm), and mean weight of the smolts at the entry of Rivers Inlet was 3.4 g (range 1.9– 6.1 g). The found weights in 2008– 2011were a bit smaller than 4.5 g but were higher than previously reported weights in Owikeno Lake (1950– 1990; 2.33 g; Shortreed and Morton 2003). However, the back-calculated weights were closer to the average weight of the smolts (4.2 g), but with a bit smaller mean fork length of 72.6 mm. The following data might suggest that Owikeno Lake sockeye fry exhibited strong density-dependent growth. The weight and length of the smolts improved in recent years, indicating that freshwater conditions might not be the determining factor that determines survival success of the smolts.

With regard to the increase in weight of the smolts, smolts did not double their weight during the outmigration unlike the statement proposed by McKinnell et al. (2001) and Buchanan (2006). However, increase in weight correlated with the duration of the outmigration. Thus, the increase in weight was the largest in 2010 and 2008 when fish spent on average 4 weeks traveling seaward through the inlet. Very small increases were measured in 2009 and 2011 when smolts spent only 2 weeks outmigrating through the inlet. The largest increase in weight happened in 2010 when fish gained 80% of its original weight, while the smallest increase was in 2011 when fish only gained 10%.

Smolt condition at catch in 2010 were highest across four years and was classified by Barnham and Baxter (1998) as "fair-good". Condition of the smolts in 2008, 2009 and 2011 was "poor-fair" with CF of 1.13– 1.14. However, very different situation was observed at the entry of the inlet. CF was the highest in 2011 (1.26) and classified as "fair-good", while 2008 smolts had extremely poor-poor condition (CF = 0.97). Smolts in 2009 and 2010 had "poor-fair" condition with CF =1.08– 1.09. The following results showed that the outmigration through Rivers Inlet had a great influence on the condition of the fish. Smolt condition greatly improved in 2008 and 2010, as well as a small improvement in 2009; however, the condition of the smolts decreased greatly in 2011 with a drop in CF by 0.12.

However, use of the condition factor on its own, could not be sufficient evidence to assess the health state of the fish and should be supported by other types of data. Thus, CF was higher in juvenile pink salmon in waters contaminated by the oil spill compared to the fish from unpolluted areas due to reduced growth rates in the oiled area. (Wertheimer and Celewycz 1996). Adams et al. (1992) also showed the condition factors of White suckers, *Catostomus commersonii*, and redbreast sunfish, *Lepomis auritus*, were higher in the polluted areas than other pristine habitats. Smolts from 2008 and 2010 spent in the inlet 2 weeks longer than smolts from 2009 and 2011, and improved their condition more in these years. Also, the outmigration in 2008 and 2010 started earlier (mid and late April respectively) compared to the 2009 and 2011 where fish started outmigrating later in May. Such observation might be an evidence that earlier migrating fish experienced better conditions for outmigration that lead to improved health condition of the fish.

No data are available for the smolt escapement from Rivers Inlet during 2008-2013 from this region. Data from "indicator-stocks" in Hecate Straight (Smith Inlet stock) also terminated the smolt enumeration in 2005 with only return numbers being available up-to-date (Figure 23). The 2008 La Niña event was followed in 2011 by high returns both in Smith and Rivers Inlet (Chandler et al. 2015); however, returns from 2010 was just slightly higher than in 2008 and 2009. Although the condition of the smolts was the best in 2010 compare to the other 4 years, the returns in 2012 dropped greatly after 2011 but were higher than in 2010 by ~50 million fish. Additional information is required (age composition, smolt survival rates, habitat condition, predation rate and etc.) to link the smolt condition at the mouth of Rivers Inlet with the survival success of salmon coming to spawn in 2 years after.
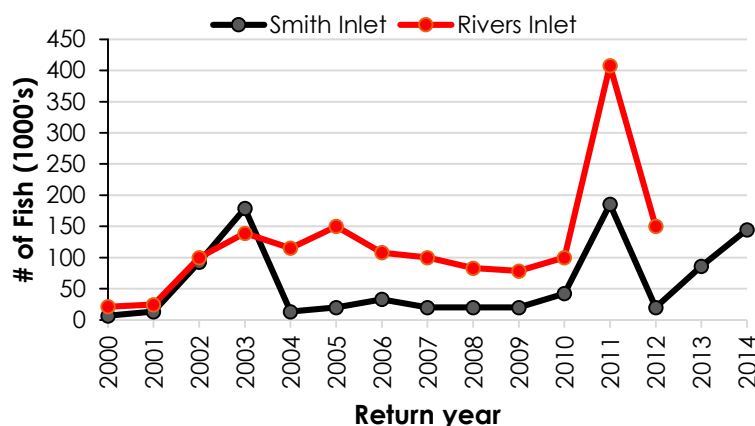
Figure 23: Total returns and British Columbia sockeye salmon in Smith (black line) and Rivers Inlets (red line) for 2000 – 2014.

Thus, the duration of the outmigration can be an important factor that can determine how much smolts will gain in size or weight traveling through the inlet, especially, in combination with the amount of food available and other environmental factors, such as water discharge. Even short outmigration periods that coincide with the high availability of the food, may lead to a greater increase in smolt weight/size compared to longer duration migrations accompanied by poor food availability (discussed in Chapter 3). The effect of the environmental factors will be considered further in Chapter 3.

2.5. Chapter 2 Summary

Regarding the methods used, Sr:Ca signal was an appropriate marker for the determination of the marine entry point compare to Ba:Ca or Sr:Ba ratios. The first goal of this study was to determine the duration of the outmigration of juvenile sockeye salmon in Rivers Inlet in 2008-2011. The outmigration rates varied over the years. The juvenile salmon spent the shortest time in Rivers Inlet in 2011 and 2009 (average ~ 2 weeks) and the longest time in 2008 and 2011 (average ~ 4 weeks).

There was no influence of the size of the smolts on the duration of the outmigration. However, the duration of outmigration had a strong inverse correlation with the day of entry. The later migration initiation date in 2009 and 2011 may, therefore, have been an important factor in the shorter inlet residence time in these years.

Based on the calculated residence time in the Inlet, average travel rates were 1.7, 3.0, 1.6 and 3.2 km/day in 2008, 2009, 2010 and 2011, respectively. Regarding BL/s, the fish traveled through Rivers Inlet slower than the theoretically determined optimal speed of active migration and varied between 0.2 and 0.5 BL/s.

Based on the back-calculated day of entry, the migration from Wannock River started as early as 17 April in 2008 and lasted for 7.4 weeks. In 2010, migration from the river started one week later than in 2008 but lasted for a similar period (~8 weeks). Migration in 2009 was initiated on 5 May and lasted for ~6 weeks. The start of migration was latest in 2011, on 19 May, and lasted only for 3.6 weeks. Also, the obtained timing of the start and end of the migration from the Wannock River was correlated with the lunar cycle. The first fish entered the inlet at the full moon in mid-April to mid-May (depending on the year) and the migration ended by the 3rd full moon after the start of the migration.

The duration of the migration correlated with the increase in size of the smolts. Fish that spent more time in the inlet (2008 and 2010) has a larger overall size gain. With the exception of 2010, the smaller smolts gained more weight compared to the larger individuals. However, the average daily gain showed the opposite trend with the highest increase in daily size in 2011 rather than 2008 or 2010. Only some fish doubled their weight in 2008 and 2010, while in 2011 and 2009 fish only gained 10 and 20% of their weight at entry respectively.

CHAPTER 3: SPATIAL AND TEMPORAL PATTERNS OF SOCKEYE SMOLT MIGRATION IN RIVERS INLET

3.1. Introduction

To assess spatial and temporal differences in the seaward migration timing and duration of juvenile salmon, it is important to understand the behavior of the fish during the outmigration period, and how it interacts with local environmental conditions. Upon entry to the estuary, juveniles of several species including pink, sockeye, steelhead, and Atlantic salmon swim actively and rapidly through coastal waters during early outmigration (Lacroix and McCurdy 1996, Lacroix et al. 2005, Hedger et al. 2009, Welch et al. 2011). Conversely, juveniles of chum, coho and chinook tend to swim at much slower rates utilizing nearshore habitats, such as shallow sublittoral areas, exposed gravel beaches, salt marshes, mudflats, contained embayments, for a longer period of time (Stober et al. 1973, Miller et al. 1978, 1980, Simenstad and Eggers 1981, Simenstad et al. 1982, Healey 1987, Bond et al. 2008, Melnychuk et al. 2010, Welch et al. 2011).

Apart from differences between species, outmigration dynamics through the estuary can vary between populations, fish origin or size (Thorstad et al. 2007, Chittenden et al. 2008, Melnychuk et al. 2010). It is possible, however, to classify the outmigration patterns of juvenile salmon (sockeye in particular) into two categories: (1) active swimming west and north towards the open ocean and (2) slow moving utilization of the habitat. Juveniles may use estuaries for feeding and growth (Healey 1980), or to improve their osmoregulatory capability, adjust to the marine environment, and avoid predators (Chittenden et al. 2008). The rate of outmigration can vary greatly from 0.53 BL/s to 4 BL/s (Lacroix and McCurdy 1996, Thorstad et al. 2007, Kocik et al. 2009) with a mean value of 1 BL/s (Martin et al. 2009), and more rapid movement during the night (Aprahamian and Jones 1997, Martin et al. 2009, Furey et al. 2016). In fjord systems, measured outmigration speeds are slower than in

estuaries, with speeds of 0.53-0.77 BL/s (Fried et al. 1978, Moore et al. 1995, Finstad et al. 2005, Thorstad et al. 2007).

Smolts move at varying rates through different parts of an estuary. In general, juveniles travel at slower rates in the inner part of the system with a subsequent increase in travel rate in the outer part of the estuary (Thorstad et al. 2007). In the coastal areas, juvenile salmon tend to use ebb tides for migration (Lacroix and McCurdy 1996, Aprahamian and Jones 1997, Martin et al. 2009) and swim together with tides during darkness (Moore et al. 1998, Chamberlin et al. 2011). Moore et al. (1995), in their study of *S. salar* smolts, observed variable behavior throughout the estuary: (a) in the freshwater-tidal and upper sections of the estuary, the smolt migration was passively aligned with the water flow closely following the tidal pattern; (b) in the middle section of the estuary, while the migration was still passive, an ebb tide transport component was identifiable; and (c) finally, in the lower estuary, smolts were clearly oriented with the flow and exhibited directed active swimming, sometimes against the current. From the above, it is apparent that tidal cycle can play an important role in determining fish migration behavior and rates in most systems (Moser et al. 1991).

In addition, research on the Central Coast of BC found differences in juvenile salmon abundance between bay, point, and channel habitats (Temple and Price 2007). In that study, more juveniles were found in channels than near points, though this difference between habitats was not significant. Smolts may actively use estuarine currents while spending extended periods of time holding in "current quiet" areas (Moser et al. 1991). During their estuarine residency, juveniles tend to utilize the top 2-4 m of the surface of the water column (Doving et al 1985, Sturlaugson and Thorisson 1995) and are even shallower during the night time (Davidsen et al. 2008). The depth of travel was found to be dependent on temperature, salinity and light conditions (Davidsen et al. 2008, Manel-La et al. 2009).

Water discharge is one of the most influential factors in smolt migration timing (Wood et al. 1993, Buchanan 2006, Carter et al. 2009, Ajmani 2011, Hodal 2011). In glacial systems in temperate North America, the snowpack melt driven freshet can cause extreme summer river flows that drive a strong estuarine circulation. Rivers Inlet represents such a system, and at the peak of the freshet surface water residence times in the inlet may be as low as 4 days (Hodal 2011), potentially significantly affecting juvenile salmon migration dynamics.

Migration dynamics may also interact with other environmental factors impacting the growth and survival rates of juvenile salmon, including temperature (McDonald 1969, Torgersen et al. 1999, Cooke et al. 2011), productivity and prey availability (Beamish et al. 1999, 2010). Evidence suggests that local effects of climate change during their early marine phase may be primary drivers of sockeye salmon decline (Rutherford and Wood 2000, McKinnell et al. 2001).

In Rivers Inlet, several studies have been conducted to explore environmental interactions with the timing and duration of the sockeye smolt seaward migration (Buchanan 2006, Wolfe 2010, Ajmani 2011, Stocks et al. 2014). Food availability was suggested to affect the duration of outmigration through Rivers Inlet, as juveniles tend to travel faster through the inlet if the prey was scarce (Ajmani 2011). Ajmani (2011) also proposed that smolts entering the inlet during the high water discharge period would have a shorter passage time than the fish entering inlet during weak river flows. This was subsequently confirmed by Stocks et al. (2014). Passage time through Rivers Inlet is thought to be important, as during this time smolt mean weight may double (Buchanan 2006). Growth rates are very important during the seaward migration because larger individuals tend to have higher survival rates during the marine phase (McKinnell et al. 2001). The pilot study of Stocks et al. (2014) was described in Chapter 2.

3.1.1. Goals for Chapter 3

Previous studies and our findings from Section 2.3 indicated that some of Owikeno sockeye smolts spend several weeks in Rivers Inlet, while others move through the inlet rapidly. According to Wood et

al. (1993), eddies may act as smolt holding places. Although the occurrence of such features has not been examined in Rivers Inlet, heterogeneity of habitat may be associated with the complexity of connecting channels, river inflows, bays, wetlands and so on. Anecdotal evidence suggests a tidally driven eddy may persist at the confluence of Darby Channel with the main inlet. Other examples of holding points could include Kilbella Bay, influenced by a high productivity wetland where the majority of smolts were caught in 2010 (Ajmani 2011), and the Wannock outflow, the major sockeye entry point to the inlet, where fish may hold to adjust to the salt water in a shallow and productive habitat. The latter is supported by visual observations during the Rivers Inlet Ecosystem Study and has been observed in other systems (Pavlov et al. 2015). Such locations may be important for smolt physiological adjustment and growth.

Previous estimates of average fish residence time in the inlet were based on active continuous migrations, and thus if fish were exhibiting holding patterns in the system, the estimated migration duration would have been underestimated (Wood et al. 1993). Our calculations suggest that if sockeye smolts in Rivers Inlet were swimming continuously, their passage time would be on the order of 6-7 days, assuming a smolt length of 100 mm and a migration speed of 1 BL/s. However, according to the results of the pilot study by Stocks et al. (2014), smolt may spend up to several weeks in the Inlet. In this study, the duration of outmigration varied from 2 to 4 weeks depending on the year (Chapter 2). This implies that either smolt swim at rates significantly slower than 1 BL/s, or they hold for an extended period in some areas.

Two environmental factors will be considered (water discharge and temporal food availability) in this chapter to explore their potential influence on the duration of smolt outmigration dynamics. These parameters have been chosen based on previous research in Rivers Inlet where water discharge and temporal food availability were proposed to be important factors for smolt survival (Buchanan 2006, Tommasi 2008, Ajmani 2011, Hodal 2011, Tommasi et al. 2013a).

Aims and associated predictions of Chapter 3 are:

3.1. Investigate spatial-temporal pattern of the sockeye smolt migration in 2009 (choice of year is discussed in Section 3.2.1.).

Prediction 2.1: Sockeye smolt migration rates vary spatially and temporally, with fish dynamically using "holding points," influenced by seasonal changes in surface current speeds.

3.2. Determine whether migration dynamics are controlled by zooplankton densities and/or water discharge.

Prediction 2.2: Freshwater discharge and zooplankton abundance are strongly correlated with the timing and rate of outmigration of sockeye salmon in River Inlet in 2009.

3.2. Methods

3.2.1. Choice of Otoliths and Sites

According to the stated predictions, samples were selected to provide spatial coverage of different locations throughout the inlet and temporal coverage of different migration times. Samples were selected from 2009 as a large number of specimens were available from that year, with good temporal coverage of the migration period across sites. Ralph, Scandinavia, Geetla, and Dimsey samples were excluded from the analysis to increase the sample size analyzed at other sites (due to the sample limittaion, more samples can be analyzed at fewer sites) and to avoid potential mixing of Rivers Inlet smolts with fish from other systems. Samples from the Bosquet sites were assumed to be representative of fish that had migrated through Darby Channel, and samples from the Mouth site were assumed to be representative of fish that had migrated through the Main Channel. To understand the spatial and temporal patterns of outmigration of sockeye smolts, time since the marine entry was determined at eight stations along the

length of the inlet (sites 1- 4, 8–10 and 14 on Figure 24). Otolith collection, extraction and storage procedures were identical to those mentioned in Chapter 2.
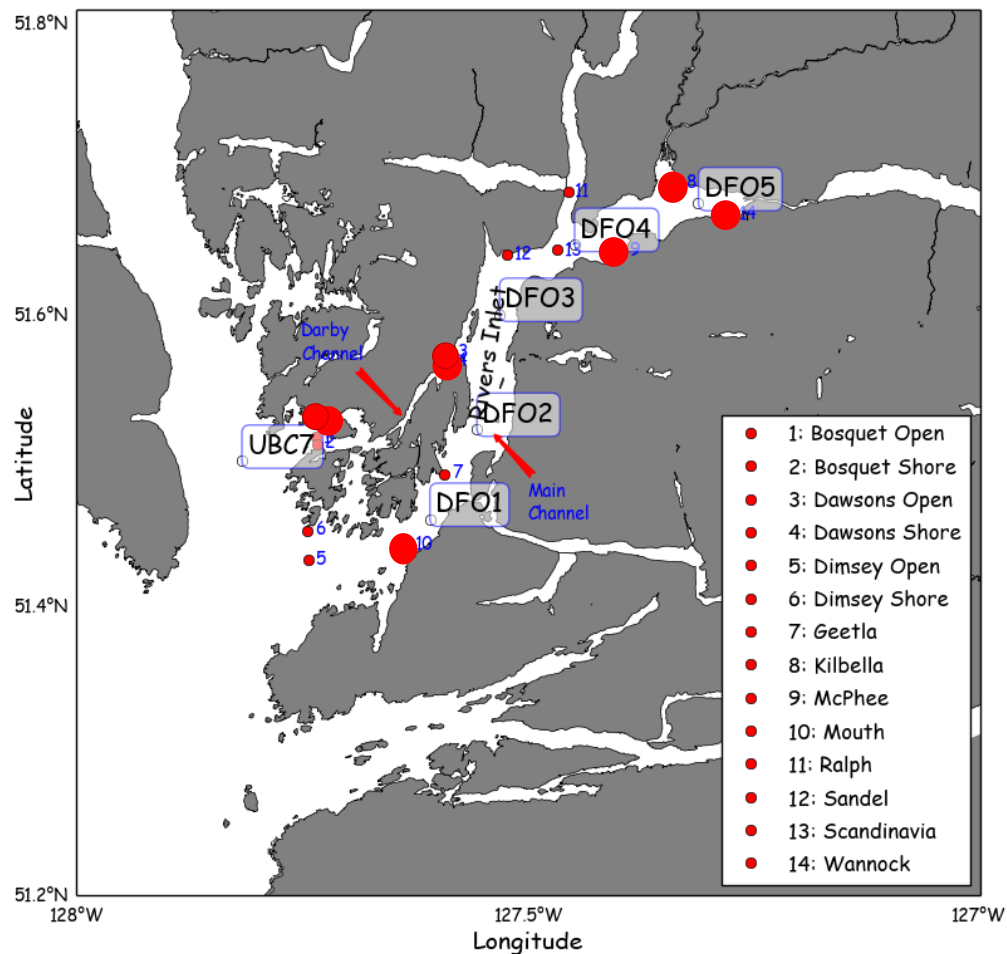
Figure 24: Map of Rivers Inlet showing the location of 14 seining stations (red dots). Arrows indicate two proposed migration routes from the Inlet. Small red circles show the locations where smolts were caught, the bigger red circle shows the sites used for analysis in Chapter 3. The text in the rectangles represents the location of 6 oceanographic stations where chlorophyll a and zooplankton samples were collected.

3.2.2.   Environmental Data

Wannock River freshwater discharge between April and September from 2008-2011 was obtained from HYDAT data archived by Water Survey of Canada (www.ec.gc.ca/rhc-wsc/). Phytoplankton and zooplankton data were collected fortnightly during the RIES program, between March and August 2008-2010. Water samples were collected with Niskin bottles at 0, 5 and 15 m depth.

Chlorophyll a (Chl a, mg/m^2) was measured by filtering 60– 300 ml through a Whatmann GF/F filter. Pigments were extracted in 90% acetone for at least 24 hours at ~ 20 ºC (Strickland and Parsons 1972). Zooplankton were collected using a bongo net equipped with 150 μm mesh which was deployed to a maximum depth of 300 m (or to 10 m above the bottom). Samples were preserved with a buffered 5% formalin-seawater solution. In the lab, zooplankton was identified and counted, and density was expressed as individuals/m^2. For the purpose of Chapter 3, each seining station was matched with the nearest oceanographic station (Table 7), as the zooplankton and phytoplankton samples were not necessarily taken at the location where fish were caught (Figure 24).

Table 7: Matching of the seining stations with corresponding chlorophyll and zooplankton stations

Chlorophyll and zooplankton station	Seining station
UBC 7	Bosquet Open & Shore
DFO1	Mouth
DFO2	Dawsons Open & Shore
DFO3	---
DFO4	McPhee
DFO5	Wannock, Kilbella

No oceanographic station was in close proximity to the Dawsons sites, and so DFO 2 in the Main Channel was used.

3.2.3.   Microchemical Analysis on LA-ICP/MS.

Sample preparation and LA-ICP/MS ablation procedures were the same as described in Chapter 2. While the Sr:Ca signal was the clearest indicator of freshwater vs. marine phases, it was sometimes problematic at the early stages of migration (first few days) and requires additional validation. For those samples where the breakpoint could be determined, the duration of stay in the Inlet was calculated using the average width of daily rings in the marine zone of the otolith (marine radius). Knowing the number of days that each smolt spent in Rivers Inlet as well as the date when the smolts were caught, the entry day into the Inlet was back-calculated. In addition, chemical profiles in rejected samples were

investigated for a potential explanation for why the determination of the marine entry point was not possible for a large number of samples (see Appendix B).

3.2.4.   Spatial Variation of the Outmigration Rate throughout Rivers Inlet.

To investigate spatial differences in outmigration rate, the duration of the outmigration in Rivers Inlet was determined for each of the 8 sites (Table 1), using the same methods as in Chapter 1. Using two methods for the determination of the size at entry (see Chapter 2, Section 2.2.6), the initial size of entry to the inlet was back-calculated for all 8 sites. Size gained during the outmigration was calculated as the difference between the size at catch and the size at entry to the marine environment. To eliminate the influence of the distance traveled, the size gained was calculated per 1 km of travel. To eliminate the influence of size on fish traveling rate, the outmigration speed was calculated in BL/s.

3.2.5.   Investigation of the Daily Growth Rates

An additional exploration was done on 3 smolts (Table 8) from 2009 where daily otolith rings were seen all the way from the edge of the otolith to the break point (marine entry), to explore how the growth rates varied with distance from the head of the inlet. The ablation paths in these otoliths were located in the same area on the otolith to ensure that the width of the rings was not influenced by the fact that the width of the same ring is different depending on where on the otolith it is located. The widths of the rings were measured with the ImageJ program. The average width of an otolith ring was an estimation of the daily growth rate of the fish. The changes in daily growth with distance were compared, assuming that the fish moved at a constant average speed. Using the available information about the width of the rings, the initial size at entry was back-calculated and using the length-weight relationship the weight at entry was determined.

Table 8: Summary information for the three smolt for which each of the daily growth rings was measured: day and location when the fish was caught, size (mm) and weight (g) at catch, the duration (days) and average speed (BL/s) of the outmigration and condition factor (CF).

Sample ID	Date caught	Site	Size, mm	Weight, g	Days in the Inlet	BL/s	CF
BO2009_01_8	29 June 2009	Bosquet Shore	84	8.10	6.9	0.72	1.37
BO2009_03_8	15 June 2009	Bosquet Shore	79.5	5.65	26.5	0.21	1.13
BO2009_03_2	16 June 2009	Bosquet Open	72	4.11	6.0	0.97	1.10

3.2.6. Influence of Environmental Factors.

As water discharge was found to be one of the major factors influencing the residence time of the smolt (Martin et al. 2004, Thorstad et al. 2008, Mantua et al. 2010), the cumulative water discharge during outmigration was compared to the duration of outmigration depending on the day of entry. In addition, since fish may experience very low discharge rates during certain periods and only a few days of very strong discharge, the average daily discharge (mean and geometric mean) was also calculated and correlated to the duration of the outmigration through Rivers Inlet.

Smolts spent different amounts of time traveling through Rivers Inlet. Each individual smolt, therefore, experienced different water discharge levels. In order to explore the effect of this, the cumulative and mean water discharge experienced by each smolt was plotted against the total days the smolt was in the inlet. To assess the differences in productivity across sites, zooplankton and phytoplankton distributions were compared.

3.2.7. Rejected Samples

Quite a large number of samples (12 out of 15) at the Wannock site were rejected from the analysis for the second part of the study (Chapter 3) due to inability to determine the break point by LA-ICP/MS. In the rejected samples, Sr:Ca profiles had an approximate V- shape, with the concentration being high at the start of the ablation, at ~ 10000 ppm, then dropping sharply at 90 μm to ~6500 ppm before returning to previous levels (Figure 56). Ba:Ca profile showed a small rise from 16 to 34 ppm. Because of this unusual behavior of the Sr:Ca signal, the Sr:Ba ratio decreased at the end of the ablation path instead of

increasing as expected. Mg:Ca profile showed a dramatic rise in concentration from 40 to 200 ppm (see Appendix B for a discussion).

3.2.8. Statistical Analysis of Data

ANOVA analysis was applied to test the significance of differences in the length of stay in different sections of the inlet. Simple regression analysis was used to examine the relationship between the size at entry and the day of entry to the inlet. ANCOVA analysis was used to explore the effect of water discharge (seasonal, cumulative and mean) and year on the duration of the outmigration.

3.3. Results

3.3.1. Spatial Variability

For all the sites, with the exception of Wannock and Kilbella, the migration rates got faster through the outmigration season. On average, fish spent 2-3 weeks at the Wannock - Kilbella region of the inlet (1 and 4.7 km from the head) until late June. Note that only one fish was available from the last period of the outmigration for these sites (Wannock and Kilbella) and may have been caught right after it exited inlet. In addition, smolts seemed to gain almost no size during their prolonged stay at the inlet's head, with the exception of later migration fish from June (discussed further in this section). Smolts that entered the inlet in April were only caught in 3 locations: Dawsons Open and Shore, and Mouth. Fish that entered the inlet in April spent the most time in the inlet and were also the smallest juveniles entering the inlet. The Mouth (not shown in the figure below) had only 4 fish available, and size at entry was not available for April and early May.
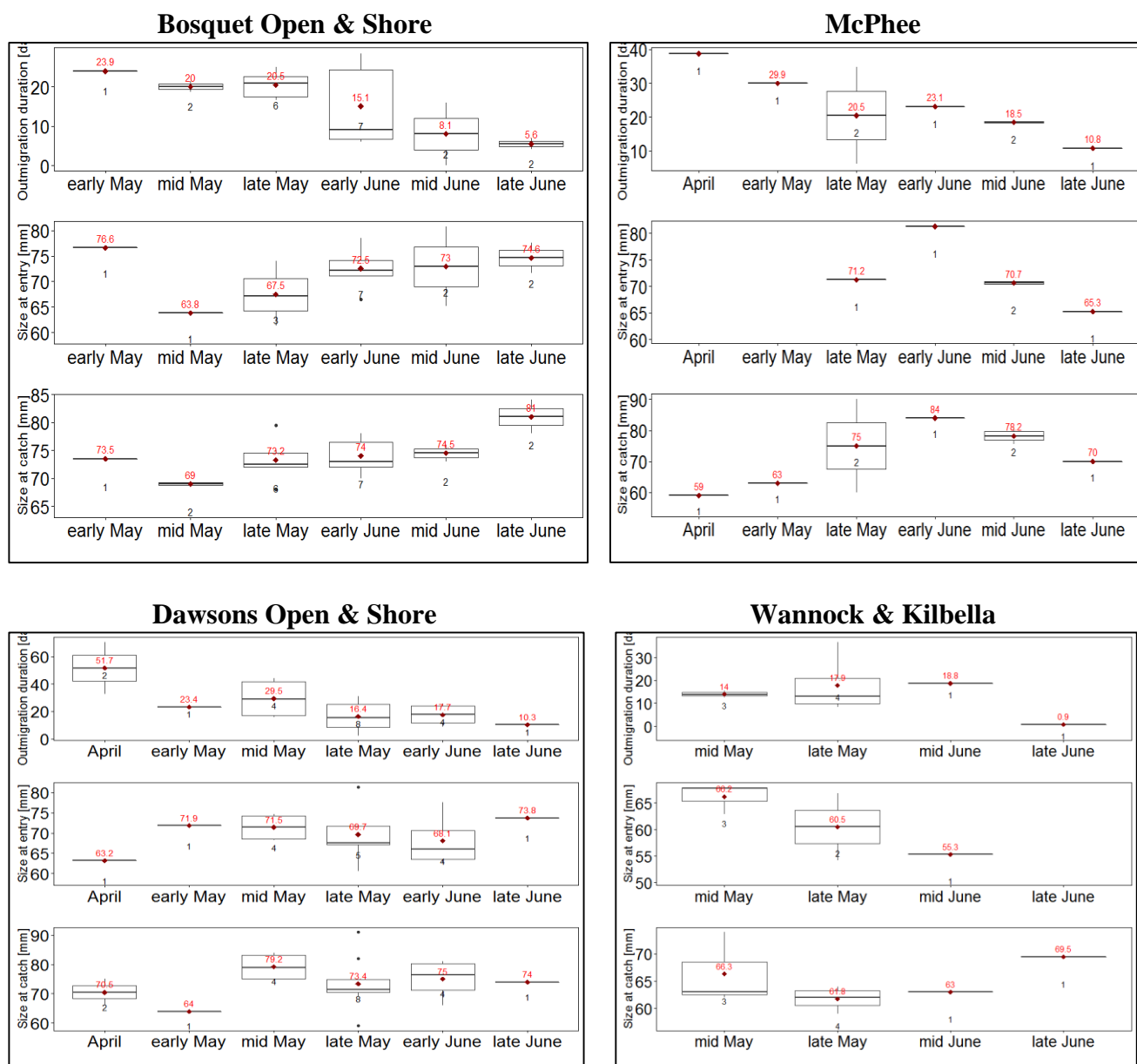
Figure 25: Number of days spent in the Inlet (outmigration duration in days), size at entry and catch (mm) at 7 locations throughout Rivers Inlet. Median values (black solid horizontal line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Black dots are the outliers. Red dots and numbers are medians, and the black numbers are the sample sizes.

In mid-May, assuming all the fish spent ~ 14 days in the Wannock-Kilbella region, some fish traveled to Bosquet in a matter of 10 days. Smolts that were caught at McPhee may have spent ~ 6 days in the region between McPhee and Kilbella. It took ~ two weeks for smolts to reach the Dawsons site after spending 2 weeks at the head of the inlet. It was equally possible that smolts swam to Dawsons in a matter of days and held at that location. After mid-May, the migration through the mid part of the

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inlet was more rapid. In late May, smolts still spent 2-3 weeks at the head but swam through the rest of the inlet in ~3 days. Mid-June fish spent almost 3 weeks in Wannock-Kilbella region. Fish caught at McPhee were also in the inlet for 18 days, while smolts that were caught at Bosquet were in the inlet for 8 days. Hence these smolts potentially did not stay at the head of the inlet but swam continuously through the inlet. The calculated travel speed for these smolts were ~ 0.7 BL/s, very close to speed of 1 BL/s.

Back-calculated sizes at entry were not constant and ranged from 54 to 83 mm in 2009 with a mean value of 70 mm. The available catch data from that year (Table 3) had smolts at Wannock ranging in size from 51.5 – 68 mm. No significant correlation was found between the day of entry and the initial size at the start of the outmigration through the Inlet ($R^2 = 0.04$; $p > 0.05$).

Due to the limited number of samples available for each outmigration period for each location, it was decided to group the samples into 3 outmigration periods: April, May and June. Only two samples were available for April 2009, so no correlation could be seen for this outmigration period (not shown in the figures below). However, for each of the outmigration periods, there was a weak correlation (R^2 of 0.1 - 0.2) between the size at entry and the distance from the inlet head (not significantly different between periods, ANCOVA; $p > 0.05$). There was no significant interaction between the distance from the head and size at entry. Size at entry did not have a significant effect on outmigration period, demonstrated by no significant difference in 'intercepts' between the regression lines of the May and June outmigration periods (ANCOVA; $p > 0.05$). It appeared though that larger smolts were caught further down the inlet compared to their smaller counterparts.

size at catch [mm] — 60 ● 70 ■ 80 ■ 90

$y = 66.2 + 0.177 \cdot x, \ R^2 = 0.0984$

$y = 64.2 + 0.204 \cdot x, \ R^2 = 0.168$
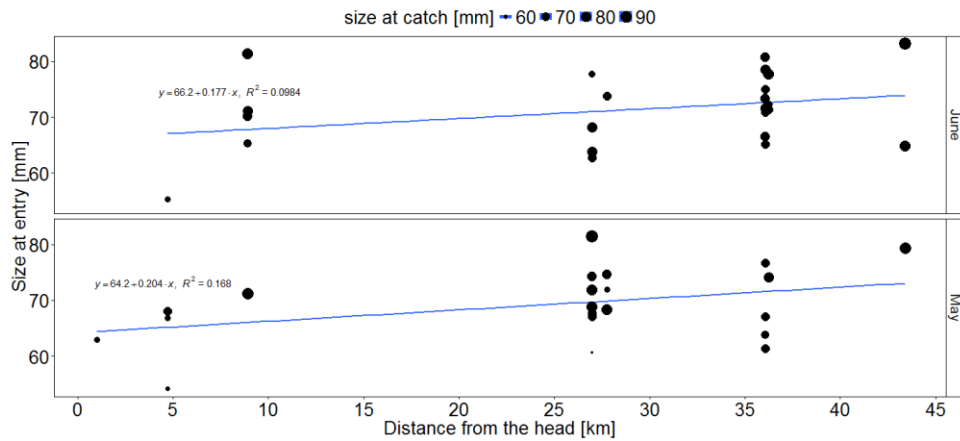
Figure 26: The relationship between the smolt size at the head of the inlet and the distance from the head in 2009. The blue line is the fitted linear regression line. The size of the circle is proportional to the size at catch.

Reconstructed growth (size gain between the day of entry and catch) at the head of the inlet (Wannock-Kilbella) in May was 0.1-0.8 mm, while in June in the Kilbella region it reached 7.7 mm. The largest growth, 19 mm (only one observation), was observed at McPhee in May. Growth at the Mouth site in the Main Channel was estimated at 8 and 13 mm in May and June, respectively, while in Darby Channel growth was 7.5 and 5.2 mm at the shore and 2.7 and 0.2 mm in the open in May and June, respectively.
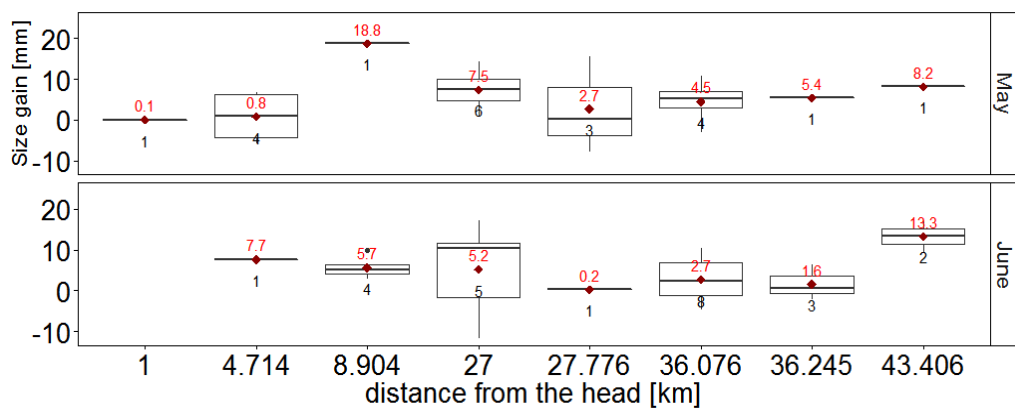
Figure 27: Size gained at each chosen section of Rivers Inlet. Distance from the head of the inlet corresponds to the following sites: 1 km–Wannock, 4.7 km– Kilbella, 9 km – McPhee, 27 and 27.8 km Dawson Shore and Open, 36 and 36.3 km Bosquet Shore and Open and 43.3 km is the Mouth sites. Median values (black solid horizontal line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Black dots are the outliers. Red dots and numbers represent the medians, and the black numbers are the sample sizes.

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At the head of the inlet (up to 9 km from the Wannock River), smolt speed ranged from 0.01-0.1 BL/s in May and June, except the Kilbella region in June where the mean speed was 0.5 BL/s (Figure 28). Beyond the inlet head region, swimming speed increased on average 0.3 to 0.6 BL/s at Bosquet, in May and June, respectively. Calculated average speed of active migration at the mouth of Darby Channel, was 0.9 (May) and 1.6 BL/s (June), while it only reached 0.1 (May) and 0.4 BL/s (June) at the Mouth site of the Main Channel.
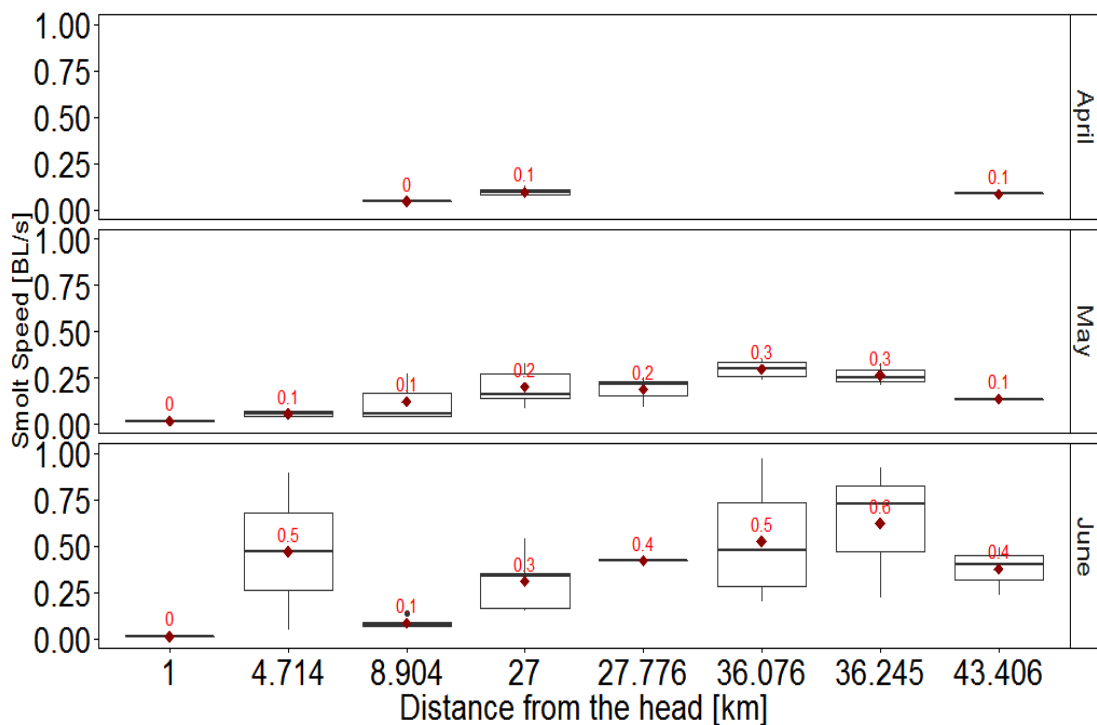
Figure 28: Average migration speed in body lengths per second (BL/s) at eight stations along the length of Rivers Inlet. Distance from the head of the inlet corresponds to the following sites: 1 km – Wannock, 4.7 km – Kilbella, 9 km – McPhee, 27 and 27.8 km - Dawson Shore and Open, 36 and 36.3 km - Bosquet Shore and Open, and 43.3 km - Mouth. Median values (black solid horizontal line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Black dots are the outliers. Red dots and number represent the median values, and black numbers are the sample sizes.

3.3.2. Daily Growth Rates

Two samples, BO09_01_8 and BO09_03_2, spent the same amount of time in the inlet (~ 1 week) yet had significantly different growth rates (Figure 29, Figure 30). BO09_01_8 started migration in late June and had otolith growth rates between 4.5 and 8 µm/day, while BO09_03_2 that migrated in mid-

June had otolith growth rates between 2 and 3 µm/day. The BO09_03_8 smolts spent almost a month in the inlet and had the largest variability in growth rate among the three samples, but generally had high growth rates in late May (up to 7 µm/day), low growth rates in early and mid-June (2-4 µm/day) with the exception of a high growth day on 13 June.
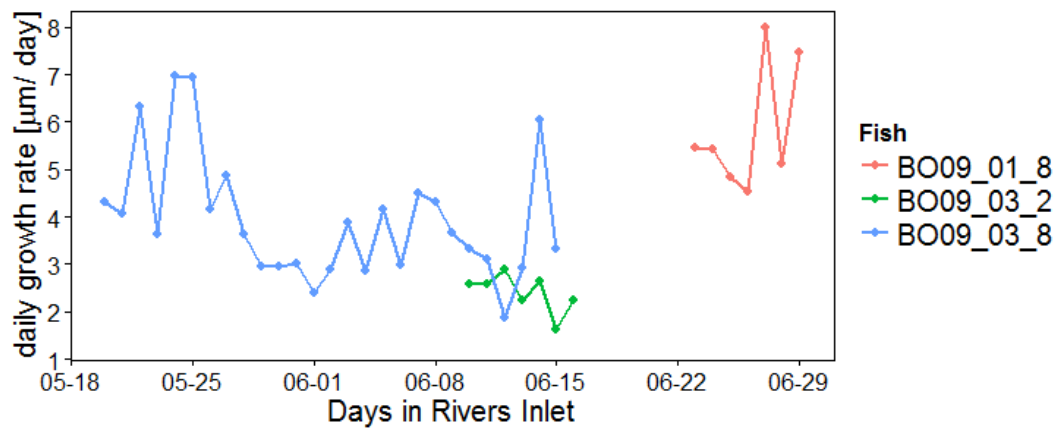
Figure 29: Change in daily otolith growth (µm) during the outmigration for three fish: BO09_01_8, BO09_03_2 and BO09_03_8.
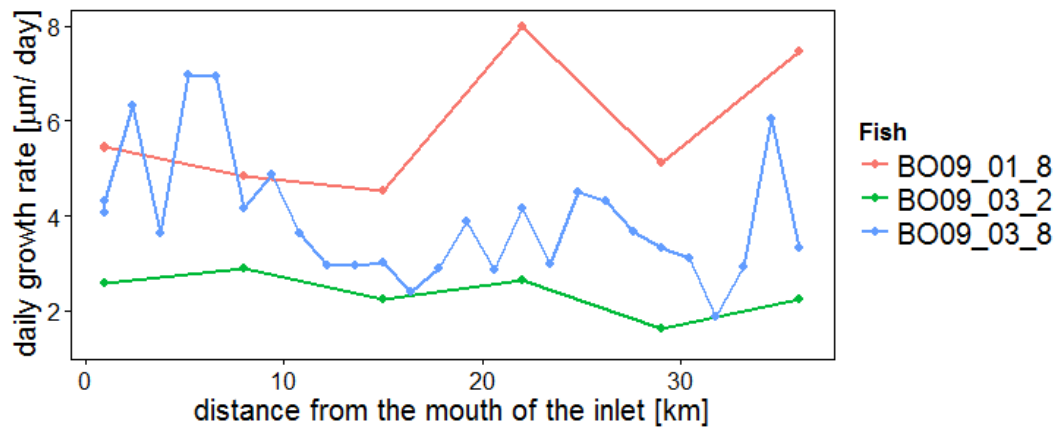
Figure 30: Change in daily growth rates of otolith with the distance from the mouth of the inlet for three fish samples BO09_01_8, BO09_03_2 and BO09_03_8.

The BO09_01_8 smolt had the largest size and weight (84 mm and 8.10 g respectively), despite spending the same number of days in the inlet as BO09_03_2 smolt (72 mm, 4.11 g), and three weeks less than BO09_03_8 (79.5 mm and 5.65 g). Using the actual ring counts, it was possible to reconstruct

the exact length of the otolith at entry and the size and weight of the smolt at the beginning of the outmigration. Each fish entered the inlet having a similar condition factor of ~1.07-1.08. Nevertheless, while the two smallest smolts, BO09_03_2 and BO09_03_8, were very similar in length and weight (72-73 mm, 4.0-4.3 g), the largest smolt (BO09_01_8) was already ~77 mm long and weighed ~5 g upon leaving Owikeno Lake (Table 9).

Table 9: Summary information for the three smolts: size (mm) and weight (g) at entry, condition factor at entry (CF) and increase in size (mm) and weight)g) during the outmigration.

Sample ID	CF at entry	Size entry, mm	Size gained, mm	Weight at entry, g	Weight gained, g
BO2009_01_8	1.08	76.9	7.1	4.93	3.17
BO2009_03_8	1.07	72.8	7.0	4.08	1.56
BO2009_03_2	1.08	73.8	-1.8	4.34	-0.22

3.3.3. Environmental Factors
Wannock River Discharge

Freshwater discharge from the Wannock River varied substantially between years (Figure 31). Overall, discharge peaked in the mid-summer, indicative of the seasonal freshet associated with snowpack and glacial melt. In 2008 the strongest measured discharge occurred in mid-May, early July and late August, with peaks exceeding 8000 m^3/s. In July, discharge increased from ~ 100 m^3/s to 1000 m^3/s in one week. The freshet in 2009 was weaker, with an increase in discharge occurring in late April, peaking at ~600 m^3/s then dropping to ~200 m^3/s. A second increase in discharge was more dramatic, occurring in early June (> 750 m^3/s), and remaining at this level until 22 June before decreasing to < 500 m^3/s. Both 2010 and 2011 showed similar patterns in river discharge, with flow increasing gradually throughout the summer months. The timing and magnitude of the 2010 freshet were similar to those in 2009. A small discharge peak occurred in mid-April 2010, with discharge rates not exceeding 300 m^3/s. The highest (> 1000 m^3/s) water discharge in 2011 was observed in late August, nearly mimicking late fall discharge increase of 2008 (Figure 31).
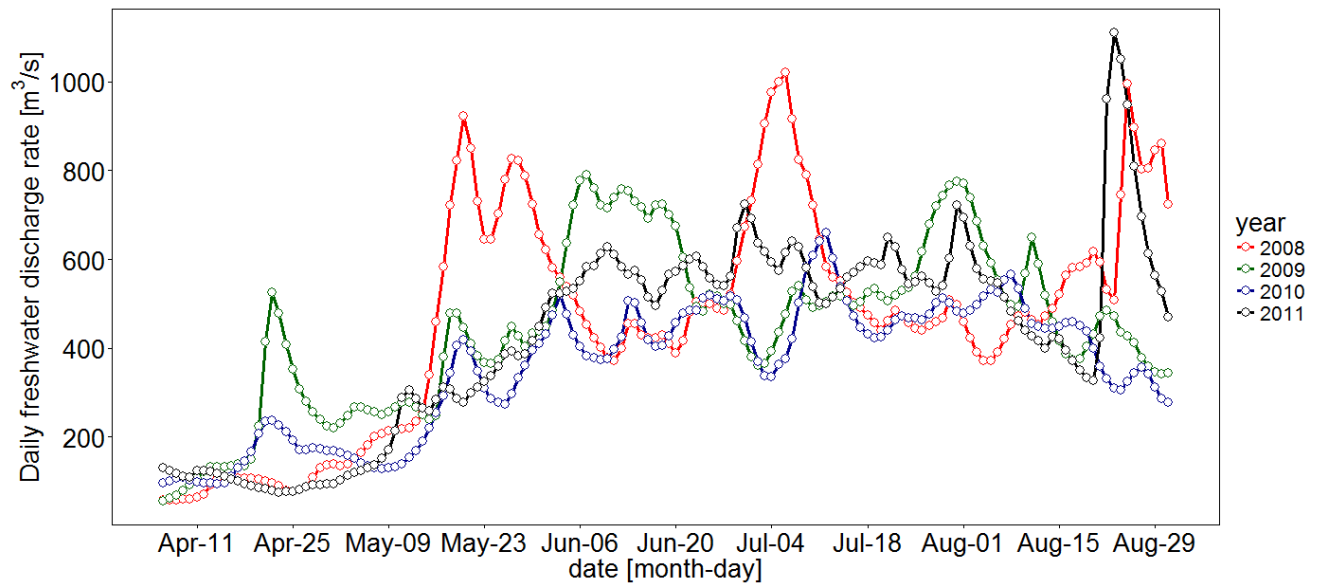
Figure 31: Wannock River freshwater discharge from April to August of 2008 to 2011.

A high correlation was found between the cumulative freshwater discharge from the Wannock River and the duration of the outmigration through Rivers Inlet (Figure 32, $R^2 = 0.75$). A significant effect of cumulative water discharge and year (ANCOVA; $p \ll 0.01$) was detected for the duration of outmigration. In addition, a significant interaction between water discharge and year was found ($p \ll 0.01$), meaning that the slopes for each year differed among years. The correlation between the geometric mean of the water discharge and the duration of the outmigration was $R^2 = 0.39$, while the correlation and between mean water discharge and the duration of the outmigration was $R^2 = 0.29$ (Figure 32). Both relationships showed a significant effect of daily discharge (mean or geometric mean) and year (ANCOVA; $p \ll 0.01$) on the duration of outmigration, but no significant interaction between them (ANCOVA; $p > 0.5$).
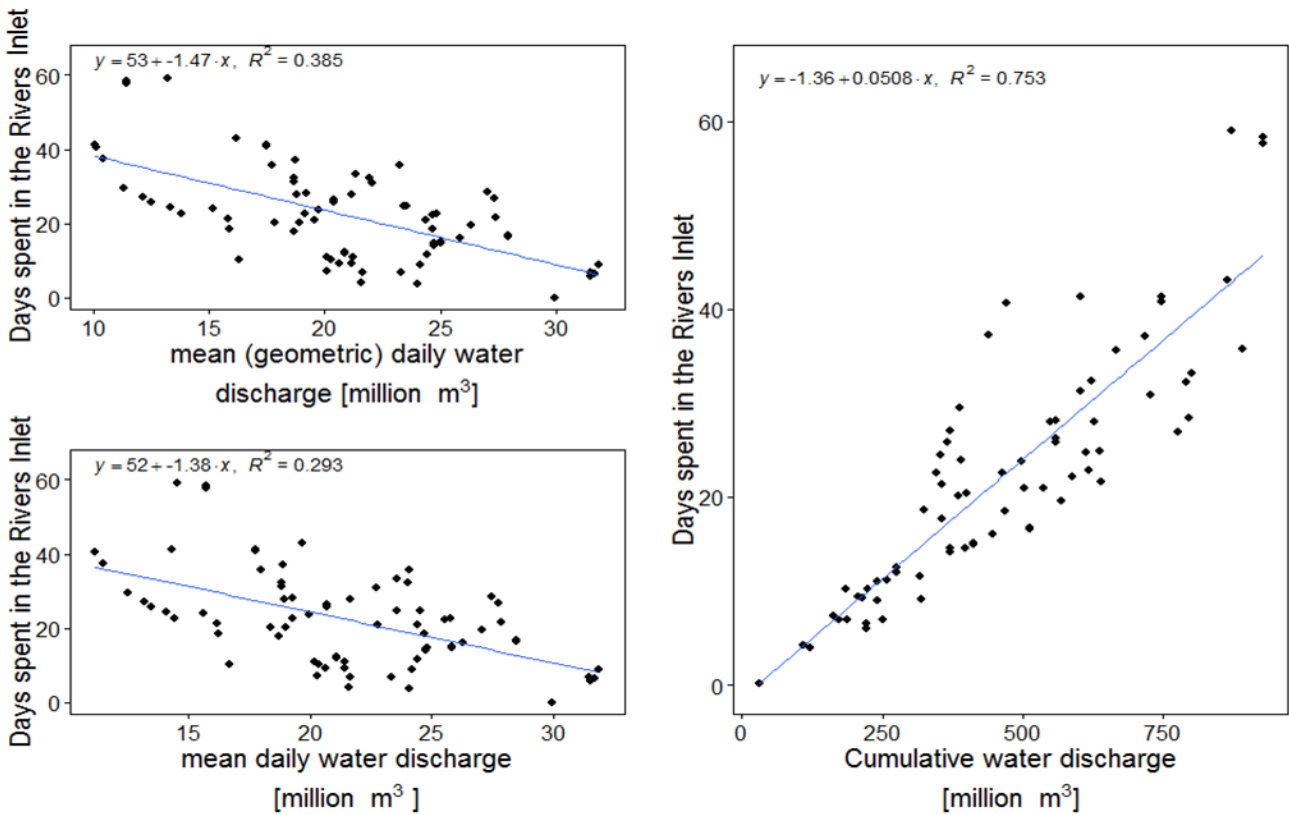
Figure 32: The relationship between days spent in the inlet and the cumulative freshwater discharge during the outmigration period for each smolt. The blue line is the fitted linear regression line.

Zooplankton and Phytoplankton Data

Zooplankton and phytoplankton standing stock and seasonal distribution varied across sites. At DFO5 (Wannock and Kilbella sites; Figure 33) phytoplankton biomass peaked in mid to late April, and zooplankton densities peaked in early May, at ~ 200 mg/m^2 and 9 x10^5 individuals/m^2 respectively.

Further down the inlet at DFO4 (McPhee site), phytoplankton peaked in mid-April reaching 60 mg/m^2. Two zooplankton peaks were recorded in late March and mid-May and peaked at about ~6 x10^5 individuals/m^2. This location was the least productive through the entire inlet.

At DFO2 (Dawsons), phytoplankton biomass peaked in mid-May with a maximum concentration of 200 mg/m^2. Zooplankton densities were rather high throughout the entire spring-summer season (6 - 8 x10^5 individuals/m^2), with a peak in early June. At DFO1 (Mouth), phytoplankton biomass was

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extremely low (peak was at 35 mg/m^2), while zooplankton densities were rather high, reaching ~8 x10^5 individuals/m^2 at two peaks, in early April and late May.

In Darby Channel, only one station was sampled (UBC7), close to the Bosquet Open and Shore seining stations. At UBC 7 phytoplankton biomass peaked (120 mg/m^2) in mid to late April while zooplankton peaked (~ 6 x10^5 individuals/m^2) in early May.
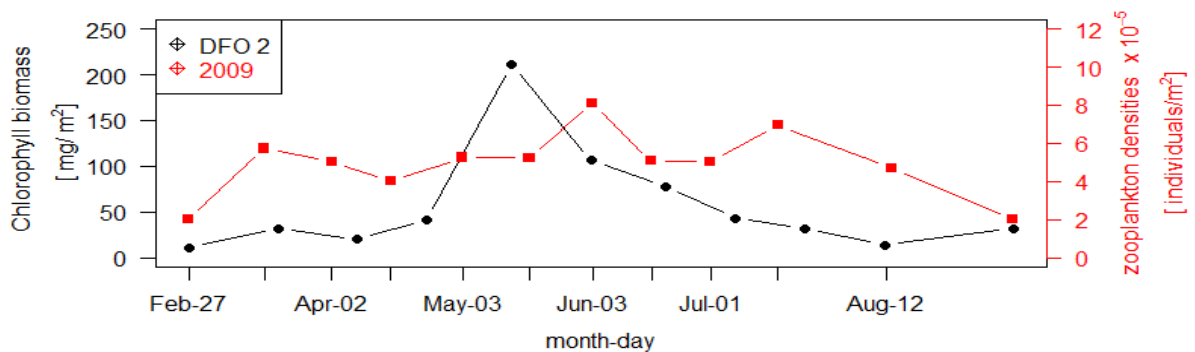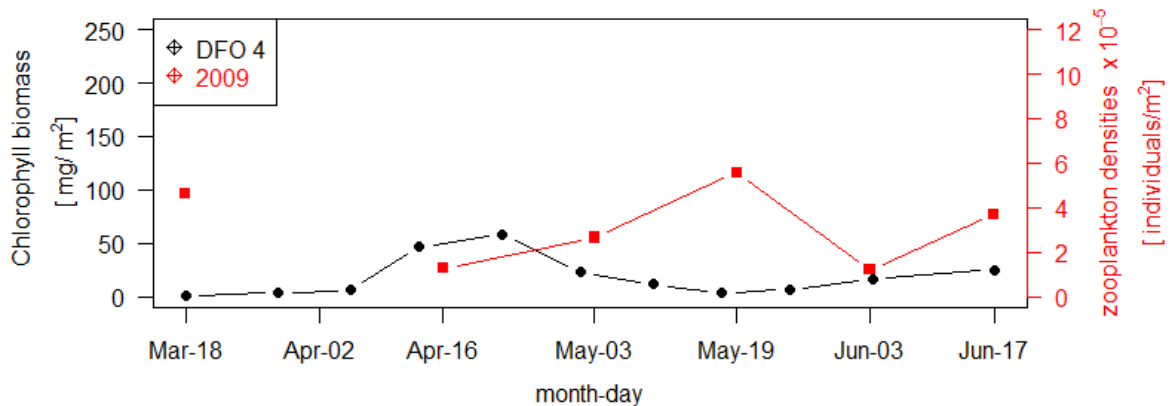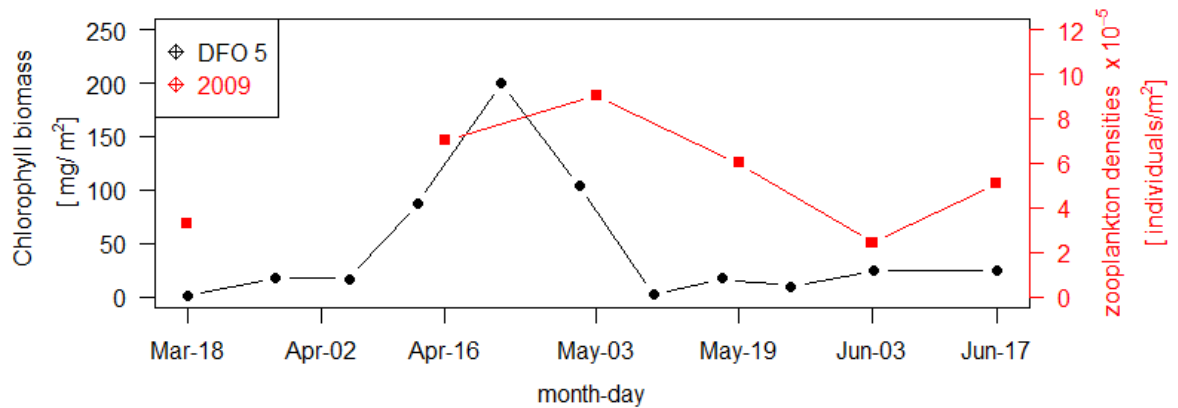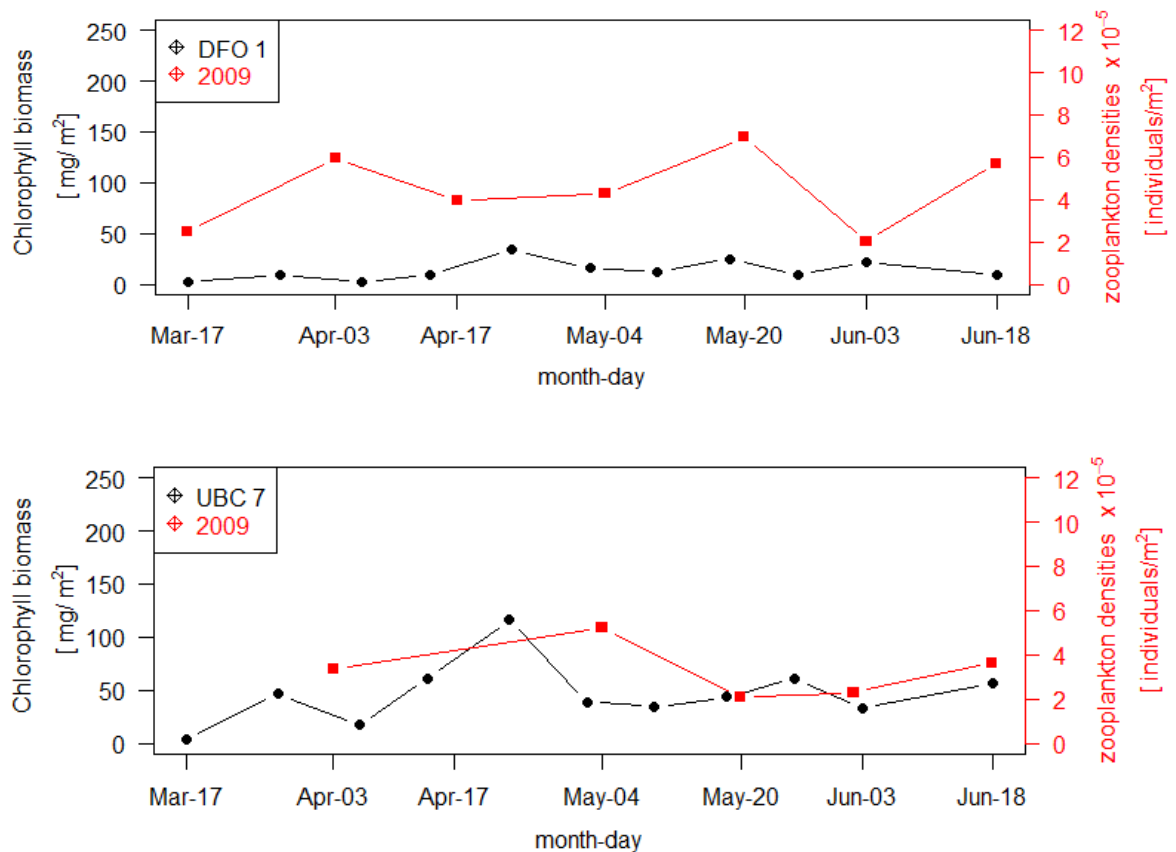
Figure 33: Change in phytoplankton biomass (mg/m²; black line) and zooplankton densities (individuals/m²; red line) and throughout the outmigration season.

3.4. Discussion

3.4.1. Spatial Variability in the Growth Rates and the Timing of the Outmigration

In Chapter 2, the estimated seasonal average migration speed of sockeye smolt in 2009 was 0.5 BL/s, which did not take into account differential swimming speed throughout the inlet or the day of entry. Comparison of outmigration duration across 8 different locations throughout Rivers Inlet (6 if the two Dawson sites and Bosquet sites are combined) demonstrated large seasonal differences in the migration patterns within the adjacent outmigration paths. Overall, throughout the season the residence time of smolts decreased in areas beyond the inlets head. While fish consistently (with some exceptions) spent 2-3 weeks at the Wannock-Kilbella area, the residence time at McPhee decreased from 40 to 10 days, at Dawsons from 51 to 10 days, and at Bosquet from 24 to 6 days from Apr-May to June, respectively. Different behaviors were observed for smaller and larger smolts. Smaller

individuals tended to migrate slower through the inlet compared to the larger fish that were able to reach the inlet's mouth while smaller smolts, although entering the inlet at the same time, were still near the head. The slower average travelling rate can be thus explained by both the fish size and the holding behavior of the smaller smolts at the head of the inlet. Indeed, not all smolts spend up to 3 weeks at the head and with some smolts traveled through the inlet in a matter of days. It is possible that towards the end of the outmigration (late June) the smolt holding time at the head could have been reduced dramatically and overall inlet's residence time decreased to ~ 10 days.

The above findings clearly show that outmigrating fish do spend variable amounts of time in different areas of the inlet, similar to previous observations by Kocik et al. (2009). Our findings showed that fish moved up to 9 km away from the Wannock River with a median travel speed of 0.01-0.1 BL/s, potentially using this time to adjust to the salt water (Thorstad et al. 2007). The calculated migration speeds at the mouth of Darby and the Main Channels included the holding time at the head of the inlet. If, however, the Wannock-Kilbella holding period is excluded from mean speed calculations, the actual traveling speeds were 0.2-0.6 and 0.9-1.6 BL/s during May-June in Main and Darby Channels, respectively. The calculated speed in the Main Channel was close to theoretically determined values (Welch et al. 2011, Martins et al. 2012), while the speed in Darby Channel was higher than in the Main Channel. The speed in Darby Channel in June appeared to be quite high suggesting fast passage due to currents, which could be further facilitated by juveniles actively swimming with the current. However, if, as suggested above, holding time at the head is reduced, the overall travel rate through the inlet should be slower.

It is very important to acknowledge that calculated weights and sizes at entry (and as a result travel rates) may be an overestimates of the actual values. Our data were obtained from the survivors and does not take into account juveniles that died earlier on their route. Smaller fish could be a subject to higher predation rate or smaller individuals might also be the fish with reduced growth due to either

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genetic make-up or being physiologically challenged. As a result, only healthier or fast growing fish was caught by the nets which may result in overestimated population calculated weights and sizes. However, based on the results of this study, due to the difference in the outmigration rate of the fish, the fish that were caught in any particular location were not necessarily representing the fish with similar behavior or characteristics. For example, smaller individuals that spend 2 weeks holding at the Wannock-Kilbella region could arrive at the mouth of the inlet 4 weeks after they start their outmigration through the inlet and thus can be found at the mouth together with larger individuals that traveled through the Rivers inlet over only 2-week period.

3.4.2. Influence of Sizes at Entry on Outmigration Process

When considering all years together, the size of the smolts at the head of the inlet varied between 51 and 80 cm. A larger range may have been observed in 2009 if sampling had been conducted in July, as in 2010 and 2011. This may account for the larger size range of smolts recorded in those years. Overall, calculated sizes at entry matched the available catch data at the head of the Inlet.

There is evidence to support fish entering the inlet at different sizes. Fish caught at Wannock were as small as 51.5 mm. However, two caught at the mouth of the inlet (Bosquet Open) were already 76 and 78 mm, despite having spent just 1 and 4 days in the inlet. Two smolts caught at Dawsons, were in the inlet for 2 days and had a size of ~72 mm. Furthermore, while fish caught at Wannock appeared to be small in 2009; smolts at Kilbella, after just spending a few days in the inlet, showed great variation in size.

No correlation was found between size at entry and the day smolts entered the inlet, suggesting that the initiation of the migration is not size dependent (see also Ibbotson et al. 2011). However, the majority of research has in fact showed that seaward migration timing is size dependent (Bohlin et al. 1996, Quinn 2005, Jutila and Jokikokko 2008). Two possible explanations for this discrepancy are 1) the result of mixing of different migrating groups and 2) rapid spring growth in the lake resulting in larger

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individuals beginning their migration later in the season (Dombrotski 1954, Burgner 1962, Foerster 1968). Nelson et al. (2003) surveyed 7 different spawning sites in Owikeno Lake and found that sockeye from Wannock and Amback Rivers gave some indication of being partially isolated from the other sites. This provides some evidence that different migration groups may co-exist. A limnological assessment of Owikeno Lake in 2001 showed that zooplankton biomass was higher in one of the lake's four basins, was highly seasonal, with peak biomass occurring in summer and with different timings between basins (Shortreed and Morton 2003). Since there was no relationship between the smolt size distribution and day of entry into the inlet, smolts' size may have been the result of the combined effects of spatial and seasonal variation in lake's productivity (Burgner 1987, Edmundson and Mazumder 2001, Rich, et al. 2009).

Although no correlation was found between the day of entry and smolt size, there was a weak correlation between smolt size at entry and the speed of outmigration, with larger juveniles traveling at a faster speed than the smaller ones. This difference probably comes from the fact that smaller individuals prefer to hold at the head of the inlet, while the larger ones may swim continuously through the inlet without a prolonged holding period.

The holding behavior of smaller individuals may be due to smolt 1) using the area to improve their osmoregulatory capability, adjust to the marine environment, and avoid predators (Chittenden et al. 2008); or 2) using this area for feeding and better growth opportunities (Healey 1980). The first factor, supported by observations by Chittenden et al. (2008), is plausible in Rivers Inlet. According to the "smolt window" concept, there is a range of certain conditions when smolt can enter and quickly adapt to the environment. In addition, chemical otolith analysis conducted in this study (Appendix A and B) suggested that the smoltification process for some juveniles was not yet completed upon entry to the inlet. The second factor, described by Healey (1980), appears to be not supported in Rivers Inlet system. In the beginning of the outmigration season (April-May), despite fish spending at least 2 weeks at the

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Wannock site, little increase in size was observed. This may have been due to fish having reduced feeding activity due to physiologically stress (or still undergoing the smoltification) or/and poor feeding conditions.

In addition, physiologically stressed smolts are more likely to have a weaker reaction to predators and as a result can be more frequently preyed upon (Handeland et al. 1996). In such situations, larger smolts that tend to move faster and exhibit reduced holding at the head had an obvious evolutionary advantage. Evidence for significant predation in Rivers Inlet is lacking. However, studies in nearby inlets (Parker 1968, 1971) and other fjord systems (Hansen et al. 2003) showed that losses due to predation over a 40-day period could reach 77% of the smolts entering the inlet. In Rivers Inlet, sockeye salmon were found in the stomachs of Lingcod and Pinnipeds (Watkinson and Pauly 1999). Of the potential predators, marine birds (Manuwal 1977, Simenstad et al. 1982, Okland et al. 2006) and mammals may be significant sources of predation mortality of juvenile sockeye (Simenstad et al. 1982). However, predation by piscivorous fishes is thought to be very low (Simenstad et al. 1982). Predation on juvenile sockeye salmon by of Pacific harbor seals (*Phoca vitulina richards*) and orca (*Orcinus orca*) is likely minimal (Simenstad et al. 1982). It is possible to speculate that the predator mortality of early migrating (often smaller smolts) is higher compared to larger smolts. Although the predation might be significant in the inlet, it could be reduced due to schooling behavior of the juveniles at the shallow habitats (such as Kilbella Bay), nocturnal migration, and presence of high turbidity waters.

However, later in the season (June), smolt growth rates were rather high at the Kilbella region. This change in growth conditions could have positively affected smolts that were holding in the Wannock-Kilbella region later in the season. The rest of the inlet showed the opposite trend with growth in May being higher than in June.

3.4.3. Daily Growth Rates

Average daily rings may be appropriate for estimating the duration of the migration, but do not provide information on the daily (or seasonal) growth pattern of smolt through the inlet. That is why for three individuals (all caught at Bosquet) all daily rings were measured. It is important to note that outmigration duration determined from the actual daily ring counts was within 1-day of the estimate using average width of the rings (a slightly larger error was observed in Appendix E), highlighting the efficacy of the latter approach. The daily growth rings varied greatly, and fish that resided in the inlet longer had a higher variability in daily growth rates (Figure 29). Out of three, BO09_03_8 smolt was in the inlet for 27 days, while BO09_01_8 and BO09_03_2 smolts spent just 6 days in the inlet. This may be attributed to behavioral differences, with BO09_03_8 likely utilizing a holding strategy while the others swam continuously through the inlet. BO09_03_8 entered the inlet in mid-May while the others entered the inlet in June. This may be evidence that timing of migration may be an important factor in establishing the growth rates of the fish.

Assuming a constant speed throughout the inlet, BO09_01_8 and BO09_03_2 smolts showed significantly different growth rates (Figure 30 and 31). The smolt that started migration in late June had a higher (nearly double) growth rate range than the one that migrated earlier (BO09_03_2). BO09_03_8 had the most variable otolith growth rates with two distinct areas of rapid growth: near the head (0–10 km) and close to the mouth of the inlet (32– 36 km). This difference may be linked to the food availability in the corresponding regions and the duration of the stay in that area and may point to the low growth potential in the middle part of the inlet.

It is thus possible that other environmental factors (river discharge and/or food availability) play an important role during the outmigration. Two smallest fish behaved strikingly differently. BO2009_03_8 smolt spent about 27 days in the inlet and increased in weight by 28%, while BO09_03_2 smolt resided in the inlet for 1 week and did not gain weight. The largest smolt, BO09_01_8, while also spending only

1 week in the inlet, increased in weight by ~40%. It is unfortunate that only three otoliths were available for this type of analysis but as preliminary as they may be, the findings indicate complex smolt behaviors as well as possible individual based smolt responses to variable conditions in the inlet. This definitely requires further research, but it was largely beyond the scope of this study.

3.4.4. Identification of Environmental Parameters That May Potentially Determine Sockeye Growth Rates/Migration Timing

An attempt was made to identify environmental parameters that may have determined the timing of the migration and its spatial patterns. Parameters investigated include freshwater discharge intensity and pattern, and primary and secondary productivity. According to the statistical model used by Furnell and Brett (1986), about 90% of sockeye natural mortality occurs during the first 4 months of the marine phase. West and Larkin (1986) established that there is a positive correlation between the size and mortality of juvenile salmon. The smaller individuals tend to have lower survival rates than bigger young fry. In addition, even if small and large smolts leave the lake at the same time, larger fish might be traveling seaward faster, as swimming performance increases with body length (Brett 1965).

Wannock Freshwater Discharge

The residence time of the freshwater surface layer in Rivers Inlet depends on the river discharge rate and can vary between 4 and 22 days (Hodal 2011). The significant correlation found between discharge and duration of outmigration points to this being a key factor in Rivers Inlet juvenile sockeye migration dynamics. In 2008 and 2009, the Wannock River discharge was highly variable with alternating periods of high/low discharge, while discharge in 2010 and 2011 was more stable. It is possible that high discharge in mid– May of 2008 caused early migrants to be advected through the inlet faster than those starting their migration in mid-June 2008. This agrees with the results from Stocks et al. (2012), as fish caught on 27 May 2008 spent 12-16 days in the inlet, while smolts caught on 9 June, spent 21– 31 days. In this study, temporally resolved outmigration growth in 2009 showed noticeably different patterns

before and after mid-May sampling, being longer before the freshet. It is also notable that at higher water flow there was less variation in the duration of migration. This may be an indication that the surface current speed was primarily responsible for the outmigration time. Overall, it is likely that variations in discharge accounted for a substantial proportion of the seasonal and interannual variation in outmigration duration.

The relationship between days spent in the inlet and mean water discharge was not as strong with only 29% variation explained. It was also confirmed that with an increase of mean daily water discharge rate the duration of the stay in the inlet decreases. Considering that fish may spend time at a low/fast discharge rate and before experiencing the opposite water conditions, the geometric mean of daily water discharge was calculated. The relationship improved slightly ($R^2 = 0.39$) and confirmed the trend of decreasing inlet residence time for fish with increasing daily water discharge. The change in cumulative river discharge each day is not the same for each day or year.

Phytoplankton and Zooplankton Abundance

The phytoplankton bloom that occurs in spring influences zooplankton dynamics both in terms of composition and quantity (Irigoien et al. 2004, Richardson 2004, El-Sabaawi et al. 2009). A mismatch between the timing of the spring bloom and zooplankton life cycles would be expected to alter zooplankton community development and in turn influence smolt survival (Tommasi 2008, Tommasi et al. 2013b). In Rivers Inlet, the spring bloom timing depends on several factors, including wind speed and direction, freshwater flux and cloud coverage, with stronger, outflow winds leading to delayed phytoplankton blooms (Wolfe 2010).

In 2009 zooplankton densities were highest in the Wannock-Kilbella region. These areas of high zooplankton densities were the areas where smolts spent most of their time. Smolts in the Wannock location showed the smallest increase in size, although the productivity of the area was high. This

potentially supports the hypothesis that smolts were using this area to adjust to the new environment rather than feeding. Data from the mid-upper inlet showed that zooplankton densities were relatively low throughout the outmigration season while densities in mid to lower Main and Darby Channels were slightly higher than upper-mid part.

In 2009, smolts that started the outmigration in April exhibiting the "holding" pattern with the least exposure to lower zooplankton densities in the mid- and mouth of the inlet from April to mid-May. However, although fish that started the outmigration in April were the smallest at entry throughout the whole season, their size at catch at the mouth of the inlet was comparable with the size at catch of later migrants. It is important to note that in 2009 the spring bloom occurred a month later compared to 2008 (Tommasi et al. 2013a). This may have been an important factor in the overall low mean size gain of salmon smolt in 2009 across the inlet. The size of smolt at the 2009 entry was the largest during the 2008-2011 period, while the size at catch (at the mouth of the inlet) was the lowest.

This chapter points to strong environmental effects on the duration of outmigration and size dynamics of sockeye smolts in Rivers Inlet. In Chapter 4 these effects will be explored further by using a modeling approach to link environmental dynamics with fish migration and growth.

3.4.5. Rejected Samples

The majority of rejected samples (due to an undefined breakpoint) in this study were from the Wannock Site. The absence of a clear break point may be due to several reasons. First, the rejected samples were from juveniles that entered the inlet just 3 days prior capture, and may not yet have developed strong otolith microchemistry changes. Second, this may have been affected by starvation associated with the adaptation phase to marine conditions and energy redirection towards supporting the physiological change. The physiological stress may have been a significant contributing factor to fish holding at the head of the inlet resulting in minimal growth. Thirdly, it is possible that the smoltification

process had not been completed in the lake, forcing smolts to stay in the freshest part of the inlet upon first entry. The additional information on Sr:Ca and Ba:Ca signal is discussed in Appendix B.

3.4.6. Chapter 3 Summary

Overall, throughout the outmigration season the smolts spent between 14-18 days at the head of the inlet in 2009, while the residence in other locations throughout the inlet decreased with the outmigration season. In general, the juveniles took 5 to 10 days to go through the mid portion of the inlet (McPhee to Dawsons) and ± 4 days to migrate from Dawsons to Bosquet. Some fish resided less than two weeks in the Inlet; these fish either spent less time at the head or continuously swam through the inlet. This was especially noticeable for the sites close to the mouth of the inlet in Darby Channel (Bosquet and Dawson's sites) at the end of the outmigration period. It is possible that fish captured at McPhee primarily migrated through the Main Channel rather than Darby Channel where the duration of outmigration was slower (one smolt was in the inlet for 65 days). Another possible explanation is that not all fish utilize the Wannock-Kilbella region, but rather swam through the inlet continuously. Migration duration at Dawson's in April-May was higher than at Bosquet indicating another potential holding point in Dawson's region.

Back-calculated smolt size at entry ranged from 54 to 83 mm in 2009, potentially implying variable (spatially and temporally) conditions in Owikeno Lake, influencing the size at entry. Although fish may spend quite a long period near the Wannock site, fish showed little size gain in this region. In contrast, at the Kilbella site, smolts grew significantly larger later in the season.

Analysis of the seasonal variation in daily growth rates through the 2009 outmigration suggested that smolt growth rates were higher for fish that started their outmigration in late June compared to their counterparts leaving the Wannock River in early June. This may have been related to higher zooplankton densities later in the outmigration period. It was also apparent that fish that started their migration sooner (before the freshet) had a longer migration duration. It would therefore appear that there is a tradeoff

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between time spent in the inlet and food availability, i.e., early entry and slow migration does not necessarily imply good growing conditions and "successful" inlet residence time. Exploring the influence of the environmental factors such as river discharge rates and plankton availability, cumulative freshwater discharge experienced by each fish explained 75% of the variation in the duration of the smolt outmigration. No evident trends were found between plankton and smolt dynamics, possibly influenced by the general mismatch between plankton sampling and seining locations. Nevertheless, our findings suggest that the fish may spend a longer time in the high productivity areas when early migrants tend to hold at the head of the inlet.

CHAPTER 4: PREDICTIVE MODELLING AND GENERAL CONCLUSIONS

4.1. Introduction:

Overall, the early marine stage of the sockeye salmon life history is a very important period that influences their survival success at sea. During the seaward migration, smolts experience a wide range of environmental conditions and undergo drastic physiological changes. The interplay between different factors can make the outmigration process very beneficial or harmful to fish. Rapidly changing climate can catalyze fast changes in the ecosystem, further complicating outmigrating smolt conditions as the fish may miss the optimal conditions for growth and survival. The behavior of the fish and environmental conditions can work together to provide better conditions for growth and survival of the fish.

4.1.1. Effect of Climate Change

Climate change triggered by rising atmospheric CO_2 is altering marine ecosystems all around the globe through, for example, shifts in temperature, timing and location of the upwelling and spring blooms, zooplankton abundance and quality, and ocean acidification (Bates et al. 2008, IPCC 2014, Chandler et al. 2015). Nearshore environments may be affected by both changes in the open ocean and the conditions on the land. For instance, North American water bodies during the past century experienced a 1 – 4 week earlier peak stream flows, 0.1 – 1.5 °C increase in water temperature and overall decrease in the extent of snow coverage and annual precipitation (Bates et al. 2008). Future projections suggest +1 to +4 ºC warming of Pacific Ocean surface waters and North American continents by 2100 (IPCC 2014). A 10% increase in winter precipitation is expected in the coming years (Salathé 2006), which will be coupled with a decrease in the proportion of precipitation falling as snow, and a significant advance in timing of snowmelt runoff (Stewart et al. 2006). In addition, a higher amount of water vapor in the atmosphere may lead to more extreme climatic events, for example, droughts in summer and more intense precipitation in winter (Crozier et al. 2008, IPCC 2014). All of these changes may lead to changes

in nearshore marine environments by changing the amount of the freshwater input in the system, affecting water temperature and chemistry.

Climate change may lead to a shift in the outmigration timing from the lake as smolts may start migrating earlier due to high water temperatures (Hayes et al. 2014). For example, Achord et al. (2007) and Quinn (2005) demonstrated that in seasons with warm fall and spring, smolts initiate their migration earlier. Although it is believed that start of outmigration is determined by a lunar calendar, Otero et al. (2014) after analyzing the seaward migration in 67 rivers over the 50-year period have found that it advanced on average by 2.5 days per decade.

Warmer water temperatures may affect differently the productivity of the lake basins (such as Owikeno Lake), leading to a larger size heterogeneity in outmigrating smolts. This may result in a delayed smoltification (Beamish and Mahnken 1998) creating physiological challenges for outmigrating juveniles, e.g., smolts have to hold in the certain area to complete the smoltification and may miss optimal conditions on its way to the open ocean. In this study, it was found that a holding period at the head of Rivers Inlet may have generated "lower quality" smolts. Even though smolts spent one or two weeks in that highly productive region, almost no gain in size was recorded. The chemical and physical analysis of the otolith showed potential signs of starvation at that location.

The other result of global warming is an increase in the freshwater discharge from the lake due to ice melt. On one hand, higher freshwater discharge leads to higher degree of stratification that causes earlier plankton bloom. On the other hand, high water discharge results in increased estuarine circulation advecting phytoplankton out of the inlet and impacting local productivity (Wolfe 2010). The resulting mismatch with zooplankton, affecting its quantity and quality, may impact smolt feeding conditions in the inlet (Tommasi 2008, Ajmani 2011). Furthermore, if the advection of the top layer is high, smaller smolts may not be able to capture enough food to grow to a larger size. This may reduce their marine

survival through increased predation risk in coastal waters. The size of juveniles is a very important factor in determining the timing of the northward migration during the fall and winter of their first year in the ocean (Beacham et al. 2014). Smaller smolts utilize coastal waters for rearing longer than larger individuals (see results in Beacham et al., 2013).

Each sockeye population has its life history uniquely tuned to local conditions and hence may respond differently to climate change (Crozier et al. 2008). Recent investigations demonstrated that every stage of the salmon life-history can be affected by climate change (reviewed in Crozier 2015). This study focusses on the first stage of the early-marine component of the sockeye salmon life cycle. Estuaries are the areas where the fresh and marine waters meet and are critical habitats for many juvenile fish and invertebrates (Bond 2006), providing nursery area, protection from predators and rapid growth opportunity (Healey 1980, Levy and Northcote 1982, Shreffler et al. 1992). After exiting their natal streams, salmon smolts move through estuaries on their way to the open ocean. The time juvenile salmon spend in the estuary and the benefits received from that habitat vary dramatically between different salmon species and/or locations (Bottom et al. 2005, Bond 2006). Fjord migrations are thought to be one of the most challenging environments for smolt outmigration due to high predation rates (Hvidsten and Lund 1988, Okland et al. 2006) or lack of food (Hansen et al. 2003).

Some salmon populations swim through the estuary in a matter of days, while others spend months (Fried et al. 1978, LaBar et al. 1978, McMahon and Holtby 1992, Ruggerone and Volk 2003, Kocik et al. 2009). In this study, it was found that the outmigration from the Owikeno Lake could be a lengthy process, lasting up to 6-8 weeks. Once smolts enter the inlet, the residence duration may vary between 1 and 59 days. Smolts that start the outmigration early may experience quite different environmental conditions during their early marine phase compared to smolts that enter the inlet later. Recent studies have demonstrated increased ocean survivorship for smolt that initiated their marine migration earlier in the season (Zabel and Williams 2002, Waples et al. 2007). However, rising river flow

early in the season can influence timing and duration of outmigration when fish may encounter suboptimal conditions for growth and survival.

4.1.2.   Goals for Chapter 4

In this chapter, the effect of estuarine residence time and the link between fish behavior and environmental conditions was explored to understand how smolts utilize the estuarine environment. Such information could provide a valuable baseline to explore the effect of climate change in estuaries and investigate the effect on the marine survival of sockeye smolts.

In Chapter 3, it was found 1) that river discharge was an important factor that can influence the outmigration duration of smolts, and 2) that smolts might spent more time in areas with high zooplankton densities. However, due to the interplay of many environmental factors, it was impossible to tease apart what effect each environmental parameter had on the outmigration duration of juvenile sockeye or their physical condition. The goal of this chapter was to construct a predictive model for the increase in weight of Rivers Inlet sockeye smolts from the head to the mouth using multiple environmental variables, including water discharge, water temperature, zooplankton densities and chlorophyll biomass.

Environmental parameters were chosen for two reasons: 1) they were recorded during the RIES surveys and 2) their ecological importance to the fish survival and development. The day length and temperature are known factors that initiate the outmigration from lakes. Water temperature can influence growth, susceptibility to parisites (St-Hilaire et al. 1998) and the degree of maturation (Davidson and Good 2015). Early maturation may result in decreased growth and feed conversion efficiency (McClure et al. 2007). The timing of the phytoplankton bloom can influence the development of the zooplankton which in its turn, along with the water discharge, is an important determinant of fish weight (Buchanan 2006, Ajmani 2011).

Two model types were constructed. The first environmental conditions at the inlet's head (DFO 5 station). The conditions at entry were chosen for two main reasons: (a) potential importance of this area (highest zooplankton densities) as smolts may spend 2-3 weeks there (see Chapter 3); and (b) great potential of predicting the whole inlet performance of smolts using the entry point information. The second incorporated conditions throughout the inlet (see Methods for more details). The chapter's prediction is as follows:

Prediction 3: It is possible to predict the increase in weight of smolts during the outmigration, using data on river discharge, water temperature, zooplankton abundance and chlorophyll biomass.

4.2. Methods

The analysis of environmental conditions was extended to the 2008-2010 samples. The year 2011 was omitted from the analysis because no environmental data were collected. The source and structure of the river discharge, zooplankton and chlorophyll data was explicitly described in Chapter 3. Water temperature was obtained from the Sea-Bird SBE25 CTD data that were collected during the RIES program. Temperature profiles were extracted with the *oce* package in R and binned to 1 m intervals. The mean temperature of the top 10 m of the water column was used in this study because smolt were found to utilize largely the top 3-4 m of the water column (Davidsen et al. 2008). Only samples from Bosquet Open and Bosquet Shore were used in this chapter and are explicitly described in Chapter 2.

To determine whether the environmental conditions explain the condition of the fish and the outmigration duration, a generalized additive regression modelling (GAM) approach was implanted using the *mgcv* package for R (Wood 2006). The condition of the fish was represented by two factors: 1) increase in weight (ratio between the weight at catch and the weight at entry), and 2) weight at catch (smolt weight at the inlet's mouth). The four predictor variables chosen in this chapter were: (i) the

zooplankton densities (ind/m^2), (ii) mean water temperature in the upper 10 m of the water column (ºC), (iii) daily water discharge, (iv) chlorophyll biomass and (v) size of smolts at entry to the inlet.

The water temperature, zooplankton availability, and the water discharge were determined for each smolt for their first day of entry into the inlet. Although the water discharge data were available daily, zooplankton and temperature data were only sampled 6, 7 and 4 times in 2008, 2009 and 2010 respectively. To determine the zooplankton density and the water temperature for each smolt on the day of entry, the data were fitted with *loess* smoothing, a non-parametric locally weighted regression, using the nearest neighbor approach. Later, the points were extracted for each day of entry using the *predict* function. To explore the covariate effect between the environmental factors, the Pearson correlation coefficient was determined for each pair of the parameters used in this chapter. However, Pearson's correlation coefficient is sensitive to skewed distributions and outliers, Spearman's rank correlation was used instead. It is important to note that during the preliminary analysis (not shown here) the chlorophyll biomass did not contribute to any of the models and thus was excluded from the analysis. This parameter was also removed from the model due to collinearity with several other parameters (see Section 4.3.2).

A program was written to run all possible combinations of GAM models using the different combinations of three environmental conditions and size at entry. There were 15 different possible combinations (n^2 -1, where n is a number of available parameters), but only the models with all significant terms were chosen for further comparison (p< 0.005). The best GAM model was considered to have (i) the lowest GCV score (Generalized Cross Validation), (ii) the highest level of deviance explained, (iii) the lowest AICs (Akaike Information Criterion) among the models, and (iv) highest R^2 values.

The relative contribution of each of the predictors in a GAM model was determined using a series of model comparisons, an approach suggested by Judd et al. (2008). Two models were used for each predictor, (1) the first model containing all of the predictors is the model (M1), and (2) the second model omitting the predictor of interest (M2). The PRE score (Proportional Reduction of Error) represented the effect size of the predictor and was calculated using the following formula:

$$PRE = \frac{\text{Residual Sum of Squares of M2} - \text{Residual Sum of Squares of M1}}{\text{Residual Sum of Squares of M2}}$$

The first type of model (Type I) used the environmental conditions at the head of the inlet. Station DFO 5 was chosen to represent environmental conditions at the time entry into the inlet. Three sets of the models were created using the environmental conditions at the head of the inlet:

1) Using conditions on the day of entry

2) the mean environmental values between the two dates ($\pm$ 7 days from the day of entry), to take into account the potential errors in determining the day of entry

3) the cumulative value for environmental parameters experienced by the smolts between $\pm$ 7 days from the day of entry, to take into account that the smolt might spend ~2 weeks at the head of the inlet (Chapter 3).

The second type of model (Type II) used values from the entire inlet. Two different sets of models were created:

1) the average of all 6 stations was used (DFO1- 5 and UBC7) and the cumulative values of environmental variables experienced by each smolt during the outmigration period were calculated; and

2) the average of the 5 stations (DFO1- 4 and UBC7) was used, and the cumulative values were calculated to see the contribution of the inlet's head (DFO 5 station).

It is important to note that for Type II models, the outmigration duration was not predicted as these models used the outmigration duration in the calculation of the cumulative values of all environmental parameters used in the model.

4.3. Results

4.3.1. Environmental Conditions at Entry
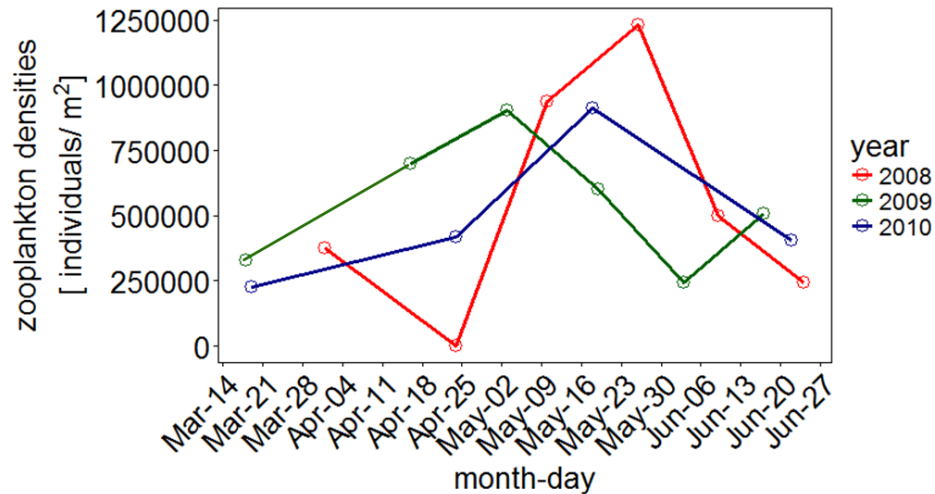
Figure 34: Zooplankton densities at the head of Rivers Inlet throughout the outmigration period in 2008, 2009 and 2010.

The peak in zooplankton density varied between the years. The zooplankton peak was the earliest in 2009 (early-May), followed by 2010 peak in mid-May; zooplankton densities in 2008 peaked only at the end of May. The largest zooplankton densities were observed in 2008 (~12.5 x 10^5 individuals/m^2) while in 2009 and 2010 zooplankton peaked at ~9 x 10^5 individuals/m^2.
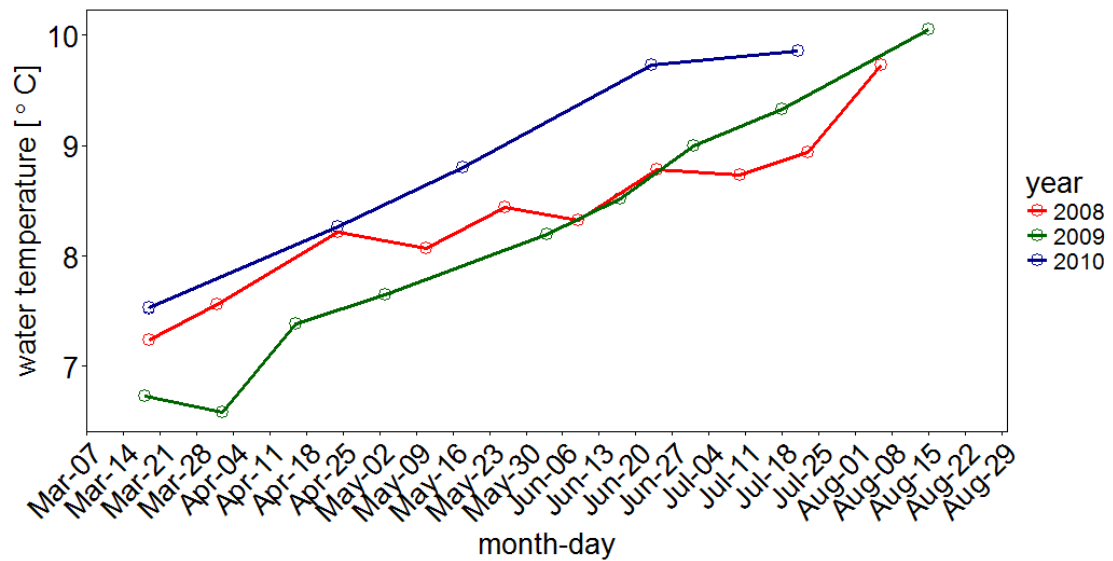
Figure 35: Water temperature (10 m integrated water column) at the head of Rivers Inlet (Station DFO5) throughout the outmigration period in 2008, 2009 and 2010.

Average water temperature in the upper 10 m water column varied between years at the entry to the Inlet (DFO5). March was the coldest (< 7 °C) in 2009 followed by 2008 and 2010. Apart from a small decrease in temperature from mid-March to April in 2009, all three years showed a gradual increase in temperature throughout the outmigration season. The rate of increase was similar in 2009 and 2010, while the rate of increase in water temperature during the seaward migration was the slowest in 2008. For a description of water discharge data and zooplankton densities refer to Chapter 3.

4.3.2. Spearman Correlation Coefficient
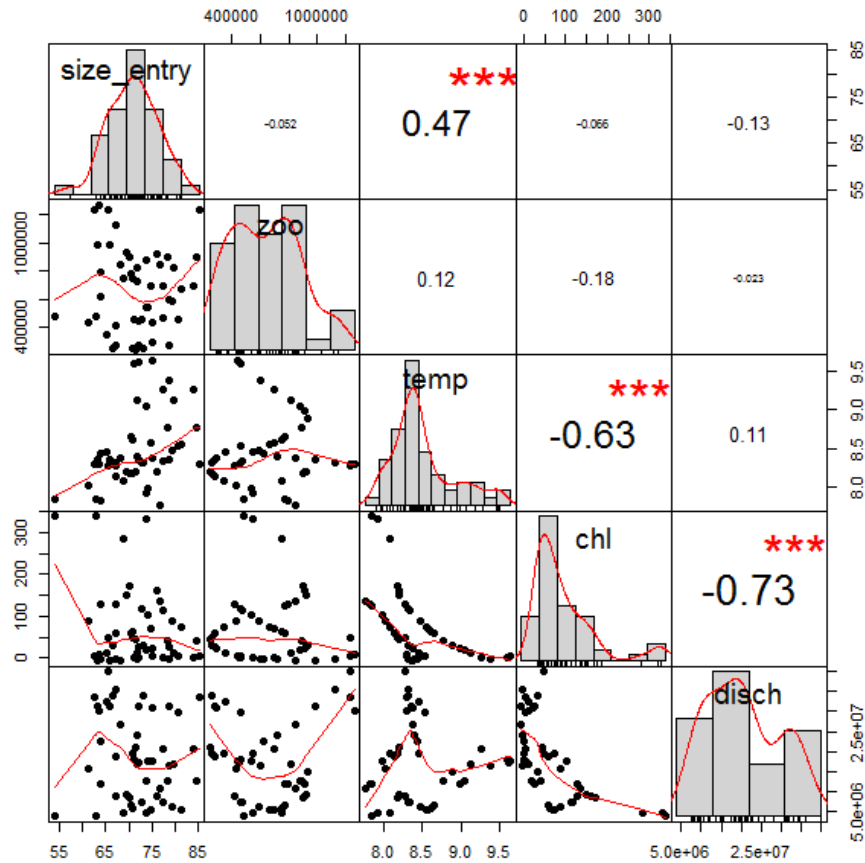
Figure 36: A chart of a correlation matrix between five parameters. Covariates represent temperature, zooplankton densities (zoo), water temperature (temp), daily freshwater discharge (disch) and size at the entry (size_entry) and chlorophyll biomass (chl). The distribution of each variable is shown on the diagonal. The bivariate scatter plots with a fitted line are displayed beneath the diagonal. The value of the correlation plus the significance level as stars are displayed above the diagonal. Each significance level is associated with a symbol: p-values 0 "***", 0.001 "**", 0.01"*", 0.05 ".", and > 0.1 " ".

Out of all possible correlations, only three correlations were significant. Chlorophyll was strongly negatively correlated with water discharge and temperature (r = -0.73 and -0.63 respectively). In addition, size at entry and water temperature were moderately positively correlated (r=0.47; p= 0.01). Other correlations were not significant (p > 0.05).

4.3.3. Type I Models

Table 10: Best-fit GAM models for a) weight at catch (g), b) weight increase and c) outmigration duration (days) using the environmental conditions at the head of the inlet (DFO5 station) at the day of entry. Generalized Cross Validation scores (GCV), deviance explained (Dev), Akaike Information

Criterion (AIC), degrees of freedom (df) and the adjusted R^2 values (Rsq) are provided. PRE scores (Proportional Reductional of Error) are displayed for each of the covariates. Covariates represent temperature, zooplankton densities (zoo), water temperature (temp), daily freshwater discharge(disch) and size at the entry (size_entry). Models only contain parameters that were significant (p <0.05), n corresponds to sample size. Refer to Table 15 for the information on all tested models.

								PRE scores		
	n	Model #	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
Weight at catch	50	1	0.78	35.14	0.84	1.97	172.2	14.6	42.0	5.10	17.5
Weight increase	56	1	0.64	35.53	0.74	0.13	34.80	44.3	62.7	9.3	38.9
Outmigration duration	58	10	0.62	43.05	0.72	84.31	419.12	-	38.2	79.6	-

CONDITIONS AT THE DAY OF ENTRY (table header)

1) Using conditions on the day of entry

Zooplankton densities and river discharge were poor predictors of the weight at catch (R^2 = 0.06 and 0.11 respectively; Table 15a). However, water temperature explained 55% of the weight variation at catch at the inlet mouth (Model 14; Table 15a). Individually, the environmental parameters were poor predictors of the increase in weight of sockeye smolt (R^2 ranged between 0.06 and 0.18; Table 15b). Combinations of two environmental variables produced better predictions of the weight at catch with the highest R^2 values of 0.57 - 0.63 (Models 10 and 11; Table 15a) and increase in weight with the highest R^2 values of 0.18 - 0.33 (Models 10, 11 and 13; Table 15b). All three factors together (Model 9) gave the highest R^2 value of 0.64 and 0.39 for weight at catch and increase in weight, respectively (Table 15a and b). Introduction of the additional parameter "size at entry", produced a significant improvement to the model. On its own, size at entry could explain 33% of weight at catch but only 2% of increase in weight (Table 15). The best model that describes both weight at catch and the increase in weight at the end of the migration is the following GAM Model 1 (Table 10):

Weight at catch OR increase in weight ~ s(zoo) + s(temp) + s(disch) + s(size_entry)

For weight at catch, Model 1 explained 84% of the variance and had an R^2 value of 0.78 (Table 10a). For the weight increase, Model 1 explained 74% of the variance and had R^2 of 0.64 (Table 10b). In both cases, the major contributor to explanatory power of the model was water temperature with PRE score of 42 and 62% as compared to the models without the temperature parameter (Model 3, Table 15). Zooplankton density and size of the smolt at entry contributed similarly to the Model 1. The lowest contribution to the explanatory power of Model 1 was the discharge parameter. The results of the GAM Model 1 are shown as plots of the best-fitting smooths for the conditional effect of the covariates on the parameter of interest (weight at catch and weight increase; Figure 37 and Figure 38).

After the effect of all other variables was removed, the residual graphs allowed to the exploration of how weight at catch changes relative to its mean (1.58 g for 2008-2010) with changes in one variable. Zooplankton densities seemed not to change much relative to mean weight until the densities reach 9×10^5 ind/m^2. The higher densities led to weight at catch being higher than the mean value (Figure 37). Water discharge showed the opposite trend, with weight at catch being higher at low water discharge and less than the mean at higher discharge levels. Water discharge of 2×10^7 m^3 was a turning point from higher than average to lower than average values of weight at catch. Temperatures colder than ~8.5 ºC seemed to be negatively correlated with weight at catch. At temperatures above ~8.5 ºC, temperature had a positive effect on weight at catch reaching a peak at 8.7-8.8 ºC and dropping after the temperatures got hotter. After the effect of other parameters was removed, the size at entry was linearly correlated with the weight at catch with a positive correlation, i.e., the bigger the smolts exiting the lake, the larger the weight at catch (Figure 37).
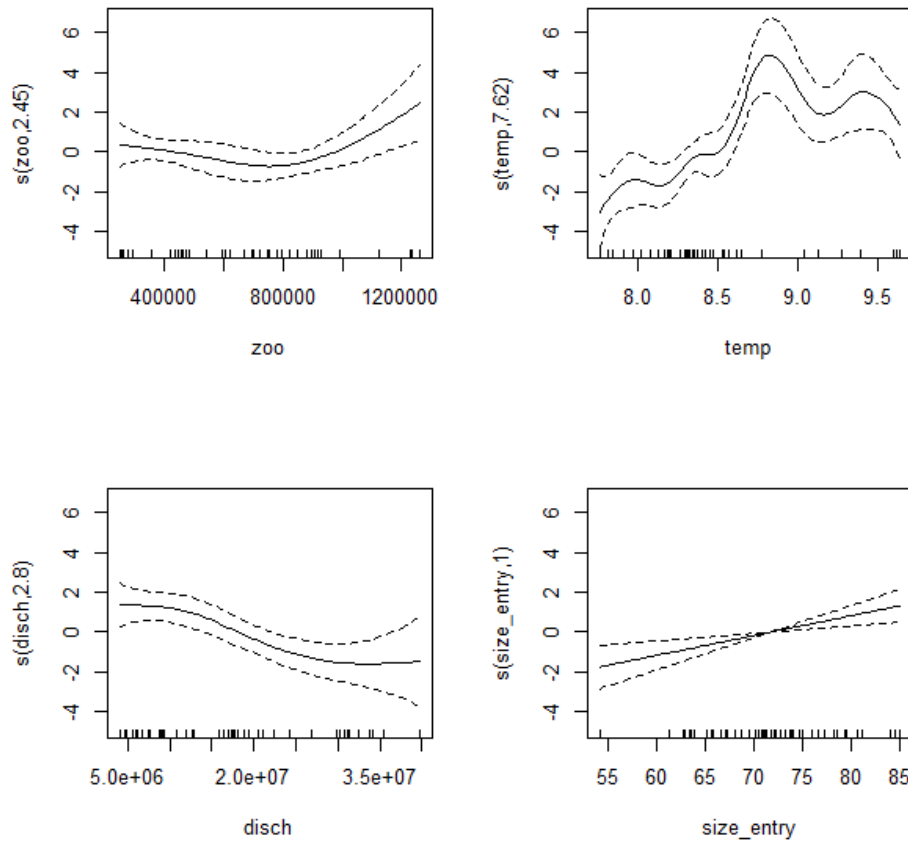
Figure 37: Fit of the GAM Model 1 showing the effect of each environmental parameter on weight at catch. The partial effects of each individual covariate (water temperature, zooplankton density, size at entry and freshwater discharge) are plotted as smoothed fits, s(•). The variables temp, zoo, size _entry and disch corresponds respectively to temperature, zooplankton density, size at entry and freshwater discharge. Zero on the vertical axes corresponds to no effect of the explanatory variable. The broken lines correspond to 95% confidence limits for the smooth. Ticks on the x-axis indicate the locations of observations. The estimated smoothers are given in brackets after the variable name on vertical axes.

Zooplankton densities had a significant positive effect on weight increase (exceeding the mean of 1.58 g for 2008-2010) above zooplankton densities of 9 x 10^5 ind/m^2 (Figure 38). Discharge had a negative linear correlation with the increase in weight with lower discharge values corresponding to higher than average increase in weight. Water discharge of 2x10^7 m^3 seemed to be a turning point above which weight increase becoming less than the average. Due to the distribution of data, the effect of size at entry on the weight increase can be only considered for the size range 63-81 mm (very few points were available outside that region). Size at entry was negatively correlated with weight increase (Figure 38).
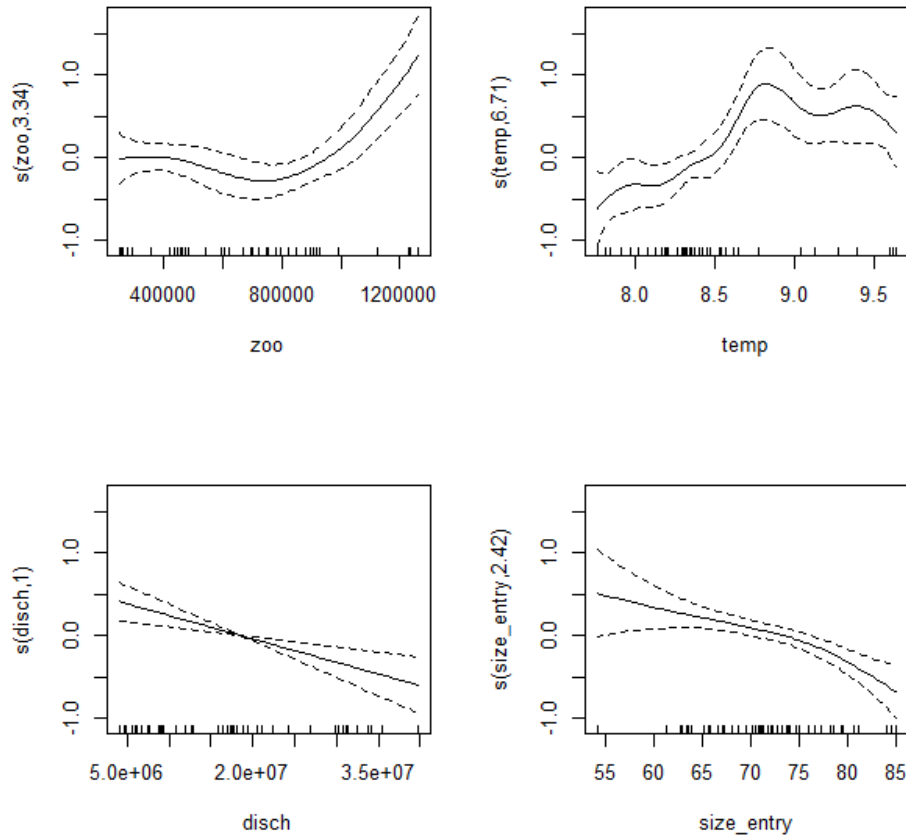
Figure 38: Fit of the GAM Model 1 showing the effect on weight increase. The partial effects of each individual covariate (water temperature, zooplankton density, size at entry and freshwater discharge) are plotted as smoothed fits, s(•). The variables temp, zoo, size _entry and disch corresponds respectively to temperature, zooplankton density, size at entry and freshwater discharge. Zero on the vertical axes corresponds to no effect of the explanatory variable. The broken lines correspond to 95% confidence limits for the smooth. Ticks on the x-axis indicate the locations of observations. The estimated smoothers are given in brackets after the variable name on vertical axes.

For the duration of the outmigration, the size at entry was never a significant parameter (p > 0.05) and zooplankton density was only significant in two models (Table 15c). The best model (Model 10) had only two parameters: water temperature and freshwater discharge. Model 10 explained 72% of the variance and had an R^2 value of 0.62:

Outmigration duration ~ s(temp) + s(disch)

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The contribution of discharge was higher (PRE = 80%) than temperature (PRE = 38%). The results of GAM Model 10 are shown as plots of the best-fitting smooths for the conditional effect of the covariates on the parameter of interest (outmigration duration; Figure 39).

Figure 39: Fit of the GAM Model 10 showing the effect on outmigration duration. The partial effects of each individual covariate (water temperature and freshwater discharge) are plotted as smoothed fits, s(•). The variables temp and disch correspond respectively to water temperature and freshwater discharge. Zero on the vertical axes corresponds to no effect of the explanatory variable. The broken lines correspond to 95% confidence limits for the smooth. Ticks on the x-axis indicate the locations of observations. The estimated smoothers are given in brackets after the variable name on vertical axes.

Discharge was negatively correlated with the outmigration duration with lower than average discharge values associated with the longer residence time in the inlet (ignoring the trend above 35 million m^3, for which only two points were available). The effect of temperature had a more complicated pattern on the outmigration duration: at temperature less than 8°C there was no or less than average deviation from the mean (mean duration is 25 days) outmigration duration; at 8 to 8.3 °C there was a positive correlation; at 8.3-8.6 °C there was a negative correlation; and from 8.6 to 9.4 °C there was no difference from the mean (or slightly higher) outmigration duration. Although such lumpiness in a general pattern could be due to under-smoothening or due to sampling error variability.

2) Using the mean environmental values between the two dates (± 7 days from the day of entry)

The models using the mean values of environmental variables between ±7 days from the entry day, had very similar values to the ones in Table 15 and models are not shown here.

3) Using the cumulative values between ±7 days from day of entry, the best model to describe the weight at catch was Model 3 with an R^2 value of 0.81 and explaining 87% of the variance (Table 11a):

$$\text{Weight at catch} \sim s(zoo) + s(discharge) + s(size\_entry)$$

Table 11: Best-fit GAM models for a) weight at catch (g), b) weight increase and c) outmigration duration (days) using the environmental condition at the head of the inlet (DFO5 station) and the cumulative values between ±7 days from the day of entry. Generalized Cross Validation scores (GCV), deviance explained (Dev), Akaike Information Criterion (AIC), degrees of freedom (df) and the adjusted R^2 values (Rsq) are provided. PRE scores (Proportional Reductional of Error) are displayed for each of the covariates. Covariates represent temperature, zooplankton densities (zoo), water temperature (temp), daily freshwater discharge(disch) and size at the entry (size_entry). Models only contain parameters that were significant (p <0.05), n corresponds to sample size. Refer to Table 15 for the information on all tested models.

| | | | | | | | | PRE scores | | |
	n	Model #	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
CUMULATIVE OF ±7 DAYS											
Weight at catch	46	3	0.81	30.71	0.87	1.94	156.37	23.1	-	12.5	21.9
Weight increase	46	1	0.64	31.87	0.74	0.13	34.53	0.45	0.10	0.09	0.39
Outmigration duration	54	12	0.58	49.84	0.61	68.05	382.80	-	-	1.00	-

The relative contribution of each factor was 23, 13 and 22% for zooplankton density, discharge and size at entry, respectively. The results of GAM Model 3 are shown as plots of the best-fitting smooths for the conditional effect of the covariates on the parameter of interest (weight at catch; Figure 40).
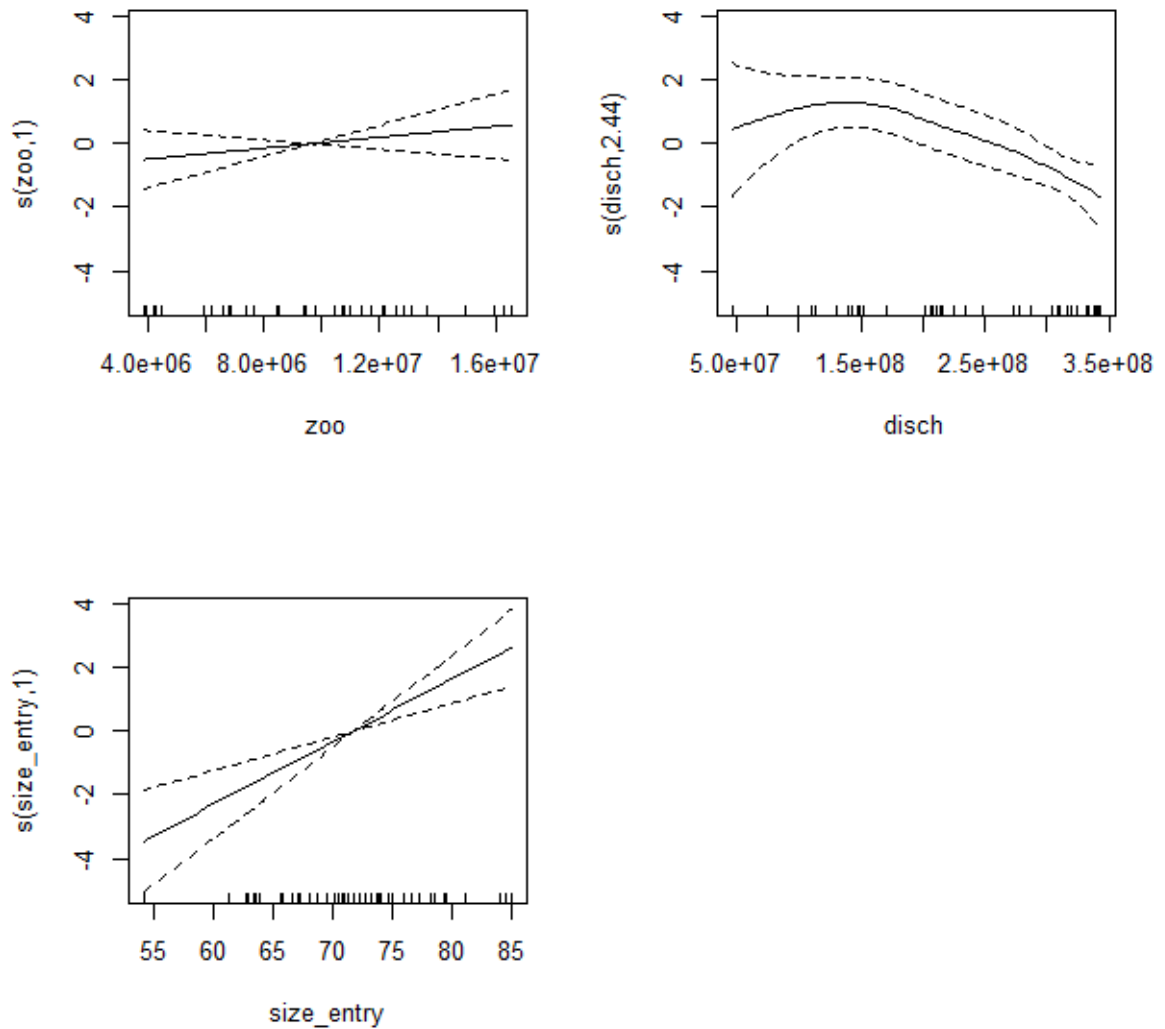
Figure 40: Fit of the GAM Model 3 showing the effect of factors on the weight at catch. The partial effects of each individual covariate (zooplankton density, size at entry and freshwater discharge) are plotted as smoothed fits, s(•). The variables zoo, size _entry and disch corresponds respectively to cumulative values of zooplankton density, size at entry and freshwater discharge. Zero on the vertical axes corresponds to no effect of the explanatory variable. The broken lines correspond to 95% confidence limits for the smooth. Ticks on the x-axis indicate the locations of observations. The estimated smoothers are given in brackets after the variable name on vertical axes.

Cumulative zooplankton densities had a small effect on the weight at catch, with weights being less than the mean at zooplankton densities less than 10^7 ind/m². In general, the weight at catch decreased with the increase in the daily discharge, with more deviation from the mean at lower cumulative discharge values. There was a positive linear correlation between the size at entry and weight at catch.

Considering the increase in weight, Model 1 gave the best results with an R^2 value of 0.64

explaining 74% of the variance (Table 11b). The relative contribution of each factor (PRE score) was

45, 10, 9 and 39% for zooplankton density, temperature, discharge, and size at entry, respectively. The

results of GAM Model 1 are shown as plots of the best-fitting smooths for the conditional effect of the

covariates on the parameter of interest (weight increase; Figure 41).
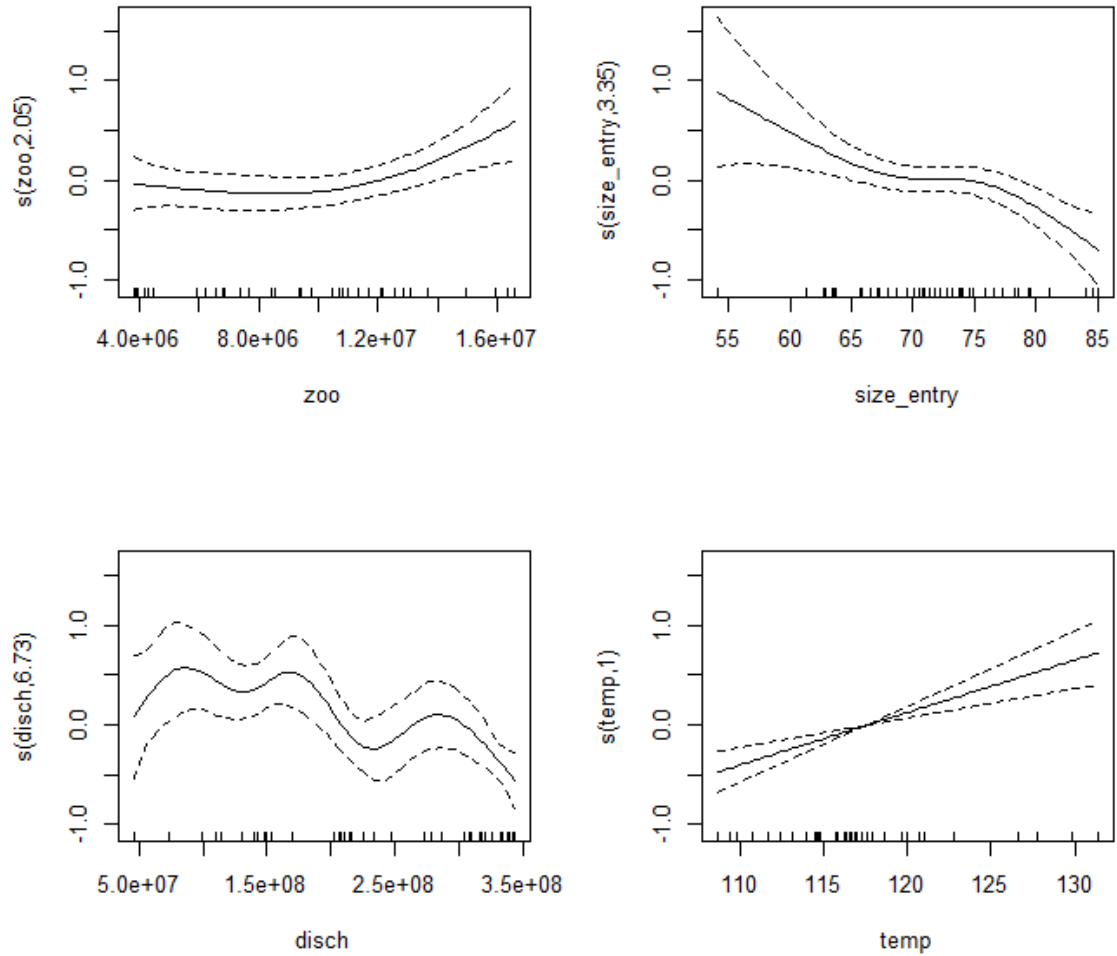
Figure 41: Fit of GAM Model 1 showing the effect on weight increase. The partial effects of each
individual covariate (zooplankton density, size at entry, water temperature and freshwater discharge)
are plotted as smoothed fits, s(•). The variables zoo, size _entry, temp, and disch corresponds
respectively to zooplankton density, size at entry, water temperature and freshwater discharge. Zero on
the vertical axes corresponds to no effect of the explanatory variable. The broken lines correspond to
95% confidence limits for the smooth. Ticks on the x-axis indicate the locations of observations. The
estimated smoothers are given in brackets after the variable name on vertical axes.

Zooplankton densities had almost no effect on the weight increase in this Model, excluding the area of high zooplankton densities (> 1.2x 10^7 ind/m^2) where the weight increase became greater than the mean value, although few data points were available at those densities. The effect of size at entry was considered between 63 and 80 mm due to the lack of data outside that size range. The size almost had no effect (size was very close to the mean value) on the weight increase until the size of the juveniles reached 75 mm after what the increase in weight became less than the mean value. In general, weight increase decreased with the increase in the daily freshwater discharge. Temperature and weight increase had a positive linear correlation.

In the case of outmigration duration, the best model was found to be Model 12 with an R^2 of 0.58, explaining 61% of the variation. The results of GAM Model 12 are shown as plots of the best-fitting smooths for the conditional effect of the covariates on the parameter of interest (outmigration duration; Figure 42). In this Model, discharge and outmigration duration were negatively correlated.
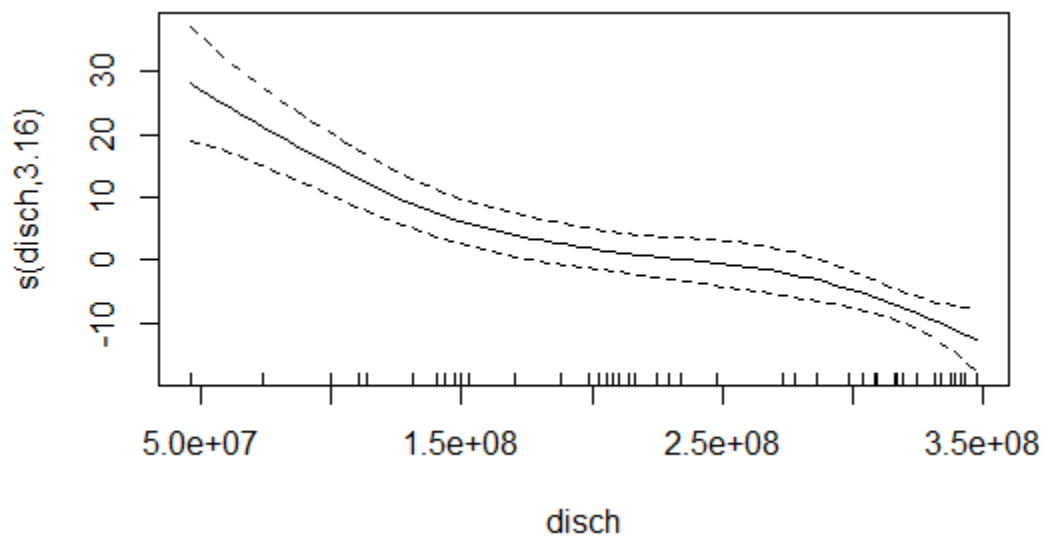
Figure 42: Fit of GAM Model 12 showing the effect on the outmigration duration. The partial effects of the individual covariate (freshwater discharge) are plotted as smoothed fits, s(•). Zero on the vertical axes corresponds to no effect of the explanatory variable. The broken lines correspond to 95% confidence limits for the smooth. Ticks on the x-axis indicate the locations of observations. The estimated smoothers are given in brackets after the variable name on vertical axes.

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4.3.4. Type II Models

1) Using the average of all 6 stations (DFO1- 5 and UBC7)

Table 12: Best-fit GAM models for a) weight at catch (g) and b) weight increase using the average environmental conditions through the whole inlet (DFO1-5 and UBC 7 stations) and the cumulative values over the outmigration period. Generalized Cross Validation scores (GCV), deviance explained (Dev), Akaike Information Criterion (AIC), degrees of freedom (df) and the adjusted R^2 values (Rsq) are provided. PRE scores (Proportional Reductional of Error) are displayed for each of the covariates. Covariates represent temperature, zooplankton densities (zoo), water temperature (temp), daily freshwater discharge(disch) and size at the entry (size_entry). Models only contain parameters that were significant (p <0.05), n corresponds to sample size. Refer to Table 15 for the information on all tested models.

WHOLE INLET CUMULATIVE											
								PRE scores			
Predictor	**n**	**Model #**	**Rsq**	**df**	**Dev**	**GCV**	**AIC**	**s(zoo)**	**s(temp)**	**s(disch)**	**s(size_entry)**
Weight at catch	52	2	0.67	38.85	0.75	2.71	197.44		0.46	0.31	0.12
Weight increase	52	5	0.43	37.24	0.58	0.19	58.70	0.44	0.72		0.74

For weight at catch, the best model was Model 2 (Table 12). This Model had R^2 value of 0.67 and explained 75% of the deviance. However, Model 5 produced similar results but had slightly higher GCV and AIC values (Table 17a). The results of the GAM Model 2 are shown as plots of the best-fitting smooths for the conditional effect of the covariates on the parameter of interest (weight at catch; Figure 43).

With cumulative temperature less than 100°C the weight at catch was smaller than the mean value. No difference from the mean weight at catch was observed at temperatures between 100 and 200 °C. There was an increase in the weight at catch when cumulative temperatures became greater than 200 °C. River discharge had the opposite effect on the weights at catch. At lower discharge values the weight were greater than the mean value, while there was almost no deviation from the mean between the cumulative water discharge levels of 3×10^8 and 7×10^8 m^3 while weights were lower than the mean when the discharge values were higher than 7×10^8 m^3. Size had a positive linear correlation with the weight at catch, but its contribution to the model was less than that of temperature and river discharge.
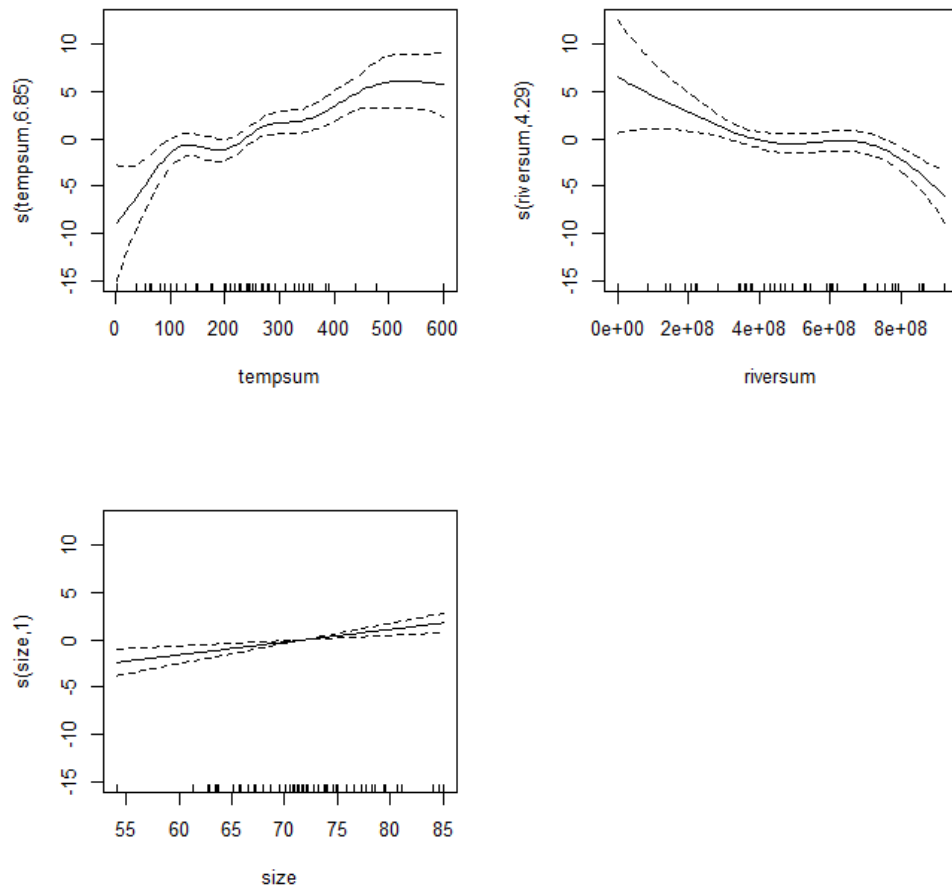
Figure 43: Fit of the GAM Model 2 showing the effect on weight at catch. The partial effects of each individual covariate (size at entry, water temperature, and freshwater discharge) are plotted as smoothed fits, s(•). The variables size, tempsum and riversum correspond respectively to cumulative values of size at entry, water temperature and freshwater discharge. Zero on the vertical axes corresponds to no effect of the explanatory variable. The broken lines correspond to 95% confidence limits for the smooth. Ticks on the x-axis indicate the locations of observations. The estimated smoothers are given in brackets after the variable name on vertical axes.

For weight increase (Table 12), two models produced similar results (Model 2 and Model 5):

Model 2: increase in weight ~ s(temp) + s(disch) + s(size_entry)

Model 5: increase in weight ~ s(zoo) + s(temp) + s(size_entry)

Both models had R^2 scores of $0.42 - 0.43$ and explained 37-38% of the variance. However, Model 5 was chosen although it gave higher AIC values, due to a slightly better R^2 value and percent deviance

explained. The results of the GAM Model 5 are shown as plots of the best-fitting smooths for the conditional effect of the covariates on the parameter of interest (weight at catch; Figure 44).
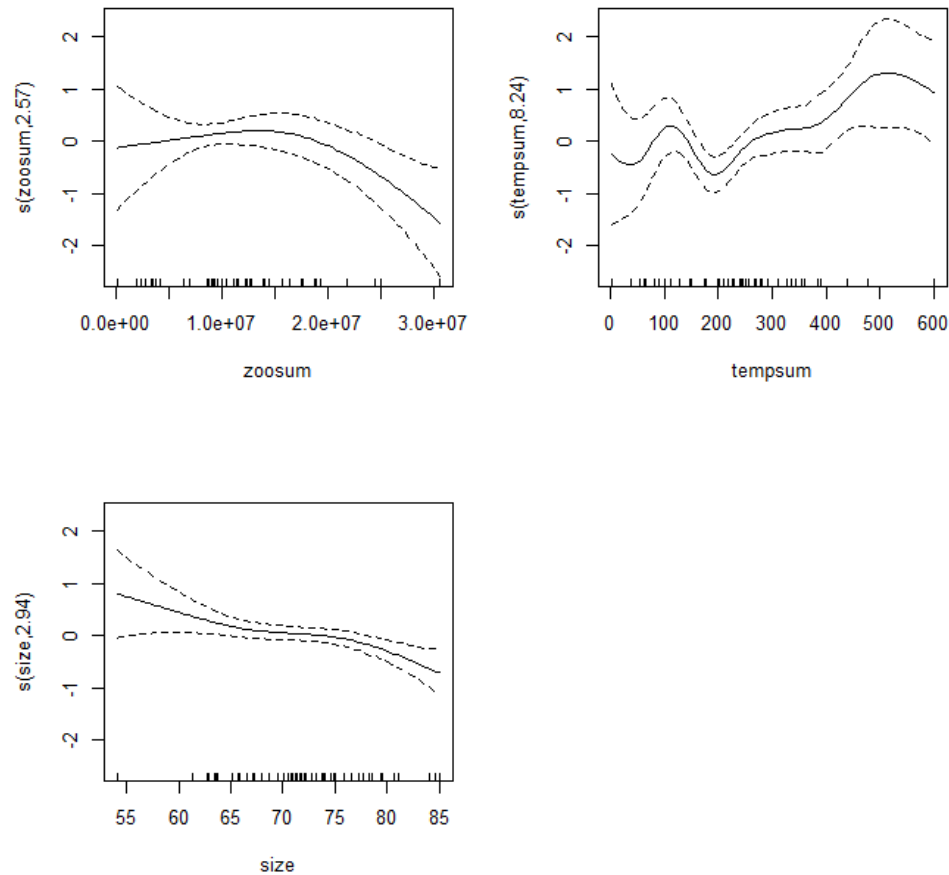
Figure 44: Fit of GAM Model 5 showing the effect on weight at catch. The partial effects of each individual covariate (size at entry, water temperature, and freshwater discharge) are plotted as smoothed fits, s(•). The variables size, tempsum and riversum correspond respectively to cumulative values of size at entry, water temperature and freshwater discharge. Zero on the vertical axes corresponds to no effect of the explanatory variable. The broken lines correspond to 95% confidence limits for the smooth. Ticks on the x-axis indicate the locations of observations. The estimated smoothers are given in brackets after the variable name on vertical axes.

Cumulative zooplankton densities had no effect on the increase in weight until they reached the $1.5x\ 10^7$ ind/m^2, after what the increase in weight decreased (relative to its mean) sharply with the increase in the cumulative zooplankton densities. Size at entry between 63-75 mm had almost no effect on the increase in weight (no difference from the mean), but above 75 mm the increase in weight decreased and was less than the mean value when the size of the smolts was greater than 80 mm.

Temperature had a more complicated pattern. Cumulative temperature of less than 100 °C had a very

high deviation from the mean. In the range of 100- 200 °C there was a decrease in the ability of the fish

to gain weight, but increasing again after the cumulative temperatures were greater than 200 °C.

2) Using the average of all 5 stations was used (DFO1- 4 and UBC7)

To explore further the role of conditions at the inlet head, DFO5 station was removed and the

models for the whole inlet rerun.

Table 13: Best-fit GAM models for a) weight at catch (g) and b) weight increase that using the average environmental conditions through the whole inlet excluding the head (DFO1-4 and UBC 7 stations) and the cumulative values over the outmigration period. Generalized Cross Validation scores (GCV), deviance explained (Dev), Akaike Information Criterion (AIC), degrees of freedom (df) and the adjusted R^2 values (Rsq) are provided. PRE scores (Proportional Reductional of Error) are displayed for each of the covariates. Covariates represent temperature, zooplankton densities (zoo), water temperature (temp), daily freshwater discharge(disch) and size at the entry (size_entry). Models only contain parameters that were significant (p <0.05), n corresponds to sample size. Refer to Table 15 for the information on all tested models.

								PRE scores			
WHOLE INLET CUMULATIVE WITHOUT DFO5											
	n	Model #	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
Weight at catch	60	9	0.68	43.27	0.77	2.51	221.84	0.76	031	0.31	-
Weight increase	52	5	0.25	44.00	0.35	0.21	68.02	0.68	0.24	-	0.56

The best model to describe the weight at catch was found to be Model 9 with R^2 value of 0.68

and explaining 77% of the variance:

$$\text{Weight at catch} \sim s(\text{zoo}) + s(\text{discharge}) + s(\text{temp})$$

The relative contribution of each factor was 76, 30 and 32% for zooplankton density,

temperature and discharge, respectively. The results of GAM Model 9 are shown as plots of the best-

fitting smooths for the conditional effect of the covariates on the parameter of interest (weight at catch;

Figure 45). Cumulative temperature of less than 100 °C had a negative impact on weight at catch (the

values were less than the mean). After that the weight at catch increased with the increase in cumulative temperature, reaching a peak at ~500 °C, although there were few points at that level. River discharge sum had a weak linear inverse relationship with the weight at catch. Weight at catch decreased slightly with the increase in cumulative zooplankton densities (until 2×10^7 ind/m^2).
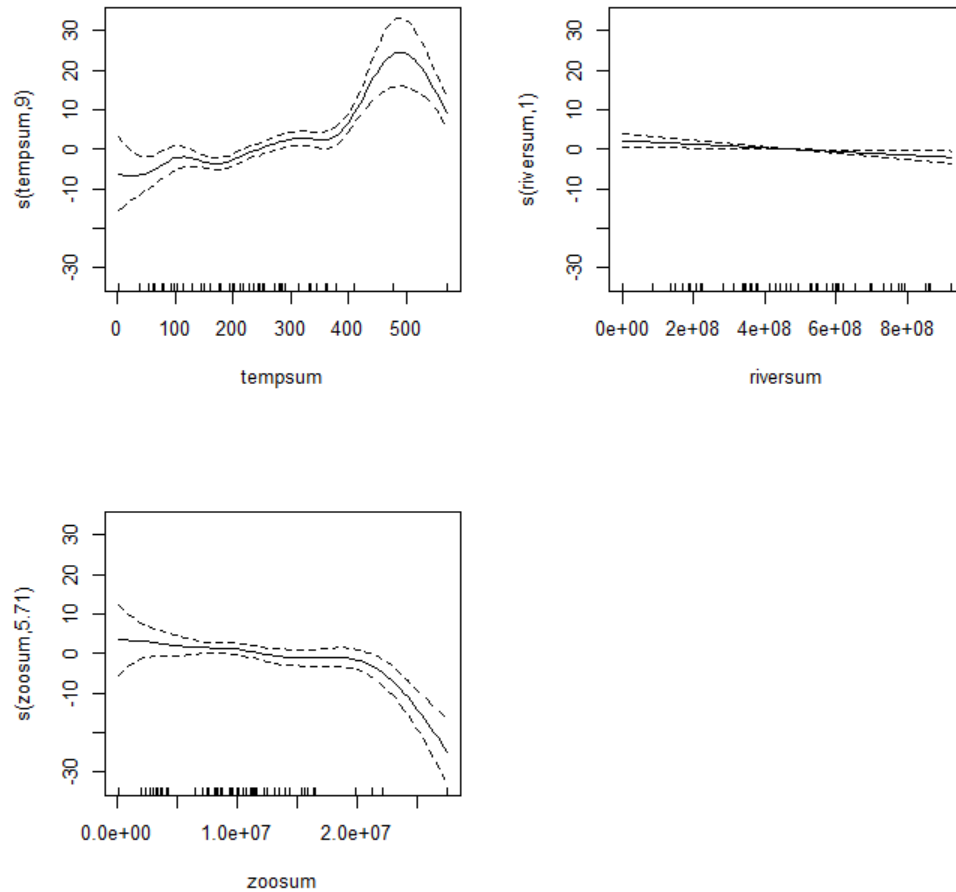
Figure 45: Fit of GAM Model 9 showing the effect on weight at catch. The partial effects of each individual covariate zooplankton densities, water temperature, and freshwater discharge) are plotted as smoothed fits, s(•). The variables zoosum, tempsum and riversum correspond respectively to cumulative values of zooplankton densities, water temperature and freshwater discharge. Zero on the vertical axes corresponds to no effect of the explanatory variable. The broken lines correspond to 95% confidence limits for the smooth. Ticks on the x-axis indicate the locations of observations. The estimated smoothers are given in brackets after the variable name on vertical axes.

For increase in weight, Model 5 gave the best results with R^2 values of 0.25 and explaining 35% of the variance:

$$\text{increase in weight} \sim s(zoo) + s(temp) + s(size\_entry)$$

The relative contribution of each factor (PRE score) was 66, 25 and 56% for zooplankton density, temperature and size at entry, respectively. The results of GAM Model 5 are shown as plots of the best-fitting smooths for the conditional effect of the covariates on the parameter of interest (weight at catch; Figure 46).
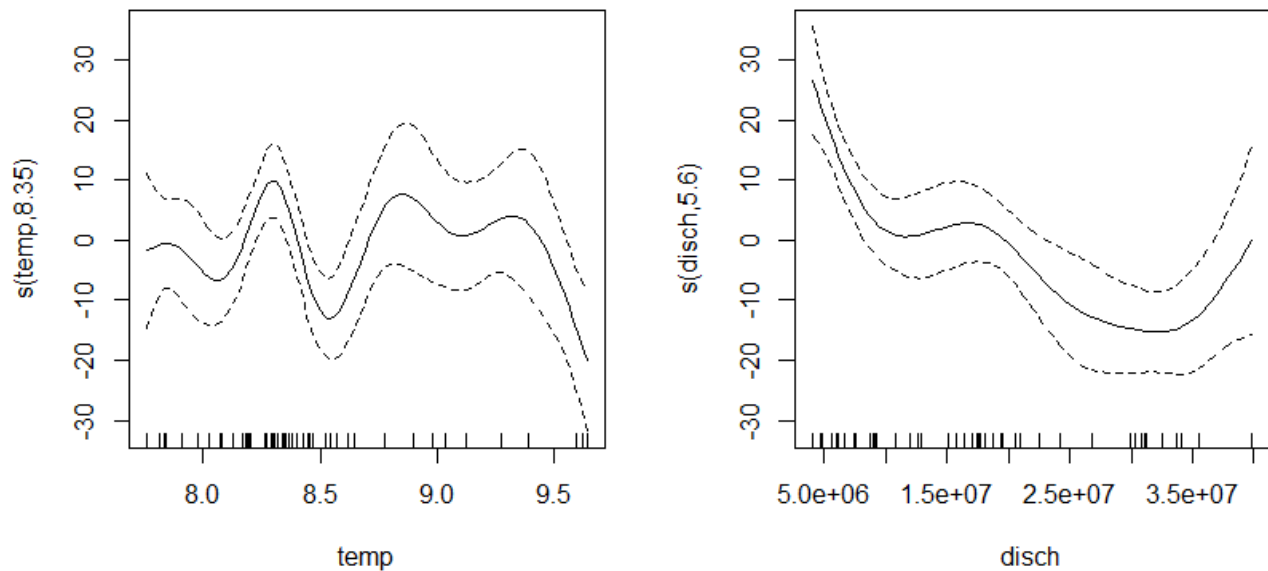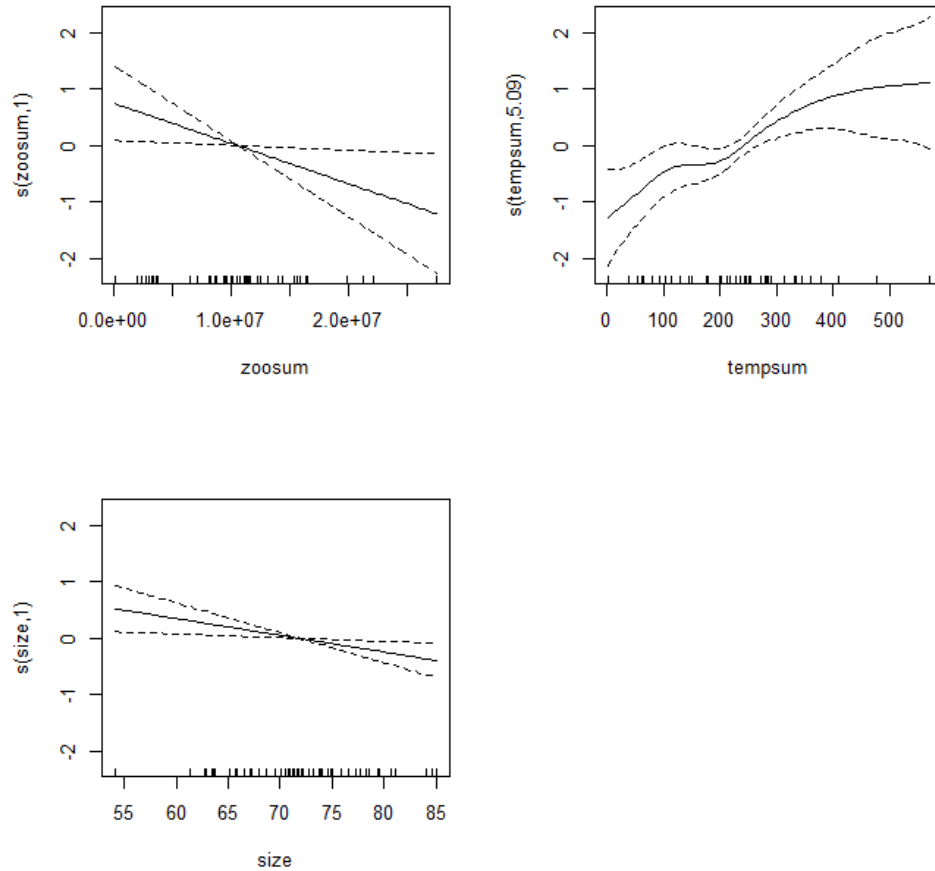

Figure 46: Fit of GAM Model 5 showing the effect on weight increase. The partial effects of each individual covariate (size at entry, water temperature and zooplankton densities) are plotted as smoothed fits, s(•). The variables size, tempsum and zoosum correspond respectively to cumulative values of size at entry, water temperature and zooplankton densities. Zero on the vertical axes corresponds to no effect of the explanatory variable. The broken lines correspond to 95% confidence limits for the smooth. Ticks on the x-axis indicate the locations of observations. The estimated smothers are given in brackets after the variable name on vertical axes.

Size and zooplankton densities had a negative linear correlation with the increase in weight in Model 5, with the zooplankton densities having a higher deviation from the mean. Increase in weight was positively correlated with cumulative temperature. At a cumulative temperature of less than 200

ᵒC, the increase in weight was less than the mean value. Above 200 ᵒC the increase in weight was greater than the mean value, but the confidence intervals were also large.

4.4. Discussion

4.4.1. Modelling Outcomes

Individual environmental parameters, e.g. water temperature, zooplankton densities, and water discharge, appeared to be poor predictors of smolt weight gain and zooplankton densities and the river discharge failed to predict the weight at catch (R^2 were 0.06 and 0.11; Table 15a). Water temperature was the main driver here explaining 55% of the smolt weight increases at the end of their outmigration (Model 14; Table 15a). The following agrees with other studies that showed a high correlation between the water temperature and fish growth (Goodlad et al. 1974, Friedland et al. 2000, Handeland et al. 2008, Reed et al. 2010). The interplay between different environmental parameters produced slightly better results in predicting smolt weight gains in the inlet (Models 9, 10, and 11; Table 15a).

Smolt size at entry on its own explained only 33% of the weight at catch and showed no predictive power for the weight gain (Models 8; Table 15). It is important to note that size at entry was found to be moderately correlated with water temperatures. However, in combination with three environmental factors the predictive power of this model increased dramatically, describing 78% of the weight at catch and 64% of the weight gains (Table 10**Error! Reference source not found.**). This indicates that the smolt size at entry in combination with other environmental factors is a critical component in the model. This was also supported by the finding of Chapter 2 that smaller smolts generally were able to gain more weight in the inlet compared to larger individuals (Figure 21).

Size at entry had opposite effects on weight at catch and weight increase: larger smolt achieved higher than average weight at catch but had the smaller weight increase. The following was also supported by Handeland et al. (2008) as they found that optimal temperature for growth increases with

the fish size and optimal temperature for feed conversion (ability to convert food into weight gain) decreases when the fish size increases. Thus, at the same temperature, smaller smolts will be able to grow less than the larger counterparts but have a higher food conversion efficiency.

Optimal water temperature for smolt to gain weight and have a high capacity for weight increase was 8.5 - 9.0 $^{\circ}$C, with the peak at ~8.7 - 8.8 $^{\circ}$C. The obtained range was low compared to the optimal temperature of juvenile Pacific sockeye salmon for growth (12 - 14 $^{\circ}$C) determined by Brett (1952), but was close to the results of Donaldson and Foster (1941) who found that optimal water temperatures for sockeye salmon from Skaha Lake (BC) ranged from 9 to 10 $^{\circ}$C. The difference between those two studies may be explained by optimal temperature being dependent on the amount of ration available (Brett et al. 1969).

The effect of the river discharge on sockeye smolt weight was not clear and was better correlated with smolt outmigration rates. Based on our model, it was found that only higher daily discharge rates (> 2×10^7 m^3) had a negative effect on smolt weights at catch and weight increase, supporting the hypothesis that at higher flow rates fish advance faster through the system preventing juveniles from gaining weight efficiently.

Zooplankton densities showed no deviation from the mean at the densities less than 8×10^5 ind/m^2 with greater densities leading to both larger than average weights and increase in weight. This finding supports the hypothesis that the density of the zooplankton is critical for fish juveniles to feed efficiently and gain weight (Edmundson and Mazumder 2001).

In addition, the conditions at the inlet head predicted reasonably well the smolt's outmigration duration in Rivers Inlet (Model 10; Table 10), with water temperature and the freshwater discharge driving this model. Our findings (Chapters 2 and 3) failed to show a correlation between smolt size at entry and the duration of migration. It was not dependent upon either the food availability or the size of

the smolt, as suggested in Chapters 2 and 3 and by other studies (Wood et al. 1993, Rutherford et al. 2005, Buchanan 2006, Carter et al. 2009, Ajmani 2011, Hodal 2011). Effect of temperature on the outmigration duration was not apparent but seemed to have a cyclic pattern. This may be due to the confounding effect of other variable(s) not included in the model. However, temperature was correlated with outmigration duration. Discharge and outmigration duration were negatively correlated, agreeing with the findings of Chapter 3.

Models that used the cumulative values of the environmental conditions within ±7 days from the day of entry did not significantly improve the available results (Table 11). The weight at catch was slightly influenced by the amount of time the fish spent at the head of the inlet, while the ability of fish to gain weight remained unchanged (Model 3 in Table 16a and Model 1 in Table 16b). The ability of these models to predict the outmigration duration was slightly lower than in the models that just used conditions at the day of entry. There are at least two possible explanations for this: (a) weight at catch was improved only for some of the fish suggesting that not all fish spent 2 weeks at the head of the inlet which is in agreement with Chapter 3 findings; and (b) juveniles used this area for adjusting to marine conditions or finishing the smoltification process resulting in minimal size gain during that period.

Previous research showed that the conditions throughout the inlet may be similar (Tommasi 2008, Wang 2014). Hence, the conditions used in the first cluster of models (DFO5) may be representative of conditions in the entire inlet, with some exceptions as observed in 2009, (Figure 33). Our second cluster of models (Type II) included averaged parameters throughout the inlet and cumulative values of water discharge, temperature, and zooplankton densities each smolt experienced during its outmigration. Models including conditions in the entire inlet overall were performing with lower certainty than models of the first model cluster. This may be explained in part by uncertainty introduced through point (spatially and temporally) measurements used in the modelling. Point observations may not be entirely

representative of conditions as juveniles will dynamically utilize the inlet and it will be hard to predict where and for how long fish will be experiencing conditions determined in station points.

Exclusion of DFO 5 station (3rd cluster) did not reduce the model ability to predict weight at catch. However, the effectiveness of the model decreased greatly (by at least two-fold) in predicting the weight gain. These results suggest that conditions at the head of the inlet appear to be disproportionally more important for some smolts and not so important for others which again agrees with the Chapter 3 findings. This does, however, indicate that the overall inlet experience is critical for predicting weight at catch. In addition, these findings can mean that although fish may not be using the head of the inlet for growth, the area is very important for establishing smolt ability to gain weight. In general, it was hard to interpret the models that include the cumulative values. Inability to tease apart the reason why the cumulative values are high or low makes the interpretation of the results less accurate.

It appears that conditions at the head of the inlet explain much of the final weight of the smolts. This potentially points to two possible conclusions: (a) the low utility of the inlet beyond it head; or (b) may suggest that the conditions at the head of the inlet are identical to the entire inlet, with would be in concordance with previous zooplankton studies (Tommasi 2008, Wang 2014). This certainly requires further investigation.

Unexpected correlation between zooplankton densities and weight at catch (or weight increase) was observed for type II models when the weight at catch decreased with the increase in zooplankton densities. The following trend might arise due to 1) an assumption that was made constructing the models or 2) shortage of available samples with high value of cumulative zooplankton densities. First, the average value for all stations was used to correlate the certain day with the set of the environmental conditions that would represent conditions over the entire inlet. However, fish can't experience conditions from all stations during the same day as it takes weeks for the smolts to travel through the

inlet. For instance, at the beginning of its outmigration, smolt most likely experienced conditions of the Wannock -Kilbella region, then moving towards through the inlet would progressively experience conditions across the inlet finally encountering conditions closer to its mouth. However, averaged parameters do not allow to accurately account for conditions of a particular inlet portion. Since cumulative values of environment parameters do not match spatial conditions fish may have experienced travelling through the inlet, the introduced assumption could lead to the model producing the results that could not be easily interpreted from the ecological point of view.

Second, the negative trend in type II models can also be due to the absence of data points in the region where cumulative zooplankton densities greater than 1.5×10^7 ind/m^2 (only 4 observations are present with densities higher than this value). Thus, to improve the quality of type II models a more detailed data of cumulative zooplankton densities are required. Nevertheless, with regard to Rivers Inlet system, very few fish were experiencing such high zooplankton densities, thus the model can be considered appropriate when comparing to other models in the range of zooplankton densities of 0.2-0.7×10^7 ind/m^2.

A significant advantage of models using the only head of the inlet conditions is their high predictive power. Alternatively, since whole inlet models had to use the duration of outmigration to calculate cumulative values, their predictive power is minimal. Because some smolts may spend up to 2 weeks at the head of the inlet, another cluster of models was designed and included the cumulative values of the environmental conditions within ±7 days from the day of entry. This, however, provided no significant improvement in the model's predictive ability.

4.4.2. Modeling the Effect of the Changing Conditions

In this study, we found that a set of the environmental conditions - river discharge, water temperature, and zooplankton density, and biological parameters (size at entry) - allowed successful prediction of the weight of the smolts at the end of their outmigration. Using the outcomes of our best

model (GAM Model 1) that used environmental conditions at the head of the inlet at the day of entry, the effect of changing environmental conditions on the weight at catch was investigated. Assuming the order at which smolts enters the inlet does not change, smolts were matched with the conditions that appeared a week later that their actual day of entry. This may effectively imitate either earlier smolt entry in the inlet or a shift in environmental conditions or both. Then, the difference between the observed weight at catch and predicted weight were compared depending on the period of entry to the inlet (Figure 47).

In 2008, when the zooplankton bloom was observed in late May, a shift in conditions negatively affected only early migrating (mid-April) smolts, while the rest of the juveniles benefitted from it (Figure 47a), especially the late-migrating ones.

In 2009, which had the earliest zooplankton peak of all years, and lowest water temperatures, the shift in the conditions benefited (and greatly so from late May onwards) smolts exiting the inlet throughout the whole outmigration (Figure 47b).

2010 was the warmest of the three years, with water temperatures approaching 10 ^{o}C by June 20. Zooplankton peak occurred between the peaks of 2008 and 2009, with zooplankton densities similar to 2009 levels. The majority of the smolts, except the early migrating ones (late April and early May), would have been negatively affected by a one week shift in environmental conditions (Figure 47c). It may be hypothesized that except relatively warm conditions, in Rivers Inlet a mismatch between "ideal" (maximized growth) environmental conditions and smolt outmigration timing is already in place. These are preliminary findings, but it appears that feeding conditions, as well as temperature stress associated with climate change (Wolfe 2010, Hodal 2011), may affect sockeye smolt performance in Rivers Inlet. In general, a shift to warmer temperatures and later zooplankton development negatively affected the early-migrating smolts, while the later migrating group may benefit from it. However, the results of 2010 showed that its conditions were perhaps close to the

critical ones, so further change of the environment might be devastating for the Rivers Inlet smolt development.

a) 2008
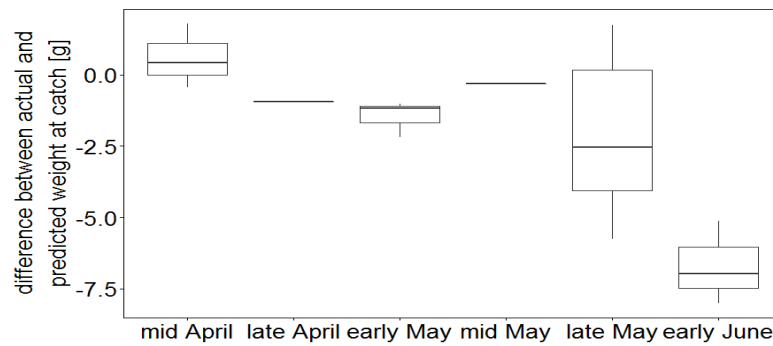
b) 2009
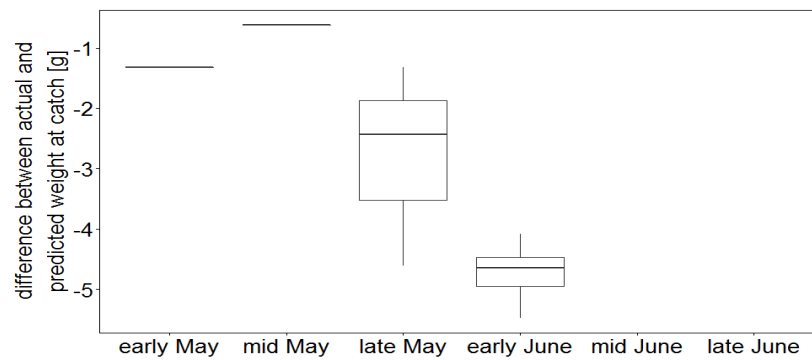
c) 2010
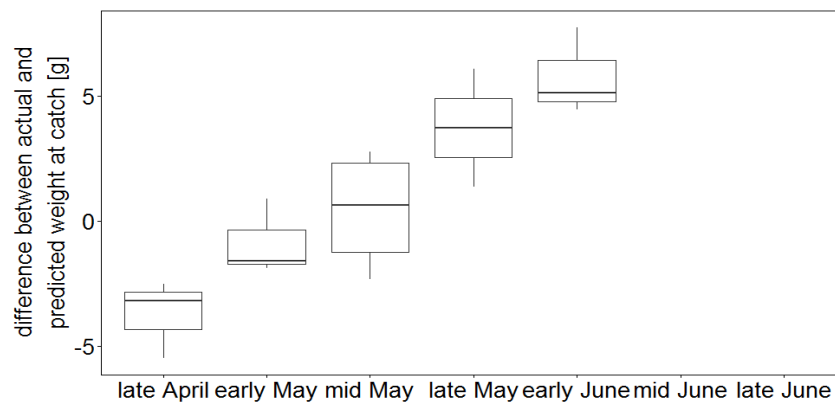
Figure 47: Predicted difference between the actual weight at catch and predicted weight at catch by GAM model for a) 2008 (n = 18), b) 2009 (n = 20) and c) 2010 (n = 21).

4.4.3.   Conceptualization of Factors Influencing Sockeye Smolts' Performance

The importance of the early-marine phase in salmon life history is crucial. The local conditions (<500 km) are very important in determining the survival success of the smolts (Pyper et al. 2001, Mueter et al. 2002). Thus, changes in the local conditions due to climate change may effect Fraser and Rivers Inlet stocks differently. Tucker et al. (2009) found that the coastal migration of the Fraser and Rivers Inlet sockeye smolts differs during the first year of their marine life. It was suggested that Fraser River sockeye salmon migrated to the north along the coast while Rivers Inlet juveniles utilized the inshore waters of central BC (Queen Charlotte Sound, Hecate Strait and Dixon Entrance) during the summer and fall (Tucker et al. 2009). Our data show that the River Inlet outmigration starts in April – May and ends in July-August. Even if fish spend four weeks in the inlet, smolts will reach the mouth by September. Potentially, Rivers Inlet smolts gather in local marine waters before migrating northward (Tucker et al. 2009, Beacham et al. 2014). Assuming similar foraging grounds, Fraser sockeye may reach the northern latitudes earlier or may migrate further north. The growth conditions are postulated to increase with the latitude due to a better quality of food (higher lipid content in zooplankton; Peterson and Schwing 2003, Mackas et al. 2004, Trudel et al. 2007). In addition, the predation rates on the shelf are higher than in the open ocean. If Rivers Inlet fish spend more time in the coastal area, they may be subject to a greater predation (Moss et al. 2005). As such, early marine experience may potentially to be critical in determining the survival success of the smolts (Hare and Francis 1995, Tommasi 2008).

To increase the survival of Rivers Inlet sockeye salmon, the juveniles should be able to grow to a larger size during their outmigration through the inlet as larger smolts are found to have higher survival rates in the open ocean (McGurk 1996, 1999, Beamish et al. 2010). Integrating the results from Chapters 2 to 4, it was found that the combination of both duration of the seaward migration and environmental conditions (e.g. prey availability, water temperature and discharge) are crucial in determining the growth performance of smolts. Following the idea of "smolt window" proposed by McCormick et al. (1998),

there is a set of optimal range of certain environmental conditions for the fish to improve their survival (see Chapter 4).

The outmigration timing plays an important role in migration experience. The day when smolts enter the inlet is very important because if the timing of the zooplankton bloom is delayed (due to changing conditions), the smolts outmigrating earlier in the season will have poor feeding conditions and hence less chance to grow to larger size. Prolonged stay in the inlet may compensate for the shortage of food. However, the longer stay in the inlet may impose higher predation rates and increased chance of parasite infection. Smolts outmigrating later in the season have a short stay in the inlet, so the timing of the zooplankton bloom can be crucial for them. If the feeding conditions are poor, juveniles will not be able to grow to a larger size during their short stay in the inlet. However, if the timing of the bloom matches with the timing of late-outmigration smolts, they may able to reach a larger size even during a short stay in the inlet.

Size at entry seems to be a very complicated factor for the fish. On one hand, the smaller the smolts at the head of the inlet the more they need to grow to reach the large size. On the other hand, smaller smolts can gain size/weight easier than their larger counterparts. The duration of the outmigration can equilibrate the poor conditions: in the low food availability scenario smolts slowly grow to the larger size by staying in the inlet for the longer period of time (early migrants). Indeed, holding behavior was observed for the smaller smolts during the early period of outmigration in 2009. Although smolts seemed not to grow much in the area of holding (at least during the early outmigration season), they were able to reach the average size of all smolts at the inlet's mouth.

As an example, consider a case when food availability is reduced during the entire outmigration season. Fish that migrate earlier in season would experience low discharge rates, colder water temperature and in general longer outmigration duration compared to the fish that travel later in the

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season (we do not consider the conditions during the freshet). As it was found in this study, the outmigration is size-independent, so both large and small smolts can start their outmigration to the open ocean earlier in the season. Poor feeding conditions is a challenging factor that fish face while traveling through the inlet. In general, it was found that the larger smolts travel through the inlet faster than the smaller counterparts with the latter also holding at the head longer. The holding behavior of smolts would provide them with the opportunity for additional growth as they would experience higher (and possibly quality-wise better) prey concentrations later in the season. The smaller smolts also are more efficient at gaining weight/size when such opportunity is available. Larger smolts gain weight slower and due to the shorter stay in the inlet may not benefit much when the feeding conditions are poor. As a result, at the end of the outmigration through the inlet, both small and large smolts can reach a similar size/weight and have an equal chance for survival in the open ocean (Figure 48).
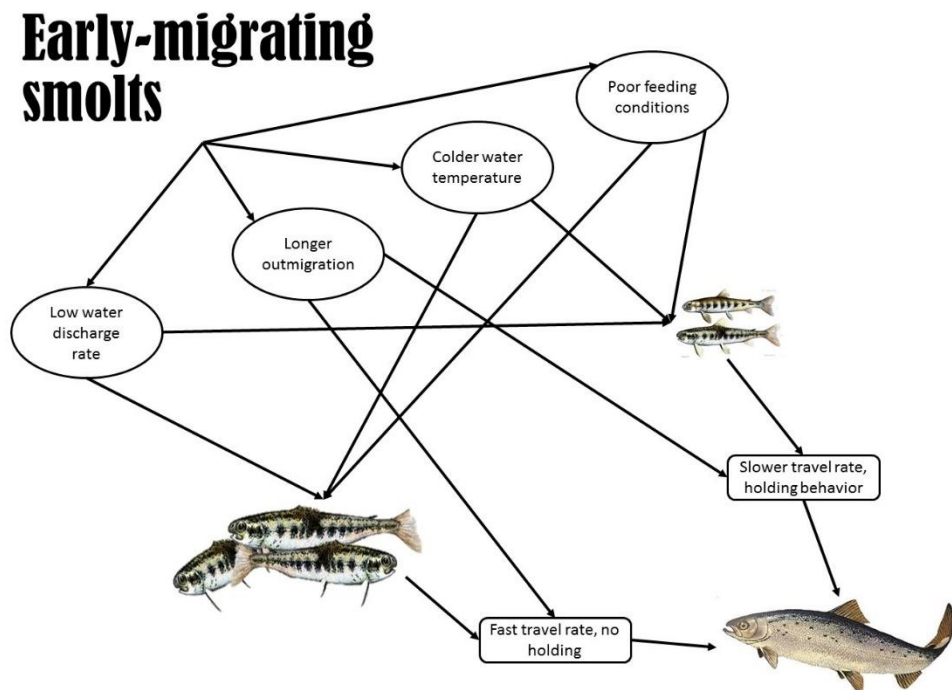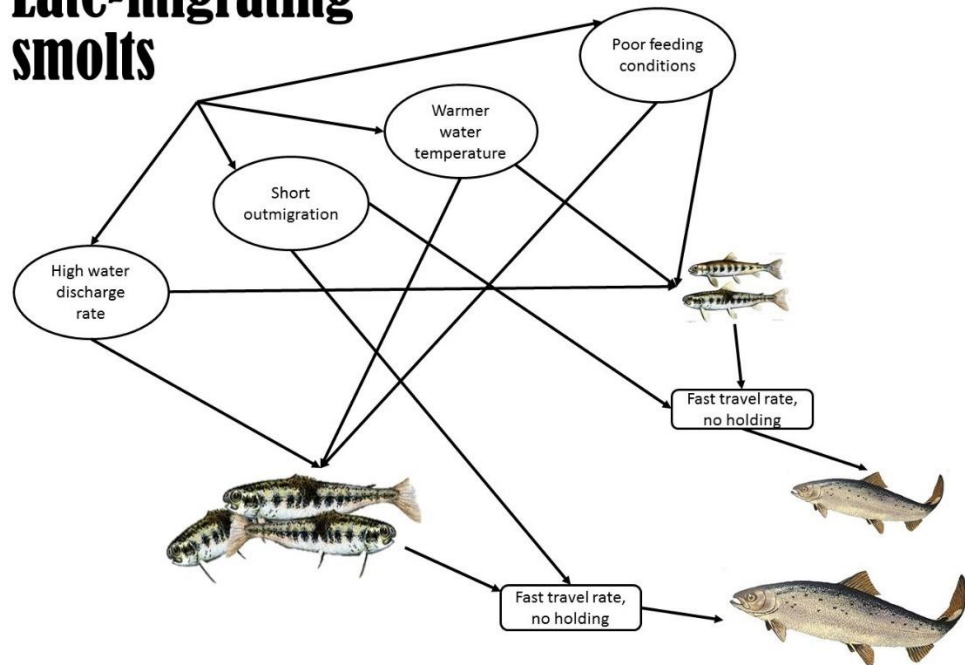
Figure 48: Two scenarios synthesizing the major findings about the factors affecting aspects of estuary migration of Rivers Inlet sockeye smolts during the present study: effect of poor feeding conditions on early- (top) and late- (bottom) migrating smolts.

Juveniles that migrate later in the season are subject to the higher water temperatures and higher freshwater discharge rates. Higher water temperature can increase the metabolic demand of the fish and make the fish more susceptible to parasite/disease development. Higher water discharge can 1) flush out the phytoplankton from the inlet that can reduce the zooplankton availability, 2) decrease the outmigration duration or 3) increase the energetic demand for juveniles holding in certain portions of the inlet as the fish have to battle with the high water velocities and elevated temperatures. Smolts that migrate late experience shorter residence time in the inlet that may negatively affect the fish if the feeding conditions are poor. Thus, small smolts that enter the inlet later in the season will not be able to hold in the area (or they chose not to hold) and travel faster through the inlet similar to the larger smolts. As a result, smaller individuals will likely not be able to gain as much weight to equilibrate with larger ones which may result in reduced survival (Figure 48).

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4.5. General Summary

The collapse of the sockeye salmon population of Rivers Inlet drew great attention to this area (McKinnell et al. 2001). A number of causes for the decline of sockeye population were proposed, such as overfishing, logging around the streams of the Owikeno Lake (Raymond 1988, Nehlsen et al. 1991, Thorstad et al. 2008, Kostow 2009), and change in ocean conditions (Hare and Francis 1995, Friedland et al. 2000, Pyper et al. 2001, Mantua et al. 2010). Recently, attention has been drawn to the early marine phase, suggesting that the timing and the duration of smolt outmigration may be a major factor determining the juvenile marine survival rate (McCormick et al. 1998). Previous estimates of outmigration duration in Rivers Inlet were found to be 1– 3 weeks with an average migration speed of 1.9– 2.9 km/day (Buchanan 2006, Ajmani 2011). The recent work by Stocks et al. (2014) determined that smolts can spend 3– 7 weeks in the inlet, yielding an average migration speed of 0.7– 4.2 km/day. Sockeye salmon survival and growth were strongly influenced by environmental parameters (McKinnell et al. 1998, Beamish et al. 1999, 2010, McDonald 1969, Torgersen et al. 1999, Cooke et al. 2011). In Rivers Inlet, several studies proposed the importance of water discharge, bloom timing, and zooplankton quality, and their variability may be factors influencing the timing and the duration of the seaward migration (Buchanan 2006, Wolfe 2010, Ajmani 2011, Stocks et al. 2014).

The first goal of this study was to determine the inter-annual variability in the duration of the outmigration of sockeye smolts between 2008 and 2011, and the role of fish size in the outmigration dynamics. It was hypothesized that migration rate differs significantly between years with sockeye salmon smolts spent more time in Rivers Inlet in 2010 and less in 2008/2011. This prediction was partially supported by our findings. Analysis of the four-year dataset showed that the duration of outmigration through Rivers Inlet varied between the years but sockeye juveniles spent the longest in 2008 and 2010 (~4 weeks) and less time in 2009 and 2011 (~2 weeks). Based on the assumption that fish swim continuously throughout the inlet, average smolt travel rates were 2.2, 4.0, 2.0 and 3.6 km/day in

2008, 2009, 2010 and 2011 respectively. In terms of BL/s, the fish traveled through Rivers Inlet at a speed of 0.2 – 0.5 BL/s which was slower than the theoretically determined optimal speed of active migration (1 BL/s). In our study, we found little influence of smolt size on the duration of their outmigration, but the day of entry and the duration of outmigration were inversely related. Thus, the later the fish entered the inlet, the less time it stayed there.

We also predicted that the increase in smolt size is related to the timing of the migration and the initial size at entry to the inlet. The duration of the outmigration was an important factor in determining the size of the smolts. Fish that spent more time in the inlet, on average, gained more in both length and weight. Smaller smolts gained more size and weight traveling through the inlet than the larger smolts. So, even smaller smolts entering the inlet have an opportunity to grow to a similar size as a larger faster out-migrating smolts. Size at entry varied greatly in Rivers Inlet (54 – 85 mm). Potentially, such a large variability in size at entry might be attributed to the different conditions (zooplankton availability and type, water temperature, etc.) in different basins of the Owikeno Lake or different growth rates in the lake throughout the outmigration season. Smolts hardly were able to double their weight in 2008– 2011. The growth rate in 2009 was low, and the majority of the fish growth occurred in the Kilbella region (~6 mm size increase), and there was almost no growth at the Wannock site (0.06 mm increase in size). The largest increase in size throughout the outmigration was in 2010 (fish gained 8 mm) followed by 2008 (~6 mm gain), 2009 and 2011 (2– 4 mm gain).

The second goal of this study was to investigate temporal patterns of the sockeye smolt migration over the entire Rivers Inlet length in 2009. Our prediction was that smolt migration rates vary spatially and temporally due to fish dynamically using "holding points." In 2009, two possible migration behaviors appeared to be present for sockeye smolts in Rivers Inlet. The first type of behavior exhibited a "holding" pattern at the head of the inlet for 2-3 weeks. The second type of smolt behavior was continuous swimming through the inlet with no holding that was very evident for the later migrating fish. On average,

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traveling times through the Main Channel were within theoretically determined range (0.9-1.6 BL/s), while speeds in Darby Channel were slightly lower (0.2-0.6 BL/s). These two types of the behavior were dependent on the day of entry to the inlet and potentially size of the fish. Fish that entered the inlet prior to the mid-June spent two weeks at the head of the inlet and were relatively smaller compared to larger smolts that entered the inlet later in the season and seemed to swim continuously throughout the inlet. In general, the movement in the upper portion of the inlet was slower than at the head that was consistent with the findings from other areas (Moore et al. 1995, Thorstad et al. 2007, Kocik et al. 2009).

The third goal of this thesis was to explore whether migration dynamics is controlled by environmental parameters in 2009. We proposed that the freshwater discharge is strongly correlated with the timing and rate of outmigration of sockeye salmon in Rivers Inlet in 2009. It was found that Wannock River discharge was an important factor influencing the duration of smolt outmigration throughout the inlet. Mean geometric water discharge that each smolt experience throughout its outmigration explained 39% of the variability in the duration of the outmigration agreeing with studies in other regions (Carter et al. 2009). Due to the poor representation of zooplankton data at seining locations (e.g., all the zooplankton samples were only collected in the Main Channel, at locations different from where the fish were caught), the spatial aspect of fish distribution with respect to zooplankton availability requires further investigation. However, fish stayed longer at the head of the inlet where the zooplankton densities were the highest (DFO5; Figure 33).

The last goal in this thesis was to explore the effect of the combination of the environmental factors on the physiological condition of the smolts. Thus, it was established that conditions in the whole inlet are important in determining the weight of the smolts at the mouth of the inlet. Based on the results of the GAM models, no difference in environmental factors was observed between different locations in the inlet, although the zooplankton densities were higher at the head of the inlet. The conditions at the head of the inlet alone explained almost 78% of the variation in weight at catch and 64% of the variation

in increase in weight for the fish. This model was based not only on the combination of three environmental factors but also on the size of the smolts at entry, agreeing with the findings in Chapters 2 and 3. The GAM model was able to predict the 64 % of the variation in the duration of outmigration through Rivers Inlet (Table 10). The outmigration duration, according to the model, was only dependent upon the two environmental factors, i.e. river discharge, and water temperature. Both these factors are expected to be highly affected by climate change and regional warming, potentially negatively influencing smolt residence time in Rivers Inlet.

In addition, based on the results of the model there is an 'optimal window' of environmental conditions to ensure the fastest growth and greatest weight gain. Based on the results of type I models, we found that in Rivers Inlet the optimal conditions were low water discharge ($< 2x10^7$ m^3) and high zooplankton densities (>8-10 x10^5 ind/m^2). Furthermore, size at entry of the smolts was very important, with larger (>73mm) smolts having a higher chance to reach larger weight but having less ability to gain weight. Outmigration season was important determining the environmental conditions in the inlet. Prolonged residence time during the early outmigration season along with the holding behavior that was observed for smaller smolts can result in additional weight gain, increasing the weight at catch and potentially higher survival. This benefit could, however, decrease throughout the season due to reduced residence time in the inlet and potential absence (or shortening) of fish holding behavior at the head of the inlet.

It is plausible to suggest that Rivers Inlet may act as the area of equilibration between variable smolt qualities produced by the Owikeno Lake. This means that fish are experiencing vastly different environmental conditions may have the similar outcomes: (a) if there is high water discharge and fish spend short periods of time in a very productive inlet; or (b) when water discharge is low, and fish spend long periods of time in an unproductive inlet.

4.6. Future Suggestions

The greatest limitation in this work came from the choice of the method for otolith preparation. The technique used in this study technique was perfect for the LA– ICP/MS study. However, it presented problems when counting the daily rings. One of the biggest assumptions of this study was the use of the average width of the daily rings. This assumption is valid over shorter distances but might be a problem if the fish spent more time in the marine phase. It is well known that width of the rings is not uniform, contrary to Campana (1992), but can vary within days. In some laboratory studies, it was shown that the rings may not be deposited daily if the fish is starving, or two rings can be deposited daily if the food is abundant (Neilson and Geen 1982).

Regarding the sample preparation, the otolith should be polished separately from both sides and then mounted together for the analysis to avoid overpolishing and to ensure a better view of the rings. Instead of a puck, the thermoresign should be used when polishing the sample. In addition, the count of the rings should be done independently by at least two people, and then the average of the counts should be taken.

The assumption that fish deposit rings daily certainly warrants further investigation. The laboratory study should determine the length of the starvation period that triggers fish to stop depositing daily rings. It is also important to know the lag time between the change in the environment and the recorded change in the otolith. As in some species, Ba:Ca ratios in otoliths appeared to be accurately recording the change in the Ba/Ca in ambient water, (Bath et al. 2000); while it requires at least 30 – 60 days for eels' otolith composition to be completely at equilibrium with ambient elemental conditions (Yokouchi et al. 2011).

With respect to the environmental conditions, this work only considered the quantity of the food. Currently, we know that variations in the Rivers Inlet zooplankton productivity and community composition may potentially influence the survival rates of the juvenile salmon (Tommasi et al. 2013a). Thus, quality of the food source might be an important factor that can improve the predictive model. In

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addition, the model should be further tested in its ability to be able to predict weight and duration of the outmigration on smolts from other years.

The obtained GAM models should be tested with additional datasets to see whether obtained weight at catch through the model matches the actual weight of the fish. The following could have been done on the fish from 2011, however not all environmental conditions were recorded in that year.

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To validate the method used by Stocks et al. (2012), the break point was determined by Sr:Ba ratio and was compared to the break points obtained by Sr:Ca or Ba:Ca ratios. Ba and Sr concentrations are affected by such environmental variables as temperature, salinity, and ambient elemental concentrations (Elsdon and Gillanders 2003, 2005, Elsdon et al. 2008) but also by fish growth and physiology (Gretchen et al. 2000, Secor and Rooker 2000). Hence, incorporation rates into the blood and then into the otolith may vary between the elements. To determine whether there is a difference between Sr and Ba and Sr:Ba ratios, the difference in break point was calculated for these ratios. Sr:Ca ratio was chosen as a reference point as most of the papers uses Sr:Ca ratio for determining the marine entry (Campana 1999, Secor and Rooker 2000, Ruggerone and Volk 2003, Wells et al. 2003, Elsdon et al. 2008, Yokouchi et al. 2011, Freshwater et al. 2015).
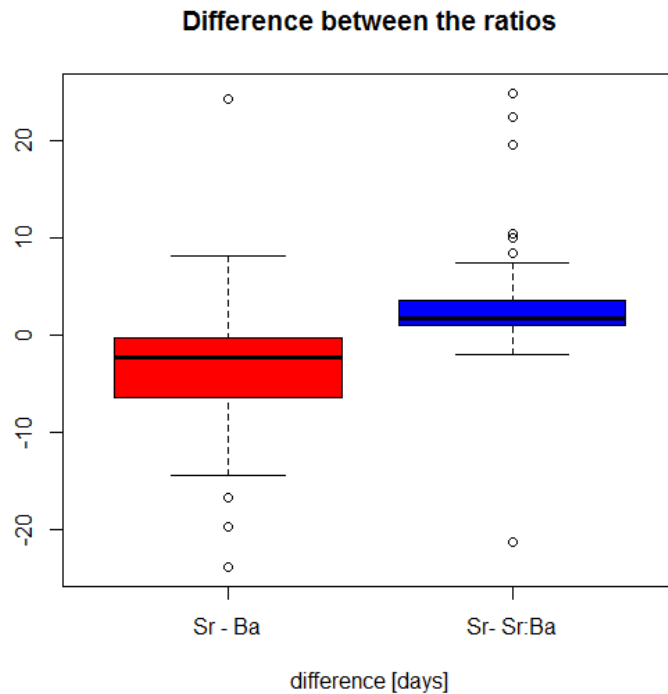
Figure 49: The difference in determining the break point (in days) between a) Sr and Ba signals (red) and b) Sr and Sr:Ba signals (blue). Median values (black solid horizontal line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Empty circles are outliers.

Compared to the Sr:Ca ratio, Ba:Ca ratio shows a break point 3 days earlier (-3.6 ± 6.7 days), while the Sr:Ba ratio underestimates the break point, occurring 3 days later (3.0 ± 5.5 days). The difference in both cases was statistically significant (p << 0.001). In both cases, the 'ouliers' may differ by 20 days (Figure 50).
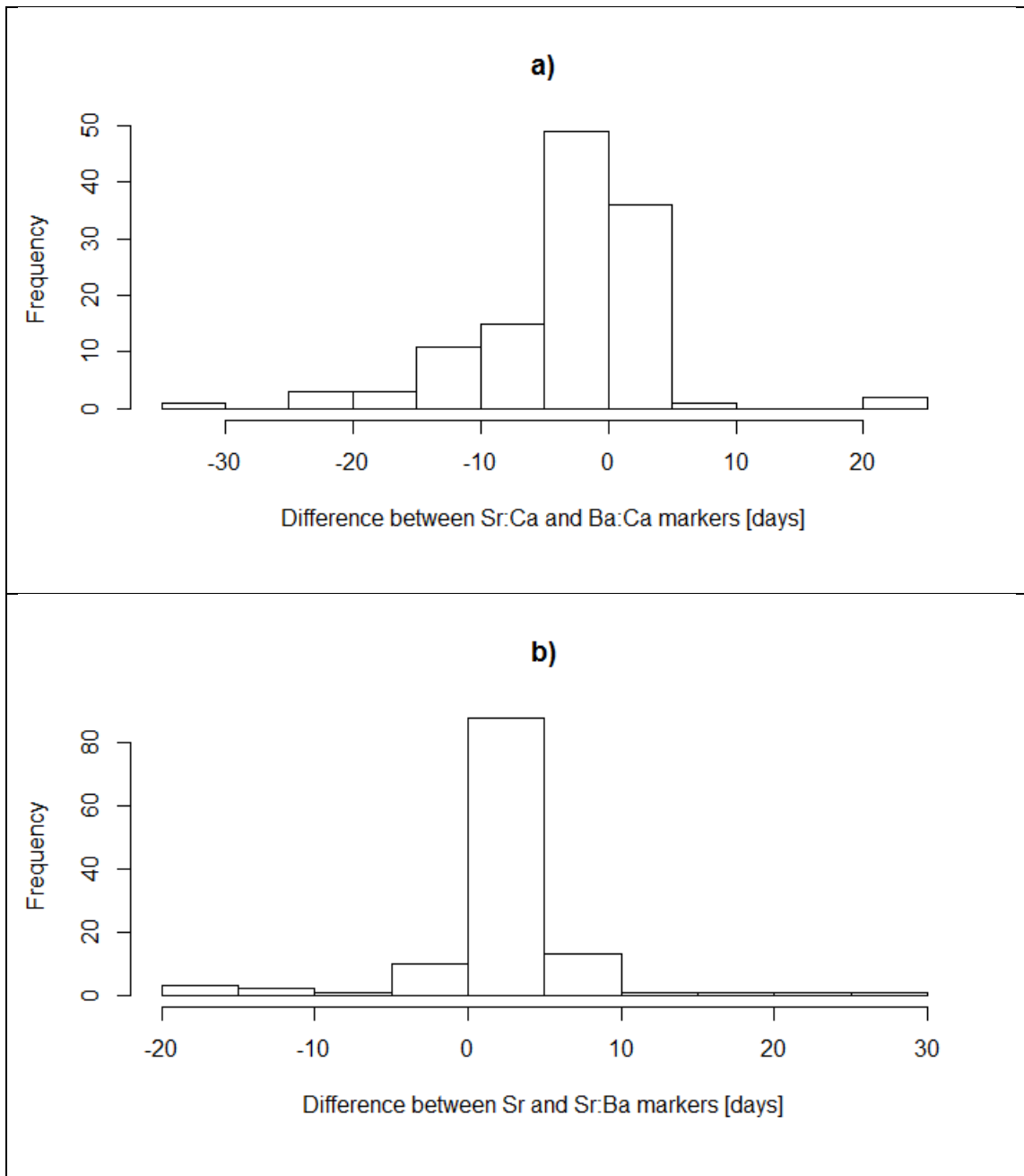
Figure 50: Distribution of the differences (in days) between reference marker (Sr:Ca) and a) Ba:Ca, b) Sr:Ba markers.

In order to determine what causes such a big difference the Sr:Ca, Ba:Ca and Sr:Ba plots were investigated. Mg:Ca plot was added for a comparison and was discusssed in Appendix B.
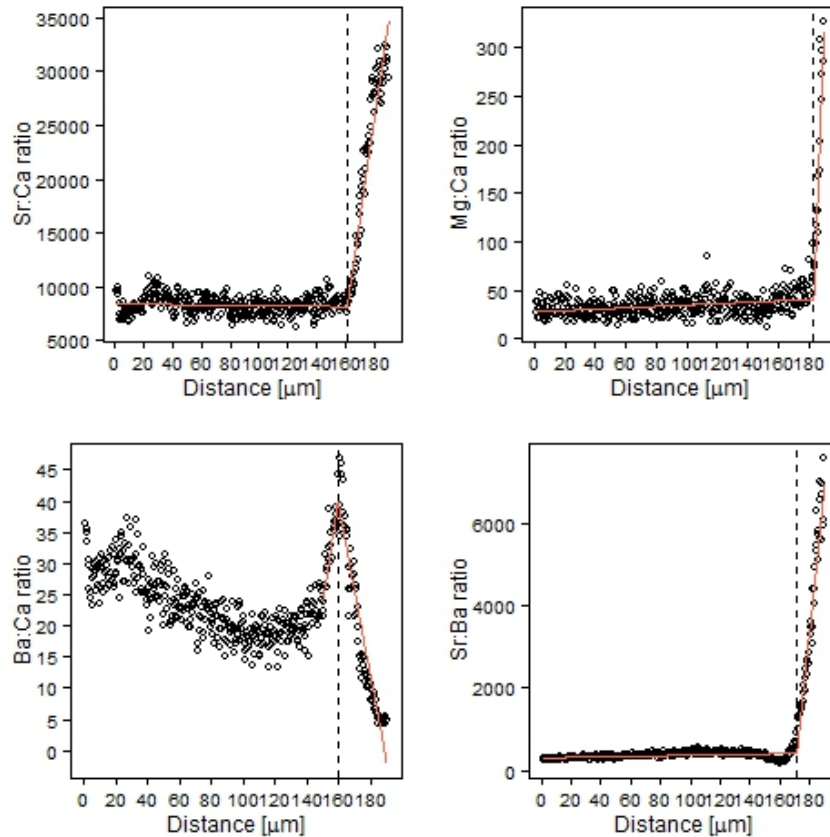
Figure 51: Example of the change in Sr:Ca (top left), Mg:Ca (top right), Ba;Ca (bottom left) and calculated Sr:Ba (bottom right) signals along the ablation path.

Figure 51 is a good example that illustrates just a slight difference in the break points for different elemental ratios. In the majority of the cases, the change in Ba signal happened earlier than in Sr (Figure 50) and as a result, the Sr:Ba ratio breakpoint happened later. Also, it is important to notice that the Ba:Ca signal was not constant prior to the break point as has been demonstrated in other studies (Gretchen et al. 2000, Elsdon and Gillanders 2005, Martin et al. 2013) but increased right before the break point. This behavior of the signal will be discussed later in this section. In the otolith analysis of *Galaxias maculatus,* Hale and Swearer (2008) found that the change in Ba:Ca signal also happened before the Sr:Ca. However, their Ba:Ca signals were quite flat prior to the break point.

Clearly, either the incorporation of elements into the otolith happens at different rates and/or the fact that change in the signal takes a longer/shorter time to show up causes the signals to appear at different times. Partition coefficients (D), the ratios between the concertation of trace element in the otolith to that in ambient water may be a good indicator of the relative incorporation rates of trace elements into the otolith. A higher coefficient shows faster incorporation rate into the otolith. Coefficients measured in Atlantic cod were 0.28 ± 0.08 and 0.18 ± 0.14 for Sr and Ba, respectively (Stanley et al. 2015). However, in the study of Atlantic salmon (*Salmo salar*) otoliths, the incorporation of Ba was more efficient than Sr. This might be linked to higher uptake and incorporation rates into otoliths (Braux et al. 2014). Different uptake and incorporation pathways could explain the observed lag in Sr incorporation in otoliths (Elsdon and Gillanders 2003) and may hint at different metabolic processing of the trace elements. In other studies of different species, D_{Sr} was also greater than D_{Ba} (Gretchen et al. 2000, Tabouret et al. 2010). Hence, it seems that Sr is incorporated more readily compared to Ba. However, the incorporation rates may also be species-specific and thus additional data of Ba and Sr concentrations in the estuary and laboratory studies is necessary to make a conclusive statement on incorporation rates of the metals into the sockeye salmon otolith. In addition, previous studies showed that otolith composition react very fast to changes in water chemistry (Ruggerone and Volk 2003, Miller 2011), thus the signal will appear at approximately the same time on the otolith as the change in the water properties. So, taking into this account, one would expect for Sr to respond earlier than Ba; however, in the example above the opposite trend is observed.

Another proposed explanation as to why Ba:Ca peaks take place earlier links the change in Ba:Ca signal with transition into an estuary-type environment, while the Sr:Ca signal marks the permanent transition to the marine environment (Hale and Swearer 2008). This statement is just a hypothesis to explain the earlier emergence of the peak in Ba rather than in Sr. To test this hypothesis the progression

of metal:Ca signals were correlated with the position of the ablation path on the picture of the otolith (the results are shown in Figure 55 and discussed later in this section).

While the Ba signal varied significantly prior to the break point, the variation in Sr signal was less pronounced due to the large difference is Sr counts between fresh and salt water; so the Sr:Ca signal looks much "cleaner". The Ba:Ca change is just only within 30 ppm units while the Sr differs within ~4,000 ppm units, so a minor change in Sr concentration is masked by these large difference.

Figure 52: Example of the "double peaks" in change in Sr:Ca (top left), Mg:Ca (top right), Ba;Ca (bottom left) and calculated Sr:Ba (bottom right) signals along the ablation path. Red errors indicate the secondary peaks in the signal profiles.

In cases when the Sr concentration does not have such a pronounced change (Figure 52), the smaller Sr "peak" can be seen at ~90 μm and a permanent change in Sr happened at ~130 μm. The

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position of two peaks for Sr matched the position of the peaks in Ba:Ca signal in this case. However, during the regression analysis, the program determined that the break point of Ba:Ca signal corresponds to the first, more evident peak, rather than the actual one that happened later due to the latter being masked by the former. This pattern was most evident when scan speed was slower (0.025 μm/s; Figure 52). However, a similar pattern was present throughout the whole analysis when scan speed was faster (2.0 μm/s; Figure 53). But because of the faster scan speed, the actual break point in Ba:Ca signal could not be detected (Figure 53). As a result, the Ba:Ca break point appeared far earlier than the one determined from the Sr:Ca ratio.
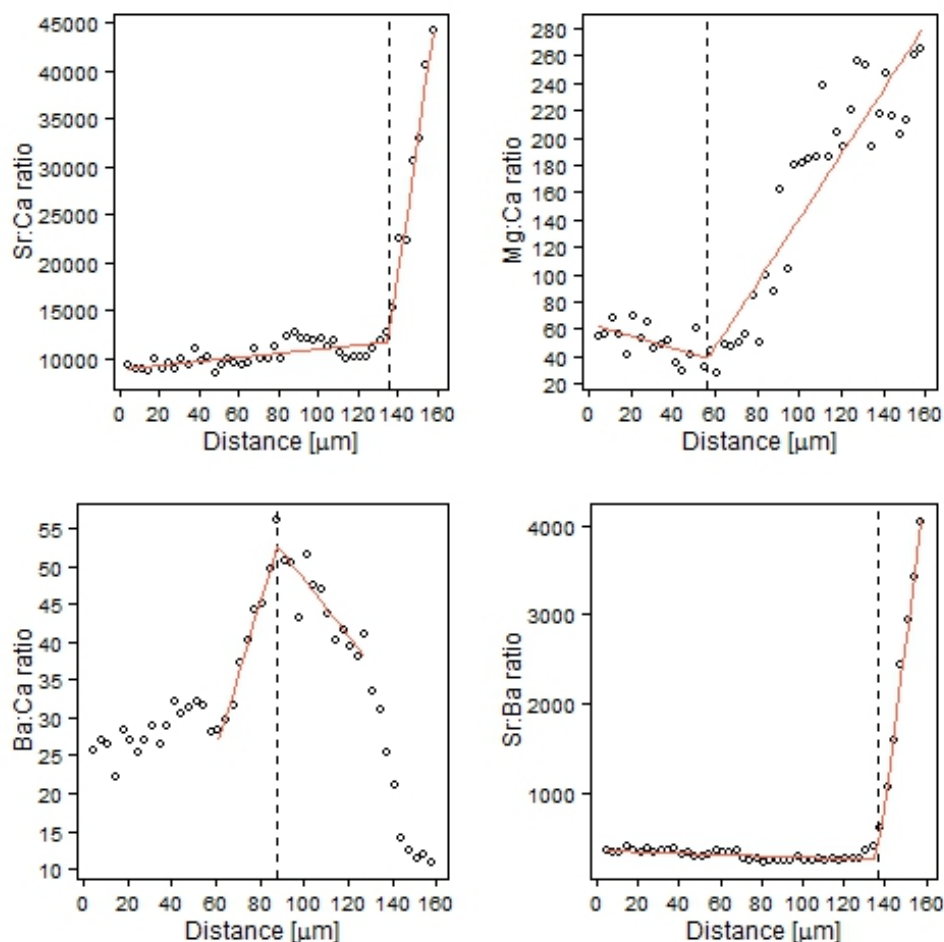
Figure 53: the example of the Sr:Ca (top left), Mg:Ca (top right), Ba;Ca (bottom left) and calculated Sr:Ba (bottom right) signals along the ablation path at higher scan speed of 2 μm/s.

This is why it seems that Ba is more sensitive to a change in the environment (Figure 53) as the peak in Ba happens at ~88 μm, earlier than the change in Sr signal that happened at ~130 μm, since due to the faster scan speed another smaller Ba peak is hardly visible at ~130 μm. As a result, large differences between Sr and Ba break points is established in this case, pushing the Sr:Ba break point to occur later on the ablation path in those instances when Ba signal changed significantly later than Sr (Figure 54).
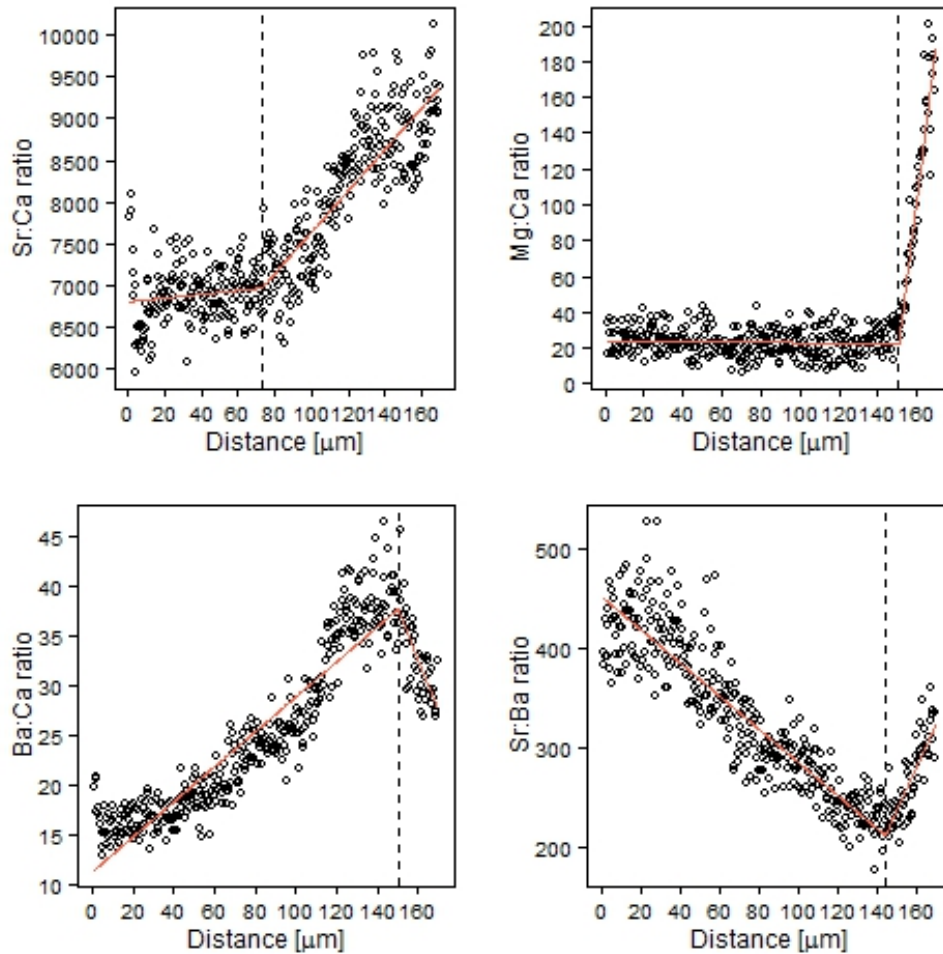
Figure 54: Example of the later change of Ba:Ca (bottom left) signal in relation to Sr:Ca (top left).

In some instances, the Ba signal changed significantly later than Sr (Figure 54). Comparing the scale between Figure 52 and Figure 54, it can be seen that the latter graph probably corresponds to the 'small' first peak on Sr:Ca plot from Figure 52. So in this case it seems that the Ba break point occurred later than the Sr one. It is also important to note the low Sr counts in this case (although the fish has

already traveled through the whole Inlet and spent 37 days in salt water). The fish also had unusual isotope values $\delta^{13}C$ of –30.73 and $\delta^{15}N$ of 7.44, while Mg showed quite a drastic change in the signal that might be related to starving of the fish (further is discussed in details in Chapter 3)

Apart from discussing the relative position of break points determined by different methods it is important to notice the unusual behavior of the Ba:Ca signal. In just in few cases the Ba peak was constant, but usually peaked at a maximum value after decreasing permanently (Figure 51 – Figure 54). To investigate this issue as well as "double" peaks in the signal, the signal profile was compared to the position of the ablation path on the otolith (Figure 55).
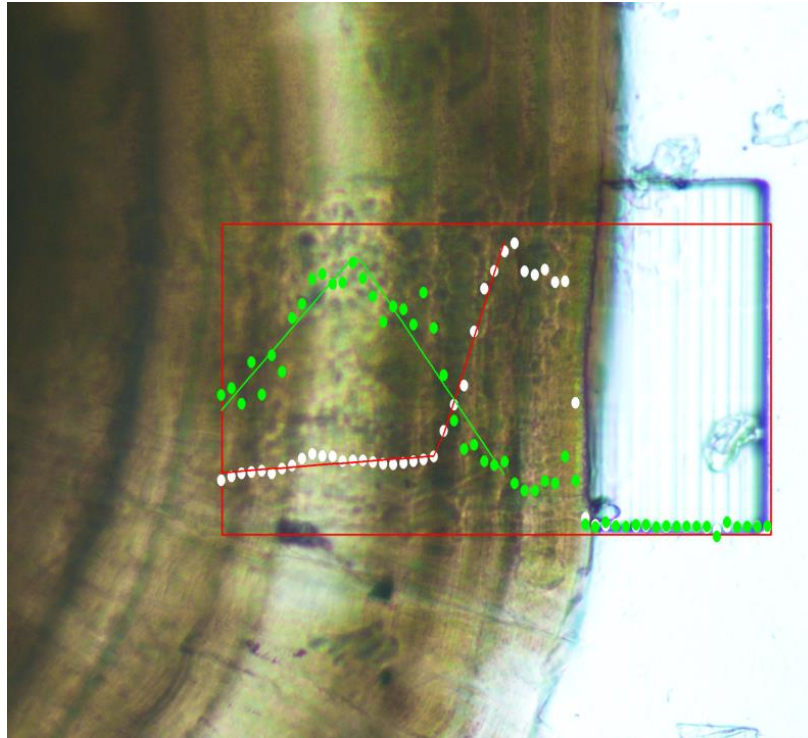
Figure 55: Example of the position of the "double" peaks on the otolith. Red rectangle represents the border of the ablation path; green dots is Ba:Ca signal and white is Sr:Ca signal .

As illustrated above, the first peak in the signal was situated on distinct translucent zone that according to Freshwater et al. (2015) represented the winter freshwater zone. So, some physiological processes that happen may be influencing the incorporation rate of the element, resulting in increase of

both Sr and Ba (i.e. potentially the rate of formation of aragonite was lower, which may have resulted in higher metal:Ca ratio (Tang et al. 2008a, 2008b).

In addition, some research has demonstrated an increase in Ba incorporation at higher levels of Sr:Ca in the water (de Vries et al. 2005). Sr and Ba might co-precipitate into the otolith where Sr^{2+} occupies multiple sites, including the normal lattice position of Ca^{2+} as well as other "nonlattice" sites. But due to the larger size, Ba^{2+} may be preferentially incorporated into these nonlattice sites. De Vries et al. (2005) suggest that the occurrence of these defects, which may be positively correlated with Sr concentration, could increase the incorporation of Ba at higher Sr concentrations. This might be true for the second peak, but not be related to the first one as the first peak corresponds to the freshwater residence stage. De Vries et al. (2005) also suggested that Ba:Ca ratio may be affected by the change in somatic growth rates. However, additional experiments to determine the effect of temperature and growth rates on sockeye smolts are needed to validate the proposed hypothesis.

APPENDIX B: REJECTED SAMPLES

Out of 15 chosen samples from the Wannock site, only 3 samples showed a change in Sr:Ca signal. The majority of the rejected samples had a V-shaped Sr profile with the trough of the profile located at the same distance where the increase in Ba profile take place (Figure 56). Since the ratio of Ba:Ca is low, this increase may be a part of the 1st smaller peak described in the Appendix A. However, previously, this peak was assigned to the smoltification process (parr– smolt transformation) that happens at the lake. However, all Wannock samples have this peak present in their profiles, thus this peak may be attributed to other physiological change that happens right after smolts exit the Wannock or the "partial" smoltification that occurring at this location (smolts finishing the parr– smolt transformation).
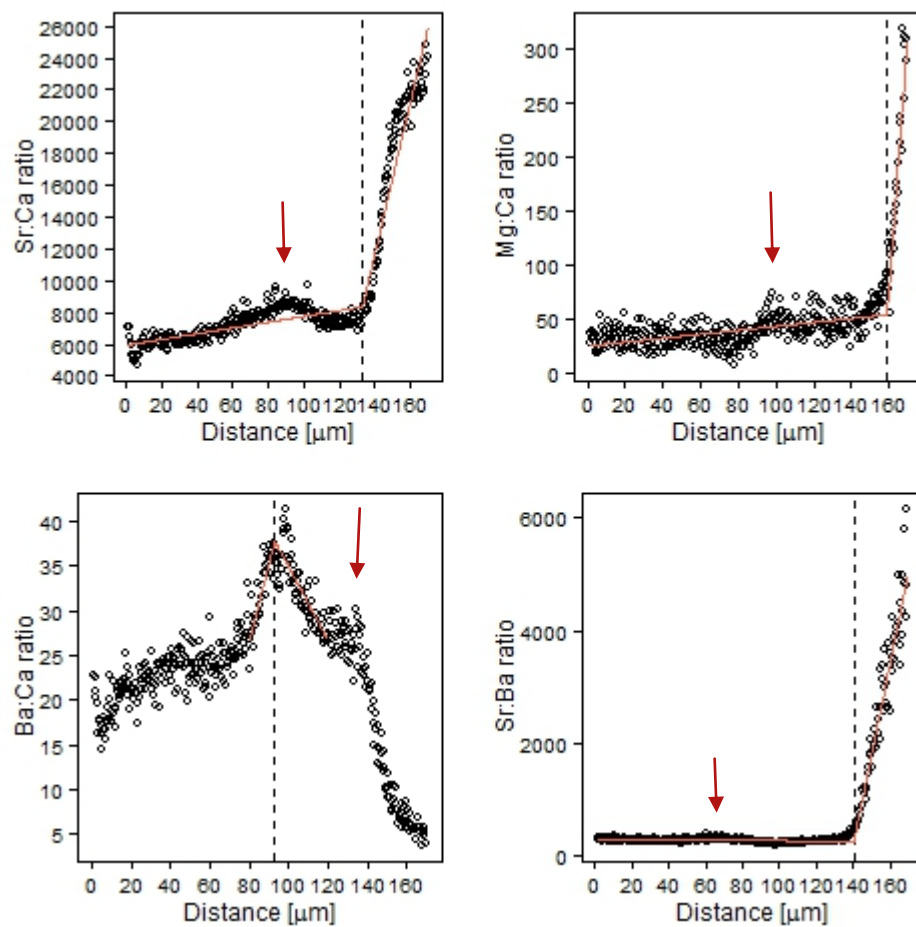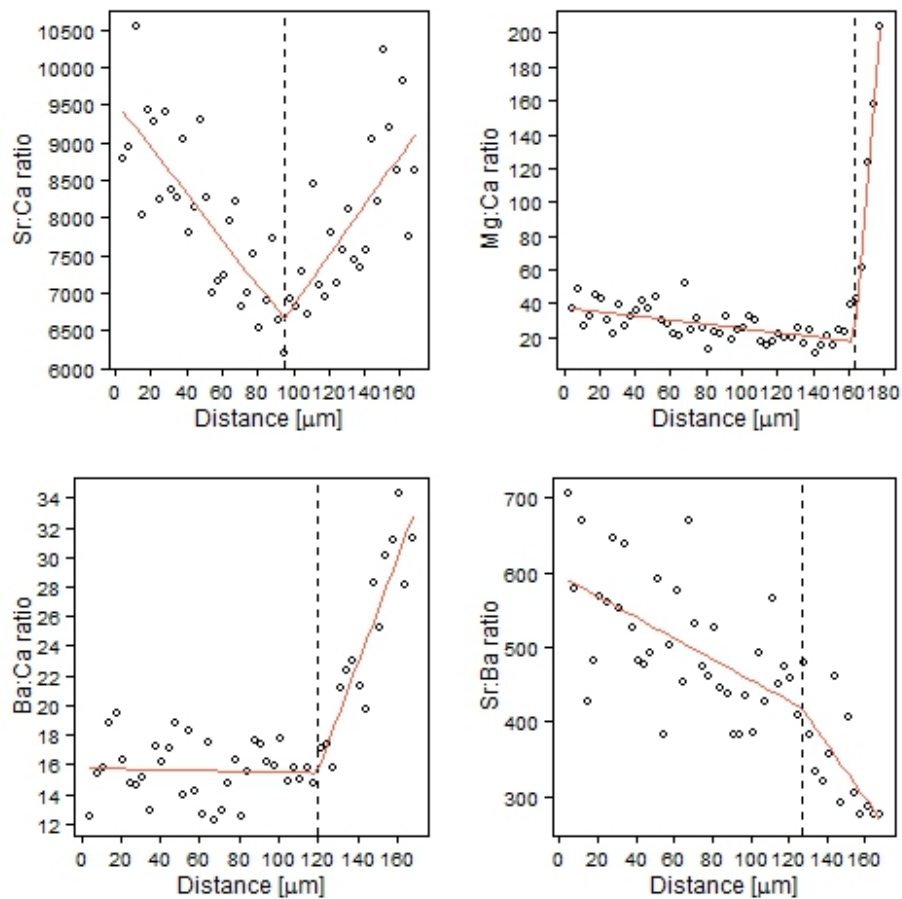

Figure 56: Example of the change in Sr:Ca (top left), Mg:Ca (top right), Ba:Ca (bottom left) and calculated Sr:Ba (bottom right) signals along the ablation path.

In aragonite, the concentration of Ca is fairly constant so it is used as a reference material for determining the minor elements concentrations. As the signal was measured as the element: Ca ratio, potentially the decrease/increase in Ca concentration might be altered due to the physiological stress/process. So the drop in ^{43}Ca:^{44}Ca signal may be an evidence of some sort of physiological process. However, examining the signals, this ratio was relatively stable, meaning that something other than the physiological change may be the cause of such Sr:Ca signal unless the physiological process does not involve the incorporation of the Ca but minor elements.

It is noteworthy that in all of the rejected samples the Mg:Ca signal increased sharply close to the edge of the otolith, as in "normal" samples (where the Sr profile shows a break point), from 20 ppm up to 200. However, the Mg:Ca signal overestimates the day of entry by 5 to 14 days in most cases, therefore it was not used as a break point estimate.

The change in the Mg:Ca is similar between the rejected and analyzed samples, while there was a drastic change in the Sr and Ba signals between these samples. Thus the process only affected incorporation of certain elements into the fish otolith. The possible explanation can be different mechanism of the incorporation of the elements into otolith. In other words, Sr and Ba incorporate preferably into the aragonite material, while Mg preferably goes into organic matrix.

One of the potential reasons why no expected pattern in Sr:Ca signal associated with the break point is the potential starvation or lack of food sources. Potentially, due to physiological stress that fish experience after first entering into marine environment, they might utilize the Wannock site as a 'holding' point to adjust to the condition and only then start to feed actively. In this case, the fish spent all their energy to make physiological changes in the organism, so no growth occurs. Thus, the otolith does not grow, and as a result no daily rings associated with the marine environment is seen.

Otoliths are made of aragonitic crystals of calcium carbonate deposited on the small amount of the organic matrix that is made of protein (otolin), glycosaminoglycans and lipids (Saitoh and Yamada 1989). So during the formation of daily increments (rings) the incremental zone is made up of needle-like aragonite crystals surrounded by the organic matrix. Campana and Nelson (1985) linked the fast growth stage with the aragonitic crystals zone and the slow stage with discontinuous phase (predominantly proteinaceous). So if towards the end of smoltification the fish were not feeding, then this period corresponds to a slow growth phase. Consequently, metal (Mg) is preferably incorporated into the proteinaceous phase, and that is why we can see such a great change in the Mg:Ca signal at the end of the ablation path. However, the proposed explanation is just a hypothesis that must be tested further in the lab studies or the information on such elements as S and P is needed that can show whether there was increase or decrease in the organic phase.

The other reason is that the fish has just entered the inlet, and thus only 1 or 2 rings belong to the marine zone, so due to limitation in the slit size in the ablation system (2 µm width), mode of the ablation (line scan) and the curvature of the otolith, the break point may be missed by the instrument. Thus, no break point can be detected in the element profiles.

APPENDIX C: METHOD VALIDATION: DIFFERENCE BETWEEN L AND R OTOLITH.

Most of the studies that use otolith to determine the age of the fish, use only left (L) or right (R) of the otolith pair in their studies for the consistency of the results (Neilson and Geen 1982, Wells et al. 2003, Hale and Swearer 2008, Yokouchi et al. 2011). However, Campana & Fowler (1994) noted that there was no difference in chemical composition between the otolith pair, thus use of L or R otolith would not contribute any difference in determining the fish age. In addition, vertebrate animals perceive the location of the sound by comparing differences in the acoustic signal between the two ears and, hence, smolts otoliths should develop symmetrically (Gagliano et al. 2008).

To investigate the proposed hypothesis, the difference between the otoliths was tested on a small sample size of 12 pairs (24 otolith) due to the scope of the work. Obviously, this is just a small attempt to investigate whether there is a differnce between the L and R otoliths; to get more statistically significant results, a larger data set should be used to exllore this issue.

To investigate whether there is a difference within the otolith pair, three physical (otoith length and radius and ring width) and one chemical (duration of the stay in the inlet, that was determined through Sr:Ca ratio and average otolith ring width) properties of the otolith were compared.
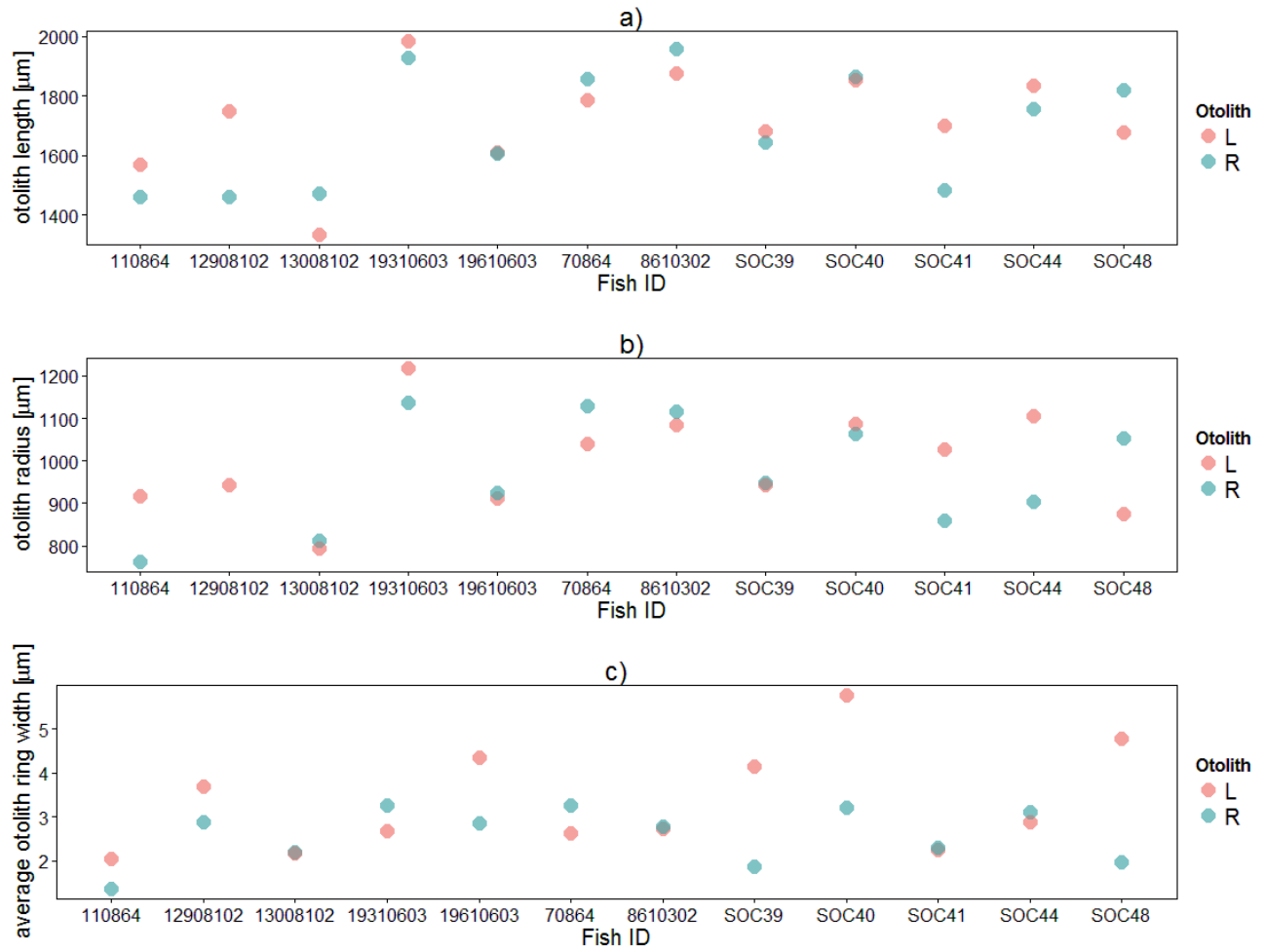
Figure 57: The difference on otolith length (a), otolith radius (b) and average ring width (c), in μm, between 12 pairs of otolith.

There was a slight difference in otolith length within the otolith pair (ANOVA, $p > 0.5$). The average difference between L and R pair was 103 ± 84 μm. In 7 pairs L otolith was larger than R. Similarly, the same trend was observed for otolith radius and average ring width with mean differences of 87 ± 75 and 1.0 ± 1.0 μm, respectively (ANOVA, $p > 0.5$).
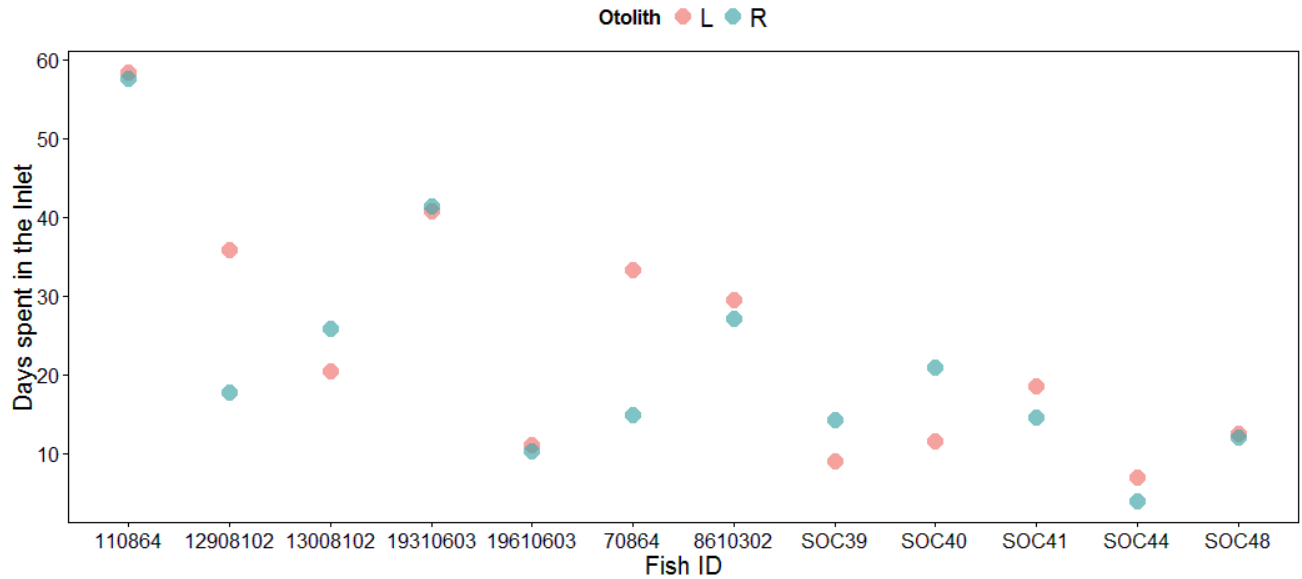
Figure 58: The difference in the duration spend in the Rivers Inlet between left (L) and right (R) otolith in 12 pairs.

For all 12 pairs the mean difference in days spent in the rivers Inlet was 5.7 ± 6.4 days. Again, in 7 cases out of 12, the L otolith showed a greater number of days in the inlet. Excluding the values for 70864 (the rings on L otolith could not be read properly, that might have introduced such error) and 12908102 (there might be potential misidentification in the raw data), the mean difference was 3.1 ± 3.0 days.

In this work, no directional asymmetry of the otoliths (i.e. is the right or left otolith usually larger) was found with either the left or the right otolith being the largest of a pair that agrees with the conclusion of the similar experiment done by Munday et al. (2011). Mérigot et al. (2007), however, found a significant difference between right and left otoliths for three of the shape indices. Several studies have demonstrated that physiological stress can lead to otolith asymmetry (Payan et al. 2004, Gagliano et al. 2008) that might be a potential explanation of the slight discrepancy between the L and R otolith length and ring width within the pair. No significant differences between right and left was found in this study in determining the duration of the stay in the inlet. For the future studies, the the size/weight of the smolts

that have a big difference between left and right otoliths and should be investigated. Potentially, if decoupling in the otolith pair is caused by physiological stress, this may result in lower survival rate of the fish.

APPENDIX D: METHOD VALIDATION: VALIDATION OF THE USE OF AVERAGE RING WIDTH IN DETERMINATION OF THE DURATION OF THE STAY IN THE INLET.

Due to the sample preparation technique, all the rings were not visible along the ablation path. To overcome this, an average daily ring width for each otolith was calculated in this study. Although the same procedure was described by Stocks at al. (2014), no validation of this method was shown in their work. The validation of this method is presented below. Ten otoliths with clearly visible daily rings over a large distance (more than 12 rings) were picked (Table 14). For each otolith, the total number of daily rings were counted and total path length was calculated as the sum of the width of all the rings on the chosen path. Then, for each sample, the path was broken into random blocks of different length. The average width of the rings was calculated for each of these sub-paths, and used to calculate the number of days along the total path as the ratio between total length of the path on the otolith and the average width of the ring. This sub-path derived value was then compared to the true number.

Table 14: The average otolith ring width for 10 otolith samples. The widths are listed in μm. Mean average value of an error and absolute deviation (MAD) is provided.

	Sample	# Of rings counted on random interval	Average ring width on interval		Total length of total path	Actual # of rings on total path	Predicted # of rings on the total path	
			Mean	Standard deviation			Mean	Error
1	BO2008_02_05	11	1.92	0.48	61.79	30	32.18	-2.18
2		16	2.08	0.41	61.79	30	29.77	0.23
3		16	1.94	0.47	61.79	30	31.89	-1.89
4		9	2.24	0.75	61.79	30	27.62	2.38
5		8	2.17	0.32	61.79	30	28.51	1.49
6		14	1.96	0.66	61.79	30	31.46	-1.46
7		23	1.95	0.47	61.79	30	31.71	-1.71
8		9	2.07	0.78	61.79	30	29.82	0.18
9	BO2008_03_05	8	1.92	0.23	28.64	14	14.90	-0.90
10		7	2.13	0.59	28.64	14	13.43	0.57
11		10	2.08	0.53	28.64	14	13.79	0.21

	Sample	# Of rings counted on random interval	Average ring width on interval		Total length of total path	Actual # of rings on total path	Predicted # of rings on the total path	
			Mean	Standard deviation			Mean	Error
12		4	2.27	0.75	28.64	14	12.63	1.37
13	BO2009_01_01	10	3.46	1.30	88.61	25	25.63	-0.63
14		11	3.76	1.61	88.61	25	23.57	1.43
15		8	3.15	1.16	88.61	25	28.12	-3.12
16		9	3.17	1.10	88.61	25	27.99	-2.99
17		11	3.90	1.43	88.61	25	22.70	2.30
18		14	3.88	1.62	88.61	25	22.86	2.14
19		6	2.98	1.10	88.61	25	29.75	-4.75
20	BO2009_02_02	9	2.50	0.59	55.42	22	22.13	-0.13
21		11	2.53	0.54	55.42	22	21.90	0.10
22		7	2.64	0.35	55.42	22	21.00	1.00
23		7	2.51	0.59	55.42	22	22.12	-0.12
24		9	2.69	0.56	55.42	22	20.62	1.38
25		4	2.46	0.67	55.42	22	22.52	-0.52
26		20	2.55	0.78	55.42	22	21.76	0.24
27	BO2009_02_05	11	1.99	0.76	57.47	24	28.81	-4.81
28		6	2.07	1.09	57.47	24	27.76	-3.76
29		6	2.26	1.27	57.47	24	25.44	-1.44
30		7	2.53	1.04	57.47	24	22.71	1.29
31	BO2010_01_02	7	1.87	0.48	25.29	14	13.56	0.44
32		5	1.73	0.55	25.29	14	14.63	-0.63
33		9	1.72	0.51	25.29	14	14.73	-0.73
34		6	1.86	0.52	25.29	14	13.63	0.37
35		3	1.77	0.57	25.29	14	14.32	-0.32
36	BO2010_01_06	7	2.14	0.59	47.30	20	22.14	-2.14
37		6	2.94	0.40	47.30	20	16.07	3.93
38		4	1.92	0.07	47.30	20	24.61	-4.61
39		8	2.74	0.61	47.30	20	17.24	2.76
40		6	2.35	0.50	47.30	20	20.14	-0.14
41		11	2.48	0.72	47.30	20	19.08	0.92
42	BO2010_01_10	6	3.71	0.78	64.37	16	17.37	-1.37
43		8	4.27	1.23	64.37	16	15.08	0.92
44		6	3.69	0.65	64.37	16	17.44	-1.44
45		9	4.15	1.26	64.37	16	15.52	0.48
46		6	3.71	0.78	64.37	16	17.37	-1.37
47	BO2010_03_06	7	3.84	0.97	77.76	19	20.25	-1.25
48		6	4.05	1.46	77.76	19	19.22	-0.22

Sample		# Of rings counted on random interval	Average ring width on interval		Total length of total path	Actual # of rings on total path	Predicted # of rings on the total path	
			Mean	Standard deviation			Mean	Error
49		5	4.64	1.04	77.76	19	16.76	2.24
50		7	4.05	1.16	77.76	19	19.20	-0.20
51		13	3.87	1.20	77.76	19	20.07	-1.07
52		10	4.01	1.13	77.76	19	19.37	-0.37
53	BO2011_01_06	5	2.26	0.22	38.08	12	16.87	-4.87
54		5	4.09	1.12	38.08	12	9.31	2.69
55		7	3.07	1.19	38.08	12	12.41	-0.41
56		5	3.73	1.24	38.08	12	10.22	1.78

Mean	**-0.33**
MAD	**1.51**

In total, 56 different intervals were measured. The average error was–0.41 rings (or days), while mean average value (MAD) was 1.81 rings/days. The obtained error was considered reasonable for the purpose of this study. The error was similar to the one produced during the ablation step where, due to the dimensions of the slit, the signal was an average for 2 μm. Thus, if the ring was 1μm, the error in determining the break point could be around 1 day.

To obtain an average value of otolith width for each sample, the picture of the otolith were examined and the width of several consecutives rings were measured. The rings were counted along the ablation path, or slightly higher or lower it if the view of the rings near the ablation path was blurry.
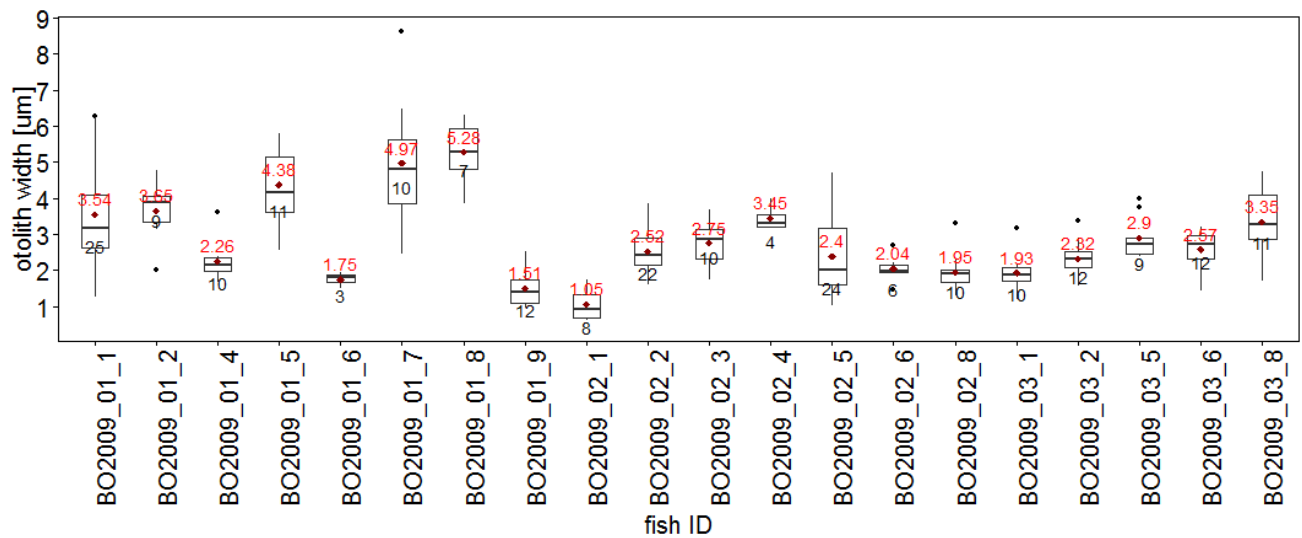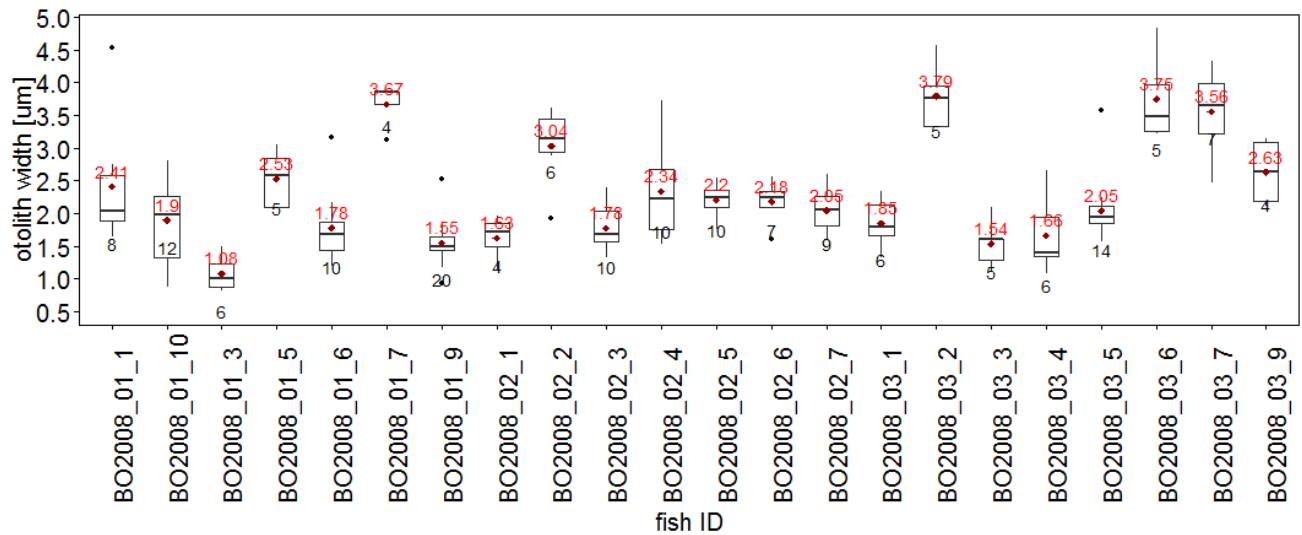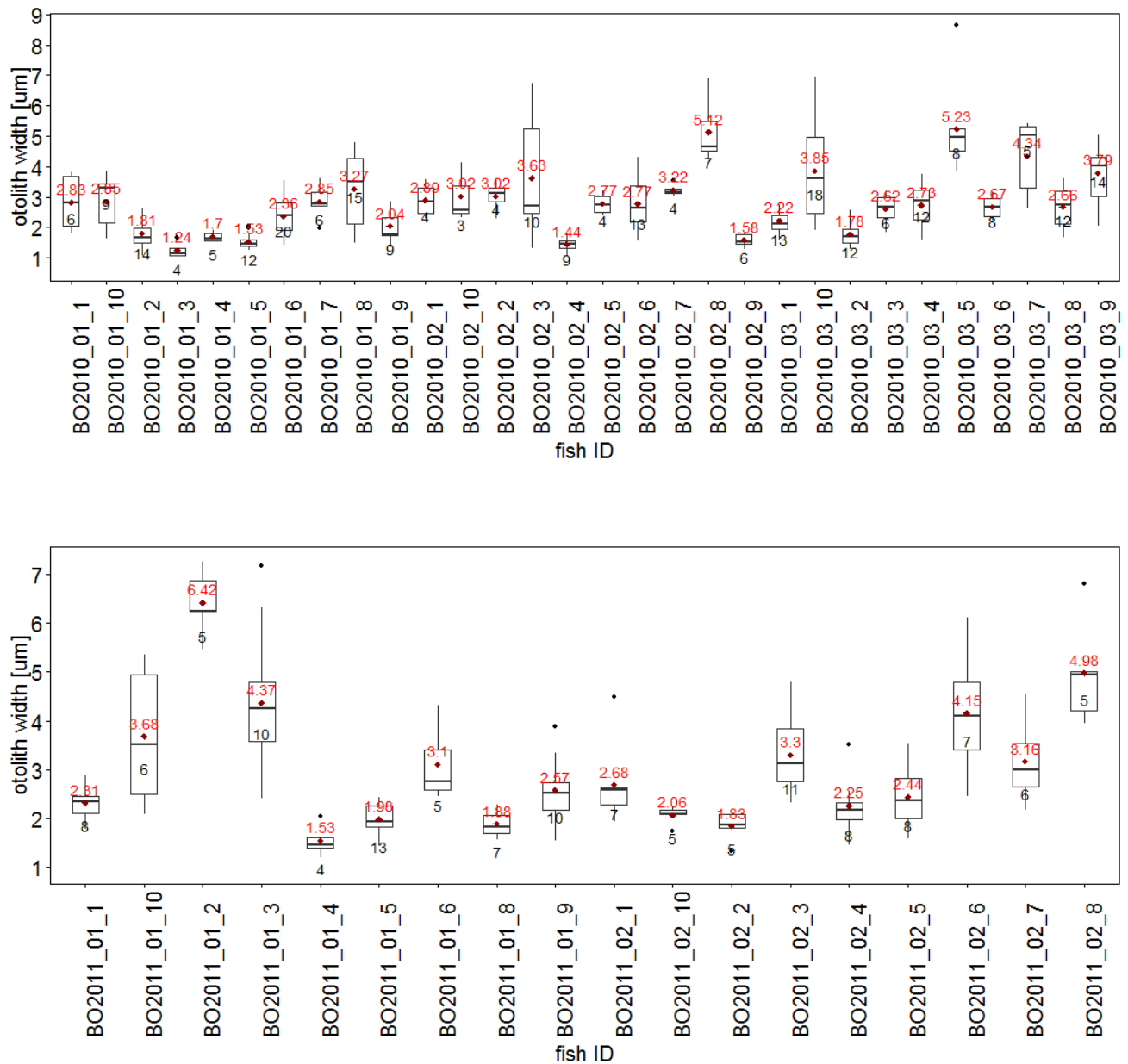
Figure 59: Variation in daily ring width for each otolith from Bosquet Open and Bosquet Shore site during 2008 (samples BO2008), 2009 (samples BO2009), 2010 (samples BO2010) and 2011 (samples BO2011). Median values (black solid horizontal line), interquartile range (box outline), and 95% confidence intervals (whiskers) are shown. Black numbers represent the number of rings measured. Black dots are outliers, red dot and number represent the mean value.
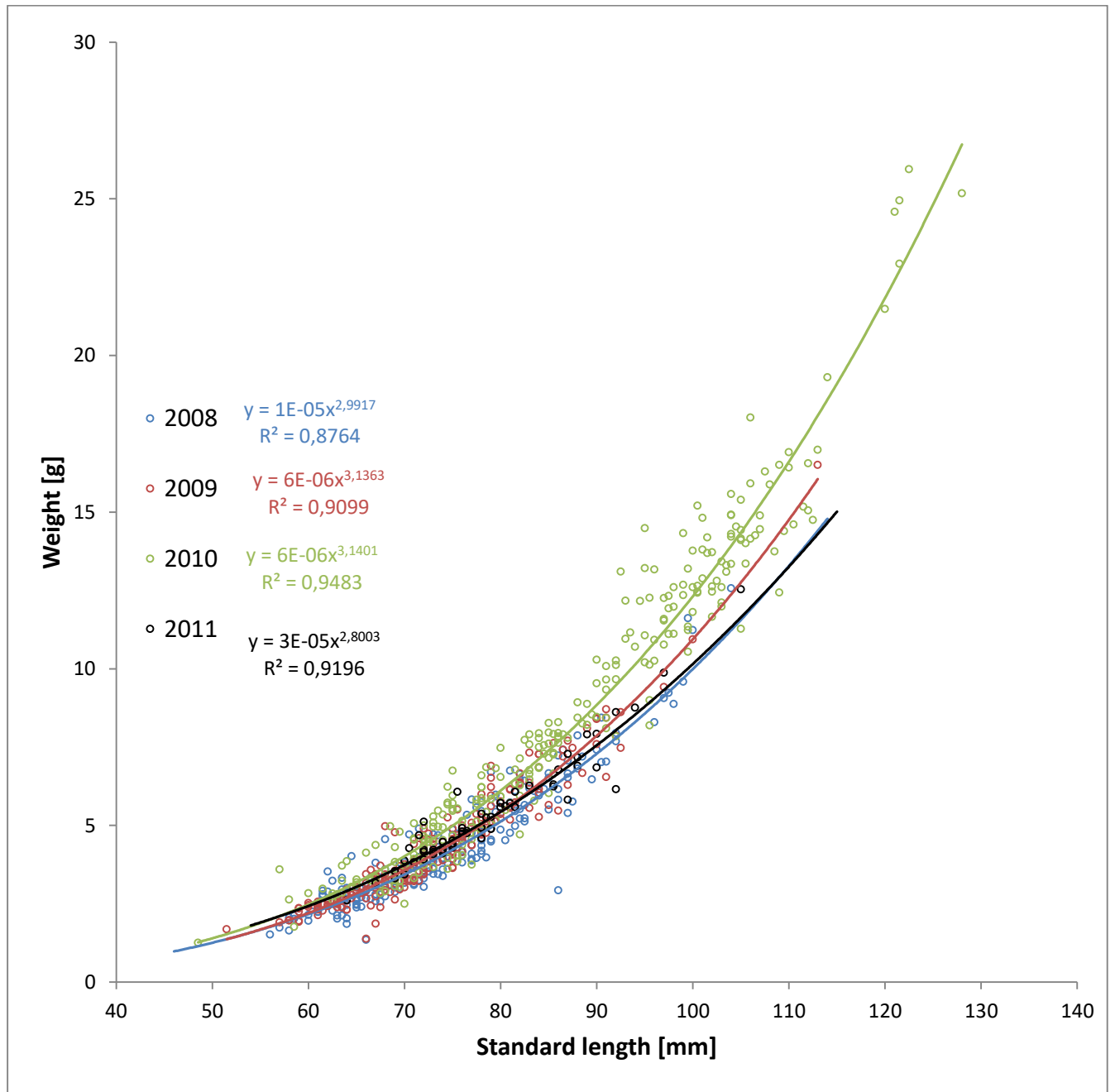
Figure 60: Weight (g) – length (mm) relationship for sockeye salmon smolts in Rivers Inlet in 2008-2011. Blue, red, green, and black solid lines are is the fitted linear regression lines for 2008, 2009, 2010, and 2011 respectively.

Table 15: Set of 15 possible GAM models for a) weight at catch (g), b) weight increase and c) outmigration duration (days) using the environmental conditions at the head of the inlet (DFO5 station) at the day of entry, letter n in breckets corresponds to the sample size. Generalized Cross Validation scores (GCV), deviance explained (Dev), Akaike Information Criterion (AIC), degrees of freedom (df) and the adjusted R^2 values (Rsq) are provided. among the models.Approximate significance levels (p-values) are displayed for each of the covariates. Covariates represent temperature, zooplankton densities (zoo), water temperature (temp), daily freshwater discharge(disch) and size at the entry (size_entry). Models in red cells were excluded from the analysis, as they contain parameters that were insignificant (p >0.05; highlighted in pink). Rsq, Dev, GCV, AIC cells are color coded with green color- the darker the color, the better the model. The best model is highlighted with a black rectangle.

CONDITIONS AT THE DAY OF ENTRY									
a) For Weight at catch (n = 50)							p-values		
Model #	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
1	0.78	35.14	0.84	1.97	172.2	0.0482	0.0000	0.0079	0.0029
2	0.67	41.53	0.72	2.53	188.6		0.0000	0.0019	0.0051
3	0.45	43.82	0.51	3.93	211.5	0.1192		0.0512	0.0000
4	0.44	44.53	0.49	3.93	211.7			0.0547	0.0000
5	0.74	35.06	0.81	2.33	180.5	0.0686	0.0000		0.0121
6	0.66	40.57	0.72	2.61	189.9		0.0000		0.0046
7	0.36	46.26	0.40	4.32	216.8	0.2405			0.0000
8	0.33	48.00	0.35	4.37	217.6				0.0000
9	0.64	45.09	0.72	2.66	219.9	0.2608	0.0000	0.0139	
10	0.57	51.60	0.61	2.79	225.4		0.0000	0.0014	
11	0.11	54.32	0.16	5.49	265.1	0.0820		0.0422	
12	0.11	54.13	0.15	5.56	265.8			0.0797	
13	0.63	42.18	0.73	2.93	223.7	0.1822	0.0000		
14	0.55	49.47	0.61	3.07	230.2		0.0000		
15	0.06	55.09	0.10	5.72	267.6	0.146733			

b) For weight increase (n = 56)							p-values		
Model #	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
1	0.64	35.53	0.74	0.13	34.80	6.53E-05	4.64E-05	1.22E-03	2.94E-04
2	0.36	43.34	0.43	0.18	58.27		0.000471	0.016391	0.000965
3	0.24	44.39	0.31	0.21	65.80	0.011996		0.026973	0.194788
4	0.09	45.03	0.17	0.25	73.94			0.238692	0.120412
5	0.58	35.03	0.70	0.15	42.79	0.001497	0.00035		0.001412
6	0.32	40.73	0.44	0.21	62.92		0.008579		0.00128
7	0.21	43.09	0.31	0.23	68.55	0.03448			0.323858
8	0.02	48.00	0.04	0.25	75.17				0.164695
9	0.39	44.25	0.45	0.17	54.71	0.00044	0.001066	0.000957	

Model #	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
10	0.18	45.46	0.24	0.22	68.62		0.00715	0.075827	
11	0.23	45.24	0.29	0.21	65.40	0.008191		0.023173	
12	0.06	46.20	0.11	0.25	74.80			0.288456	
13	0.33	40.06	0.45	0.21	62.86	0.011039	0.089257		
14	0.06	48.00	0.08	0.24	72.87		0.042271		
15	0.18	45.23	0.24	0.22	68.78	0.035003			

c) **Outmigration duration** (n = 58) — p-values

Model #	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
1	0.74	31.06	0.83	74.37	349.62	0.120529	0.000252	0.000123	0.469379
2	0.71	34.13	0.80	75.73	353.81		4.47E-04	1.23E-07	1.60E-01
3	0.45	43.85	0.51	110.52	378.33	1.12E-01		9.19E-06	1.61E-01
4	0.45	44.22	0.50	109.91	378.15			1.35E-05	1.27E-01
5	0.50	35.64	0.63	124.73	380.07	0.003042	0.000815		0.333326
6	0.20	43.80	0.29	161.15	397.17		0.04725		0.660337
7	0.13	45.08	0.20	170.22	400.23	0.14656			0.233424
8	0.06	46.91	0.10	177.01	402.51				0.253028
9	0.69	37.88	0.80	78.43	410.43	0.125816	0.000433	0.000138	
10	0.62	43.05	0.72	84.31	419.12		8.18E-04	3.03E-07	
11	0.41	52.35	0.46	108.93	438.07	1.83E-01		8.68E-06	
12	0.39	53.91	0.42	110.16	439.01			6.90E-06	
13	0.46	44.17	0.58	118.79	439.74	0.001076	0.000224		
14	0.13	53.56	0.18	157.50	459.68		0.075381		
15	0.05	54.87	0.09	166.56	463.11	0.217572			

Table 16: Set of 15 possible GAM models for a) weight at catch (g), b) weight increase and c) outmigration duration (days) that use the environmental condition at the head of the inlet (DFO5 station) using the cumulative values between ±7 days from the day of entry, letter n in breckets corresponds to the sample size. Generalized Cross Validation scores (GCV), Deviance explained (Dev), Akaike Information Criterion (AIC), degrees of freedom (df) and the adjusted R^2 values (Rsq) are provided. among the models. Approximate significance levels (p-values) are displayed for each of the covariates. Covariates represent temperature, zooplankton densities (zoo), water temperature (temp), daily freshwater discharge(disch) and size at the entry (size_entry). Models in red cells were excluded from the analysis, as they contain parameters that were insignificant (p > 0.05; highlighted in pink). Rsq, Dev, GCV, AIC cells are color coded with green color- the darker the color, the better the model. The best model is highlighted with a black rectangle.

CUMULATIVE OF ±7 DAYS

a) **Weight at catch** (n = 46) — p-values

Model	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
1	0.81	30.16	0.87	2.03	158.02	8.61E-05	7.07E-01	1.27E-02	5.03E-02
2	0.54	40.56	0.59	3.60	190.70		2.89E-01	4.90E-03	8.92E-05

Model	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
3	0.81	30.71	0.87	1.94	156.37	7.13E-05		6.59E-03	2.67E-02
4	0.63	35.98	0.70	3.31	185.05			0.001184	0.000911
5	0.77	31.58	0.84	2.27	164.41	6.57E-06	3.50E-02		4.86E-03
6	0.42	42.10	0.45	4.41	200.43		1.62E-01		5.99E-06
7	0.71	33.78	0.78	2.72	174.59	3.41E-05			1.87E-02
8	0.37	44.00	0.38	4.57	202.31				3.70E-06
9	0.75	36.57	0.82	2.37	194.61	0.000247	0.052315	0.006468	
10	0.38	48.50	0.43	4.38	234.40		0.630802	0.000113	
11	0.64	47.39	0.67	2.62	206.33	3.63E-07		7.85E-05	
12	0.38	49.49	0.43	4.23	232.68			3.34E-05	
13	0.65	39.16	0.74	3.04	210.27	5.34E-07	1.32E-01		
14	0.09	50.99	0.13	6.05	252.26		0.086432		
15	0.55	46.85	0.60	3.28	218.37	8.06E-08			

b) Weight increase (n = 46)

						p-values			
Model	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
1	0.64	31.87	0.74	0.13	34.53	6.35E-05	4.02E-02	2.63E-04	2.47E-03
2	0.35	39.39	0.43	0.19	56.18		0.05047	0.010056	0.097874
3	0.58	33.10	0.69	0.15	40.93	0.000463		0.000171	0.006721
4	0.28	39.71	0.37	0.21	60.41			0.007392	0.041177
5	0.58	32.18	0.70	0.15	41.12	0.000902	0.001476		0.010709
6	0.17	41.07	0.24	0.24	65.78		0.03736		0.341858
7	0.33	36.17	0.46	0.22	60.18	0.013932			0.008432
8	0.00	44.00	0.02	0.27	71.76				0.32517
9	0.39	40.74	0.45	0.18	51.88	0.016375	0.004612	0.000408	
10	0.30	42.14	0.34	0.20	57.23		0.009193	0.002286	
11	0.38	37.94	0.48	0.19	55.06	0.031403		0.004001	
12	0.23	40.41	0.31	0.22	62.88			0.025319	
13	0.40	36.09	0.52	0.20	55.17	0.047281	0.005588		
14	0.18	41.94	0.23	0.23	64.69		0.034234		
15	0.07	42.05	0.13	0.26	70.19	0.280781			
PRE						0.45	0.10	0.09	0.39

c) Outmigration duration (n = 54)

						p-values			
Model	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
1	0.67	35.45	0.74	66.92	323.04	1.65E-01	9.80E-02	2.04E-09	5.86E-01
2	0.61	39.83	0.66	69.39	326.66		2.08E-01	9.78E-10	6.96E-01
3	0.66	36.44	0.72	67.39	323.91	1.32E-01		2.68E-10	2.03E-01
4	0.61	40.59	0.65	68.62	326.36			1.84E-09	5.56E-01
5	0.46	34.80	0.58	110.83	345.84	0.454262	0.00021		0.952158
6	0.46	35.82	0.57	106.93	344.81		0.000208		0.809916
7	0.09	40.94	0.18	157.66	364.72	0.303558			0.546827
8	0.07	42.77	0.11	155.70	364.51				0.274207

Model	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
9	0.61	45.77	0.67	68.67	382.23	3.39E-01	7.71E-02	2.34E-10	
10	0.59	48.87	0.62	68.11	382.67		1.80E-01	6.51E-11	
11	0.58	48.71	0.62	70.12	384.20	6.25E-01		1.37E-10	
12	0.58	49.84	0.61	68.05	382.80			1.05E-10	
13	0.35	44.02	0.46	120.32	411.81	0.536439	0.00119		
14	0.35	45.13	0.45	117.04	410.78		0.00107		
15	0.04	50.87	0.08	153.36	426.83	0.322444			

Table 17: Set of 15 possible GAM models for a) weight at catch (g) and b) weight increase that use the average environmental conditions through the whole inlet (DFO1-5 and UBC 7 stations), using the cumulative values over the outmigration period. Letter n in breckets corresponds to the sample size. Generalized Cross Validation scores (GCV), Deviance explained (Dev), Akaike Information Criterion (AIC), degrees of freedom (df) and the adjusted R^2 values (Rsq) are provided. among the models.Approximate significance levels (p-values) are displayed for each of the covariates. Covariates represent temperature, zooplankton densities (zoo), water temperature (temp), daily freshwater discharge(disch) and size at the entry (size_entry). Models in red cells were excluded from the analysis, as they contain parameters that were insignificant (p>0.05; highlighted in pink). Rsq, Dev, GCV, AIC cells are color coded with green color- the darker the color, the better the model. The best model is highlighted with a black rectangle.

WHOLE INLET CUMMULATIVE

a) Weight at catch (n = 52) p-values

Model	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
1	0.69	37.66	0.77	2.59	194.25	4.27E-02	8.55E-05	1.27E-01	5.79E-03
2	0.67	38.85	0.75	2.71	197.44		7.97E-05	2.58E-03	1.46E-03
3	0.43	44.25	0.50	4.12	221.96	2.07E-01		4.76E-01	8.71E-06
4	0.36	48.74	0.39	4.19	223.86			2.00E-01	1.09E-06
5	0.67	38.11	0.75	2.76	197.75	0.001064	0.000401		0.020784
6	0.46	49.00	0.48	3.52	214.89		1.02E-03		2.91E-07
7	0.44	45.16	0.50	3.99	220.56	1.06E-01			6.04E-06
8	0.34	50.00	0.35	4.22	224.41				2.99E-06
9	0.66	44.92	0.74	2.60	225.14	1.20E-05	9.69E-09	1.53E-02	
10	0.59	46.42	0.68	3.02	234.97		5.66E-08	8.65E-06	
11	0.19	53.06	0.27	5.23	270.69	0.026357		0.113162	
12	0.05	54.81	0.12	5.92	278.47			0.434315	
13	0.61	46.26	0.70	2.85	231.34	9.81E-07	2.05E-07		
14	0.23	52.03	0.32	5.06	268.38		0.012829		
15	0.15	55.66	0.20	5.23	271.16	0.038503			

b) Weight increase (n = 52) p-values

Model	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
1	0.43	37.46	0.58	0.19	58.37	0.341653	0.009479	0.251923	0.000814

Model	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
2	0.42	38.75	0.56	0.19	58.60		0.001742	0.03697	0.001148
3	0.12	46.69	0.20	0.23	73.63	0.132527		0.35272	0.147315
4	0.04	48.85	0.08	0.25	76.50			0.245212	0.273546
5	0.43	37.24	0.58	0.19	58.70	0.020095	0.009814		0.009989
6	0.24	42.76	0.36	0.22	69.58		0.03669		0.074151
7	0.12	47.67	0.18	0.23	72.81	0.090535			0.170957
8	0.02	50.00	0.04	0.25	76.36				0.161518
9	0.18	42.55	0.32	0.24	73.30	0.832154	0.352143	0.282287	
10	0.18	44.51	0.28	0.23	72.08		0.128437	0.180641	
11	0.09	48.15	0.14	0.24	74.33	0.187012		0.487715	
12	0.03	49.88	0.05	0.24	75.80			0.145623	
13	0.11	49.00	0.14	0.23	72.56	0.48335	0.077381		
14	0.11	50.00	0.13	0.22	71.09		0.008197		
15	0.09	49.28	0.12	0.23	73.08	0.067589			

Table 18: Set of 15 possible GAM models for a) weight at catch (g) and b) weight increase that use the average environmental conditions through the whole inlet excluding the head (DFO1-4 and UBC 7 stations) using the cumulative values over the outmigration period. Letter n in breckets corresponds to the sample size. Generalized Cross Validation scores (GCV), Deviance explained (Dev), Akaike Information Criterion (AIC), degrees of freedom (df) and the adjusted R^2 values (Rsq) are provided. among the models.Approximate significance levels (p-values) are displayed for each of the covariates. Covariates represent temperature, zooplankton densities (zoo), water temperature (temp), daily freshwater discharge(disch) and size at the entry (size_entry). Models in red cells were excluded from the analysis, as they contain parameters that were insignificant (p>0.05; highlighted in pink). Rsq, Dev, GCV, AIC cells are color coded with green color- the darker the color the better the model. The best model is highlighted with a black rectangle.

WHOLE INLET CUMULATIVE WITHOUT DFO5

a) **Weight at catch** (n = 60)   **p-values**

Model	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
1	0.62	41.05	0.69	2.95	203.09	0.000629	0.009235	0.342925	0.004272
2	0.38	47.20	0.43	4.18	223.52		2.91E-01	6.02E-01	2.05E-06
3	0.61	40.24	0.69	3.12	205.52	0.001256		0.018462	0.000763
4	0.36	48.74	0.39	4.19	223.86			2.00E-01	1.09E-06
5	0.58	45.38	0.63	2.94	204.76	0.000462	0.000374		0.001907
6	0.39	48.35	0.42	4.05	222.08		1.08E-01		1.20E-06
7	0.45	49.00	0.47	3.61	216.16	1.95E-03			2.83E-07
8	0.34	50.00	0.35	4.22	224.41				2.99E-06
9	0.68	43.27	0.77	2.51	221.84	1.95E-08	2.57E-05	1.33E-02	
10	0.16	53.04	0.24	5.41	272.73		0.021299	0.080025	
11	0.47	52.80	0.53	3.42	245.04	1.68E-08		1.30E-05	
12	0.05	54.81	0.12	5.92	278.47			0.434315	

Model	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
13	0.47	54.19	0.51	3.37	244.64	6.47E-07	3.34E-06		
14	0.11	55.87	0.16	5.43	273.48		0.08605		
15	0.28	51.21	0.38	4.81	265.03	0.003883			

b) Weight increase (n = 52)

Model	Rsq	df	Dev	GCV	AIC	s(zoo)	s(temp)	s(disch)	s(size_entry)
								p-values	
1	0.32	40.21	0.46	0.21	65.92	0.039471	0.250625	0.145503	0.004841
2	0.08	46.97	0.16	0.24	75.59		0.259754	0.470043	0.203175
3	0.34	39.72	0.48	0.21	64.61	0.01396		0.109433	0.004984
4	0.04	48.85	0.08	0.25	76.50			0.245212	0.273546
5	0.25	44.00	0.35	0.21	68.02	0.043121	0.027108		0.013593
6	0.08	48.20	0.14	0.24	74.48		0.148794		0.238406
7	0.19	44.60	0.29	0.23	71.53	0.098222			0.114859
8	0.02	50.00	0.04	0.25	76.36				0.161518
9	0.10	47.92	0.15	0.23	73.92	0.051679	0.502739	0.537071	
10	0.06	48.34	0.11	0.24	75.67		0.303495	0.607361	
11	0.11	48.74	0.15	0.23	72.35	0.042357		0.273964	
12	0.03	49.88	0.05	0.24	75.80			0.145623	
13	0.11	49.00	0.14	0.23	72.33	0.048336	0.279011		
14	0.07	49.53	0.09	0.24	74.17		0.104914		
15	0.11	50.00	0.12	0.22	71.58	0.010697			