Salmonid migration and recruitment patterns: fishery monitoring using natural trace element and isotope markers

By

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Abstract

Complex life histories and a need to spawn in running water make salmonids susceptible to human activities such as dam construction, flow regulation, and water abstraction. Several aspects of salmonid migration and population dynamics are poorly understood including the dispersal dynamics, reproductive success of dispersers, and their combined influence on metapopulation dynamics and population resilience. The objective of this thesis is to improve our understanding of salmonid population dynamics by examining migration and recruitment patterns in a large regulated New Zealand river system.

The research presented here examines the utility of natural trace element (e.g. Strontium, Barium, Magnesium, Manganese and Calcium) and isotopic markers (the radiogenic strontium isotope ratio; $^{87}Sr/^{86}Sr$) for fishery monitoring, using experiments and field studies on freshwater and sea migratory (anadromous) salmonid populations. Experiments expanded the utility of natural chemical markers in eggs and otoliths by testing (1) the temporal stability of chemical markers in salmonid eggs during incubation, and (2) the influence of diet on otolith composition, in order to provide realistic boundaries for applications to wild populations. To improve our understanding of salmonid population dynamics, field surveys investigated the natal origin and movement patterns of lake migratory brown trout (*Salmo trutta*) and anadromous chinook salmon (*Oncorhynchus tshawytscha*) in the Clutha River / Mata-Au catchment. Variations in otolith $^{87}Sr/^{86}Sr$ ratios reconstruct key life history events such as exit from natal habitats and ocean entry. Linear discrimination analyses, calibrated on samples from known sites, were used to select predictive models for assigning adult fish of unknown origin to likely natal sources.

The first experimental study (Chapter 2) confirmed, for the first time, that distinctive chemical markers in incubating trout eggs (high Sr, low Ba concentrations) remain unchanged for seven weeks after fertilisation, demonstrating that the chemical composition of eggs from spawning redds

provide a reliable method for surveying migratory salmonid spawning distribution. The second experiment (Chapter 3) established that consumption of food sources that differ chemically from local environments (e.g. marine-derived forage fish) can have measurable effects on otolith chemistry within three weeks. Field surveys investigating population dynamics identified a significant lack of breeding success and recruitment contribution from a nursery stream where chemical analysis ($^{87}Sr/^{86}Sr$) confirmed approximately 30% of a lake migratory trout population spawn annually (Chapter 4). These surveys also classified 61% of all sea-run chinook salmon harvested below a hydropower dam blocking passage as progeny from self-sustaining landlocked lake populations above dams, and revealed that, on average, 82% of sea-run adults had adopted a stream-type life history (Chapter 5).

Field surveys integrating traditional and contemporary monitoring techniques suggest that water abstraction practices negatively impact a lake migratory trout population by causing a nursery stream to act as a recruitment sink. The surveys also demonstrated that metapopulation dynamics connect landlocked and anadromous chinook salmon populations. Combined, these outcomes illustrate (1) that habitat connectivity is essential to avoid ecological traps; and (2) that dispersal of migrants can be an important recruitment source that increases population resilience. This thesis highlights the need for water resource managers to consider the effects of anthropogenic water use on population dynamics in order to implement conservation actions for rebuilding supressed populations of migratory fishes. While anthropogenic use of water poses many challenges to migratory fishes, dispersal of progeny from landlocked populations above dams represents an important source of recruits that can help maintain anadromous populations in regulated river systems. Collectively, this body of research contributes to scientific knowledge by expanding the utility of natural chemical markers for fishery monitoring and advancing our understanding of salmonid metapopulation dynamics.

Dedication

For my father Lars Åke Gabrielsson who ignited and nurtured my interest in fishing and taught me to value nature while taking me handlining for Baltic cod.

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Chapter 1 : General introduction

Migrations of animals have long captured the imagination of people, and continue to fascinate as new insights into the biological mechanisms behind these movements are uncovered (Hobson et al., 2010). Understanding migration is important because it is a powerful force that shapes the distribution of animals across space and time, and influences processes at all scales, from individuals to entire ecosystems (Chapman et al., 2012). Many types of fish migrate in order to complete their life cycle, but the ecological variability of life histories exhibited by salmonids makes them particularly interesting (Kendall et al., 2015). Furthermore, the mixture of complex life cycles and a need to spawn in running water make salmonids highly susceptible to human encroachments in rivers. Successful recovery of salmonid populations impacted by fragmented riverine landscapes therefore requires an understanding of the scale and extent of dispersal within and among populations, which are important elements that can determine the population dynamics of salmonids (Rieman and Dunham, 2000; Willmes et al., 2018). From an ecological perspective, migration is the prerequisite for dispersal and exchange of individuals among populations. Yet, several aspects concerning dispersal are still poorly understood, including the spatial scale of dispersal movements and the reproductive success of dispersers, and need to be examined in further detail on scales relevant to salmonid conservation (Schtickzelle and Quinn, 2007; Johnson et al., 2012, Johnson et al., 2016; Lennox et al., 2019).

1.1 Variations in life histories and migratory behaviours

Migratory salmonids make excellent study species for studies in behavioural and evolutionary ecology, conservation biology, and as model systems for studies of population dynamics for several reasons. As a species group, salmonids are characterised by a great diversity of complex life cycles and flexible ecologies that are heavily influenced by their environments (Klemetsen et al., 2003; Quinn, 2005; Jonsson and Jonsson, 2011, Lobón-Cerviá and Sanz, 2017;

Lennox et al., 2019). Anadromous [1] salmonids are keystone species that link freshwater and marine ecosystems by carrying marine-derived nutrients to lakes, rivers and streams during their spawning run (Cederholm et al., 1999; Kendall et al., 2015). The movements of potadromous[2] salmonids within freshwater systems are often less conspicuous, but can be equally common and extensive (Northcote, 1997). Consequently, salmonid life histories frequently include a variety of anadromous and (or) potadromous migrations between juvenile rearing, adult feeding and spawning areas, in order to feed or breed, often with these different strategies occurring within a single population, i.e. partial migration (Chapman et al., 2011). Partial migration occurs when just a fraction of a population migrates and the remainder stay resident (Jonsson and Jonsson, 1993; Chapman et al., 2012). These differences in migratory behaviour demonstrate that a high degree of life history plasticity is a persistent feature within most salmonid populations (Jonsson and Jonsson, 1993; McDowall, 1997). The "decision" of fish to migrate is often described as a complex interaction involving genetic control of phenotypic plasticity and physiological (energetic) and environmental (food supply, physical habitat) correlates (O'Neal and Stanford, 2011 and references therein). While the drivers and selection pressures that determine life-histories and movement patterns can be subtle and complex, particularly for partially migratory populations within freshwater environments, a broad body of literature considers the ability to migrate to be important both for individual fitness and population resilience (Klemetsen et al., 2003; Carlsson et al., 2004; Jonsson and Jonsson, 2011).

1.2 Influences of human activities

Migratory species that rely on multiple habitats to complete their life cycle can be particularly susceptible to population declines if one or more of their habitats are degraded. Numerous studies have shown that the ecology of

[1] Fish that feed in the ocean but migrating up rivers to breed in fresh water, e.g., salmon.
[2] Fish that migrate within fresh water only, e.g., between lakes and tributary spawning areas.

salmonids can be greatly affected by human activities. For example, mining, logging, agricultural practices, and urban development frequently affect the quality of freshwater habitats and survival of embryos and fry (Quinn, 2005; Jonsson et al., 2011; Lobón-Cerviá and Sanz, 2017). Dam construction, flow-regulation and water abstraction are capable of interrupting the ecological connectivity in riverine landscapes, which may in turn affect the ecology and distributions of migratory salmonids (Elliott, 1994; Greenberg and Calles, 2010; Lange *et al.,* 2014; Quiñones et al., 2015; Phillis et al., 2018). Many of these anthropogenic impacts on the aquatic environment also have the potential to create ecological traps, which can cause source-sink population dynamics to develop (Hickford and Schiel, 2011; Jeffres and Moyle, 2012). Consequently, protecting and maintaining connectivity between high value habitats has long been the principal conservation strategy of freshwater fishery managers worldwide. However, protecting and effectively maintaining connectivity between habitats requires three fundamental but poorly understood questions in applied ecology and fisheries management to be answered: (1) what are the recruitment sources for populations with highly dispersive offspring?; (2) which of these recruitment sources are the most productive; and (3) what role does dispersal and migration play in the resilience and persistence of local and regional populations?

1.3 Defining population dynamics

Regional populations of many species with complex life cycles are believed to persist in variable environments as collections of local populations that interact through dispersal: that is, as a metapopulation (Hanski and Simberloff, 1997). The metapopulation concept has greatly advanced our understanding of the consequences of habitat fragmentation (Hanski 1989; Hanski and Gilpin, 1991), but was developed using mostly terrestrial organisms and ecosystems. However, the importance of salmonid population structure has been recognised for decades (Ricker's 1972 review). As metapopulation theory is intuitively appealing it has attracted the interest of many people involved in the conservation and

management of salmonids, and prompted some to propose the concept may hold a particular relevance for threatened or sensitive populations of migratory salmonids (Rieman and Dunham, 2000). A more recent review confirms the concept is a suitable framework for salmonid conservation, but indicates it is rarely used, and barely tested (Schtickzelle and Quinn, 2007). Possible explanations for the lack of application of metapopulation theory include: (i) the strong tendency for salmonids to home, which implies they are primarily structured as a collection of nearly isolated populations; and (ii) the lack of published data on metapopulation structure in aquatic ecosystems (Smedbol et al., 2002; Kritzer and Sale, 2004). The latter may partly be due to the difficulty of acquiring the data required to assess the potential existence and/or importance of metapopulation dynamics for salmonids. Nevertheless, long-distance dispersal of freshwater fish through straying from natal sources is increasingly becoming recognised as an important factor for genetic exchange between local populations (Johnson et al., 2012; Radinger and Wolter, 2014; Johnson et al., 2016). Many researchers also stress the need to identify recruitment sources in order to: (1) facilitate habitat protection or restoration actions; (2) understand the long term persistence of fish populations; and (3) determine to what degree local populations are replenished via self-recruitment or dispersal. Hence, determining the natal origin of fish is a critical piece of information that allows for the matching of fishery catch with production (Cadrin and Secor, 2009; Johnson et al., 2016), and is considered fundamental to understanding population dynamics and population structure (Secor, 2010). However, identifying the natal origins of individuals that migrate throughout river basins and or the ocean where multiple populations mix poses many challenges for fishery managers.

1.4 Reconstructing fish movement and migration patterns

Several methods have been used to determine the extent of migration and natal origin of fishes, but most of the information available on fish migration comes from mark-recapture studies that have used artificial tags to reconstruct

movement patterns (Dadswell et al., 1987; Hendry et al., 2004; Walther and Thorrold, 2008; Walther and Limburg, 2012). Although the tags employed have become increasingly sophisticated over time (e.g. Block et al., 2005; Lacroix, 2013), the approach can only yield information about movements subsequent to tag application, and after the fish reaches a minimum threshold size (Webster et al., 2002). As a result traditional tags are unable to provide data about early life history movements and natal origins of fishes, both of which are crucial aspects of population dynamics (Metcalfe et al., 2002). Hence, recent studies of fish population connectivity and (meta)population dynamics increasingly rely on alternative methods that use natural markers, such as otolith chemistry (e.g. Gillanders, 2002; Chittaro et al., 2006; Bradbury et al., 2011; Veinott et al., 2012; Phillis et al., 2018). Otoliths are calcium carbonate structures forming part of the inner ear of teleost fishes (Campana, 1999). Otolith chemistry involves measuring spatial variations in the chemical signatures recorded by hard tissue within the fish, post mortem, which in turn reflect temporal variations in the environmental conditions experienced by the fish during its entire life history.

Chemical analysis of otoliths at the micro- (or sub-millimeter) scale (i.e. otolith microchemistry) has the potential to provide unprecedented insights into movement and population mixing at multiple scales, and help unravel the complex ecological dynamics of many fishes (Secor, 2010). As an individual fish ages, new material is continuously accreted on the exterior surface of the otolith, and chemical elements from the surrounding environment are incorporated together with the major constitutes (carbon, oxygen, and calcium) of the otolith (Veinott et al., 2014). Natural chemical tags recorded in otoliths have several advantages over applied tags, provided they are unique and distinct at appropriate geographical scales. Firstly, all fish are marked from early life (many as embryos); secondly the tags are usually permanent; and thirdly, tags can be related to fish age (reviewed in Elsdon et al., 2008). Strontium (Sr) and barium (Ba) are by far the most commonly used, and perhaps the most informative, elements in otolith chemistry because they are easily measured in otoliths, heterogeneously distributed throughout the aquatic environment, metabolically inert, and due to their

ubiquitous distribution in natural settings have been found to faithfully reflect environmental parameters (Campana, 1999; Elsdon et al., 2008; Brown and Severin, 2009). Thus, interpreting fish movements through geochemical markers and profiles (or isoscapes) that are recorded in otoliths provides a method for reconstructing a fish's environmental history (i.e. natal origin, spatial distributions, movement patterns, and spawning locations) within large stream networks at population levels (e.g. Muhlfeld et al., 2012; Johnson et al., 2016).

1.5 Challenges and knowledge gaps for using natural tags to assess fish movement

There is little doubt that otolith microchemistry both compliments and extends the utility of conventional tagging and fishery monitoring techniques. However, while the chemical composition of otoliths often reflects environments occupied by a fish, a growing body of literature indicates that a range of intrinsic (i.e., physiological and genetic) and extrinsic (i.e., environmental: food, temperature and salinity) factors can potentially affect otolith chemistry (see reviews by Elsdon and Gillanders, 2003; Elsdon et al., 2008; Jaecks et al., 2016). Moreover, species-specific responses tend to make generalized predictions of environmental effects on otolith chemistry difficult to predict (Barnes and Gillanders, 2013). As the knowledge of how different factors influence otolith chemistry is still limited for many species, including salmonids, it can be challenging to translate the observed variability in natural tags into an interpretable pattern or movement. For example, several studies have attempted to examine the influence of diet on otolith chemistry across a range of freshwater species through diet manipulation, but report widely conflicting results (Limburg 1995; Kennedy et al., 2000; Gibson-Reinemer et al., 2009; Doubleday et al., 2013; Jaecks et al., 2016). Such discrepancies imply further research is required to better understand the mechanisms and potential drivers behind the observed level of variation in how diet may affect otolith chemistry, and to address the potential limitations of such influences may have on otolith chemistry.

Whilst numerous studies have successfully used otolith microchemistry to further our understanding of salmonid ecology, this approach frequently requires the capture of highly mobile adult fish (e.g. see Johnson et al., 2016; Phillis et al., 2018). This can, at times, present difficulties, particularly when assessing catchment-scale movement and distribution over a short-time interval, such as the relatively brief period during which adult fish are undertaking spawning migrations (Rustadbakken et al., 2004; Willmes et al., 2018). Female salmonids build and lay their eggs in a spawning nest, called a redd, which can often be identified for several weeks after spawning has occurred. Sampling and analysis of the chemical composition of eggs deposited in spawning redds has therefore been proposed as an alternative method for determining and separating the spawning location of anadromous and river resident salmonids (Waite et al., 2008; Kristensen et al., 2011). The success of this approach is based on the assumption that eggs acquire and then retain a distinctive chemical signature reflective of their maternal origin. If this is indeed the case, it may also be possible to utilise this method to track and differentiate between the spawning areas of lake migratory and river resident fishes in some freshwater systems. However, whether eggs do retain strontium (and other trace elements) at concentrations that accurately reflect their maternal origin over the course of their entire development has not yet been rigorously tested.

1.6 Thesis aim and outline

The research presented in this thesis aims to improve our understanding of salmonid population dynamics by examining migration and recruitment patterns in the Clutha River / Mata-Au catchment, a large regulated river system in Otago (New Zealand). In order to achieve this I first needed to define the limits of reliability of natural geochemical tags in otolith and eggs, to provide realistic boundaries for their application to wild populations within freshwater environments. Consequently, the research is driven by four specific objectives

which are addressed in separate chapters. I used both experimental studies (Chapters 2–3) and field based surveys (Chapters 4–5) to address these questions. The data chapters are all written as individual manuscripts for journal publication. However, in order to remove replication I have collated all references at the end of the thesis.

Objective 1: Investigate the temporal stability of chemical markers in salmonid eggs during incubation (Chapter 2), which is a prerequisite for determining if eggs deposited in spawning redds can be used to separate and map the spatial distribution of spawning by migratory and resident salmonids.

Objective 2: Evaluate the influence of diet on otolith composition in piscivorous salmonids (Chapter 3), to resolve if dietary effects may cause misinterpretations of movement patterns in freshwater migratory salmonids.

Objective 3: Examine if source-sink dynamics occur in a lake migratory brown trout population? (Chapter 4)

Objective 4: Quantify if metapopulation dynamics link three partially migratory (lake-rearing) populations with one anadromous chinook salmon population (Chapter 5).

The following five chapters summarise studies concerning the use of chemical analysis techniques to monitor salmonid migration and recruitment patterns, and population dynamics.

Chapter 2 describes a laboratory experiment that investigates the temporal stability of trace elements concentrations in brown trout (*Salmo trutta*) eggs during incubation. The hypothesis tested is that the trace element concentrations in eggs and newly hatched larvae are reflective of their maternal anadromous origin and will remain constant during the rearing period in fresh water, prior to feeding.

Chapter 3 investigates the effect of diet on otolith composition in brown trout, testing the hypothesis that seasonal prey sources can influence otolith composition of piscivorous freshwater fishes.

Chapter 4 examines the population dynamics of lake migratory brown trout by combining the use of egg chemistry to determine spawning location, otolith chemistry to determine natal origin of adults captured in the lake, and bioenergetics modelling to determine some of the drivers behind observed migratory patterns.

Chapter 5 investigates whether metapopulation dynamics link three partially migratory (lake-rearing) populations and one residual anadromous chinook salmon (*Oncorhynchus tshawytscha*) population. Results are discussed with regards to how metapopulation dynamics explain the resilience and persistent viability of a small residual anadromous salmonid population blocked from all traditional spawning areas by a hydro-electric dam without fish passage facilities.

Finally, in the General Discussion (Chapter 6), results from all previous chapters are integrated to discuss the role alternative life-history strategies, partial migration and source-sink dynamics play in the population dynamics, management, and conservation of both anadromous and non-anadromous salmonids in the Clutha River / Mata-Au (hereafter Clutha) catchment. Chapter 6 also contains an outline of future research directions and discusses some of the broader implications of my findings. As such it specifically addresses the potential for using natural trace-element and isotope markers to track fish migration and recruitment patterns to aid management and conservation of salmonid populations and fisheries in New Zealand.

Chapter 2 : Does the trace element composition of brown trout eggs remain unchanged in spawning redds?

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Author contributions

RMG conceived, designed, conducted experiments/field work, analysed the data and wrote the paper. PGC and CHS assisted with study design, creation of publication quality figures, editing and paper submission. JK, MRR, CHS and NM assisted with development of analytical protocols, chemical analysis and data reduction.

2.1 Abstract

The temporal stability of trace element concentrations in fertilised, artificially incubated anadromous brown trout (*Salmo trutta*) eggs and newly hatched fry was investigated. The anadromous status of the parental fish was confirmed using strontium isotopic analysis of otoliths. Whilst manganese concentrations in eggs varied over time, concentrations of aluminium, potassium, magnesium, strontium, barium and calcium were all unchanged 1 week and 6 weeks post-fertilisation as well as in recently hatched larvae. The results clearly suggest that the distinctive trace element signature present in the eggs and newly hatched larvae of anadromous brown trout (typically characterized by high strontium, low barium) is stable over time. Therefore analysis of the trace element composition of eggs is concluded to be a cost-effective and reliable method for determining the spatial and temporal extent of upstream spawning migration by anadromous salmonids. The temporal variability of at least one element in this study suggests the stability of untested multi-element signatures cannot automatically be assumed.

2.2 Introduction

Salmonids exhibit flexible life history strategies with resident and anadromous stocks coexisting in many species (Elliot, 1993; Zimmerman & Reeves, 2000, 2002; Olsson et al., 2006). Understanding the status of different stocks and their patterns of movement is essential for effective management and conservation (Elliot, 1993). Various approaches can be used to track and resolve the migratory status of fish stocks including coded tags, radiotelemetry and natural chemical markers in flesh, otoliths and scales (Zimmerman & Reeves, 2000, 2002; Kennedy et al., 2002; Elsdon & Gillanders, 2003; Rustadbakken et al., 2004; Acolas et al., 2008). All of these approaches require the capture of highly mobile and often difficult-to-catch adult fishes. Given the short duration and spatially extensive nature of many salmonid upstream spawning migrations (Rustadbakken et al., 2004), designing and resourcing sampling and tracking programmes that adequately represent catchment-wide patterns of movement across entire populations is logistically challenging.

An alternative method for determining the upstream extent of spawning migrations by anadromous salmonids is the trace element analysis of eggs deposited in spawning redds (Waite et al., 2008). Spawned eggs can remain buried in stream substrata for 2 months or more thus allowing for a relatively extended period of time for catchment-wide sampling and mapping of upstream migration. The success of this approach is based on the assumption that eggs acquire and then retain a trace element signature reflective of their maternal origin. Waite et al. (2008) observed a close relationship between the strontium (Sr) content of the outer layers of otoliths obtained from large female brown trout and their mature roe, indicating that the Sr content of eggs reflects patterns of maternal residency in marine, estuarine or freshwater habitats. The presence of high Sr levels in otolith primordia of the progeny of anadromous fishes has also been interpreted as retention of maternally derived Sr in eggs followed by its incorporation into the otolith of the developing embryo (Kalish, 1990; Volk et al., 2000; Zimmerman & Reeves, 2000, 2002). Whether eggs do retain Sr (and other trace elements) at

concentrations that accurately reflect their maternal origin over the course of their development has not been rigorously tested. In this study, the stability of multiple trace elements, including Sr, in brown trout eggs that were obtained from anadromous maternal fish and then fertilised and incubated in fresh water was investigated. The hypothesis tested is that the trace element concentrations in eggs and newly hatched larvae are reflective of their anadromous origin and will remain constant during the rearing period in fresh water, prior to feeding.

2.3 Materials and methods

Three large female and one male brown trout were captured by electrofishing from the Water of Leith, Dunedin, New Zealand, whilst fish were migrating upstream to spawning areas. Fish ranged in total length (LT) from 590 to 695 mm and in body mass from 2.6 to 3.6 kg. This urban fishery is dominated by large anadromous brown trout due to low summer flows, lack of adult habitat and close proximity to the Otago Harbour (< 3 km). The lower reaches of the Water of Leith are confined within a concrete channel which drains at low tide thus eliminating any permanent estuarine habitat. Hence, high Sr values reflecting a marine environment could be expected in adult brown trout body tissues collected from this system, with little opportunity for any extended period of estuarine or freshwater residency.

2.3.1 Experimental design

Three female and one male fish were anaesthetized with 2-phenoxyethanol and stripped of roe or milt. Eggs were fertilised and incubated following normal hatchery procedures (Stead & Laird, 2002), with the eggs of all three females fertilised by the same male and placed in separate hatchery incubator trays. Eggs were incubated in vertical incubator stacks, under running water and in constant darkness. Weekly egg samples (100 eggs per fish) were collected and stored frozen at −20° C until analysis. To investigate the overall stability of egg trace element concentrations over time, samples were collected

just after fertilisation (week one), 1 week prior to hatching (week six), and from larvae 1 week after hatching (week seven).

Water for the experiment was obtained from a spring water tank at the Zoology Department, University of Otago, and pumped into each incubator tray. To monitor the variability of water chemistry over time water samples were collected in a trace metal-clean manner for analysis using methods described in Kim & Hunter (1997). All reagents used were of ultra-high purity, comprising HNO_3 purified by sub-boiling distillation in a quartz still and >18 MΩ Milli-Q H_2O (www.millipore.com). Three 50 ml sub-samples were collected during weeks one, three and six and filtered through an acid washed 0.45 µm filter directly into acid washed sample tubes. Samples were then stored frozen at −20°C until analysis. Data loggers were used to measure ambient water temperature while pH and dissolved O_2 levels were manually measured to assess water quality and maintain relatively constant experimental conditions.

2.3.2 Analytical methods

Trace element composition of water samples and acid-digested tissue samples (*S. trutta* eggs and larvae) were determined by solution nebulization inductively coupled plasma mass spectrometry (ICP-MS). To confirm the morphological field identification of brood fish as anadromous, the Sr isotopic composition across the sagittal otolith was determined using laser-ablation multiple-collector inductively-coupled-plasma mass-spectrometry (LA-MC-ICP-MS). Present day marine water has a uniform global Sr isotopic signature ($^{87}Sr/^{86}Sr$ = 0.70918) and therefore provides a precise marker of the timing and duration of marine residence when observed in otoliths (Kennedy et al., 2002). The $^{87}Sr/^{86}Sr$ for the marine phase of the brood fish life cycle was estimated from the data collected from the last observed annuli to the outer edge of the otolith.

All trace element and Sr isotopic compositions were determined at the Centre for Trace Element Analysis, University of Otago. Egg and larval solutions for analysis were prepared using methods described by Waite et al., (2008). An Agilent

7500ce ICP-MS instrument (www.chem.agilent.com) equipped with a quadrupole mass analyser and operating in solution nebulization mode was employed to analyse both the digested egg and water samples for Sr, Ba, K (only eggs), Mg, Al, Ca and Mn concentrations. These elements were chosen as they have all been previously used to either track migration or locate features in tissue samples (Elsdon & Gillanders, 2003; Brazner et al., 2004; Waite et al., 2008). The instrument was calibrated with a series of solutions prepared from The National Institute of Standards and Technology (NIST) traceable standards (www.nist.gov) with elemental concentrations spanning several orders of magnitude, encompassing the range of concentrations in the unknown samples. Instrument performance was verified with certified reference materials [CRC SLRS-4 (National Research Council – Canada; www.nrc-cnrc.gc.ca/eng/services/inms/referencematerials.html) and USGS T167 (United States Geological Service – Analytical Evaluation Program for Standard Reference Samples; http://bqs.usgs.gov/SRS/)]. All solutions were spiked with scandium and indium as reference elements to correct for any instrument drift. The assay sequence was also randomized across samples to eliminate analytical bias, and analytical consistency was checked by analysing duplicate samples. Mean egg or larval elemental concentrations per time period were standardized as nmol g^{-1} egg or larva dry mass and a one-way ANOVA using SPSS version 17 (www.ibm.com/software/analytics/SPSS) compared the difference in concentrations among samples. Throughout, statistical difference was accepted at $P = 0.05$.

A Nu Instruments Nu Plasma-HR MC-ICPMS instrument, coupled to a NewWave UP-213 Nd:YAG deep UV (213 nm) laser-ablation system (www.esi.com), was utilized for the in situ Sr isotopic analysis of otolith samples. Individual otoliths were sectioned in the sagittal plane, mounted on glass slides and polished. Ablation was performed under a pure helium atmosphere whilst the laser was operated in continuous scan mode using a spot size of 65 µm, a repetition rate of 10 Hz and a scan rate of 5 µm s^{-1}. Potential surface contaminants were initially removed from the sample during a pre-ablation step using a spot size of 100 µm, a repetition rate of 10 Hz, and a scan rate of 30 µm s^{-1}. The ablated

aerosol was then transported to the MC-ICP-MS plasma source via an Ar carrier gas connected downstream of ablation using a standard T-piece configuration.

Strontium isotopic data were collected as a single static measurement by simultaneously monitoring ^{84}Sr, ^{86}Sr, ^{87}Sr and ^{88}Sr on an array of Faraday collectors operating with 10^{11} Ω resistors, yielding ^{88}Sr signal intensities ranging from 4.5×10^{-11} to 1.8×10^{-10} A. All $^{87}Sr/^{86}Sr$ were corrected for instrumental mass fractionation using the measured $^{86}Sr/^{88}Sr$ normalized to the true value of 0.1194 and the exponential mass fractionation law (Hart & Zindler, 1989; Habfast, 1998), as well as for potential isobaric interferences from krypton and rubidium, and molecular interferences from calcium argides and calcium dimers. In detail, Kr interferences on masses ^{84}Sr and ^{86}Sr were monitored by performing an 'on-peak' baseline prior to each analysis. An interference on ^{87}Sr from ^{87}Rb was then corrected for by monitoring ^{85}Rb during the course of the measurement, adopting an $^{87}Rb/^{85}Rb$ of 0.3856. Additional interferences across the Sr mass range from Ca argides and dimers were subsequently corrected for by monitoring the $^{42}Ca^{40}Ar$ dimer interference at mass 82, then peak stripping the contributions from Ca argides and dimers from the peak intensities at strontium masses 88, 86 and 84 accordingly (Woodhead et al., 2005). The veracity of this approach was evaluated by continuously monitoring $^{84}Sr/^{86}Sr$, which has a generally accepted value of 0.0565 ± 0.0001 (mean ± SD). An in-house *Tridacna* carbonate standard (Australian National University) was used to assess the performance of the analytical protocols for $^{87}Sr/^{86}Sr$ measurement. Strontium isotopic data acquired for this standard yields a mean (± SD) $^{87}Sr/^{86}Sr$ of 0.709212 (± 0.000046) which is in excellent agreement with the $^{87}Sr/^{86}Sr$ composition determined for present day open-ocean water of 0.70918.

2.4 Results

Strontium isotopic analysis of the otolith material deposited after the last annulus in each brood fish yielded $^{87}Sr/^{86}Sr$ ranging from 0.709033 ± 0.000045

(mean ± 2 SD) to 0.709138 ± 0.000045. There was no consistent change in values evident between the last annuli and the outer edge of the otolith.

Eggs from all three females were viable and hatching began 51 days after fertilisation. During the experiment pH ranged from 8.2 to 8.5, water temperature between 6.4 and 11.4°C and dissolved oxygen between 9.6 and 10.9 mg l^{-1} (Figure 2.1). The ambient water chemistry of the spring water used during egg incubation trials varied only slightly for most elements measured over the study period, but a small significant difference was found for Mn and Ba (Table 2.1). Compared to seawater, the water used during egg incubation samples had lower Sr and Ca concentrations, Ba was similar, and higher Al and Mn concentrations (see Table 2.1).

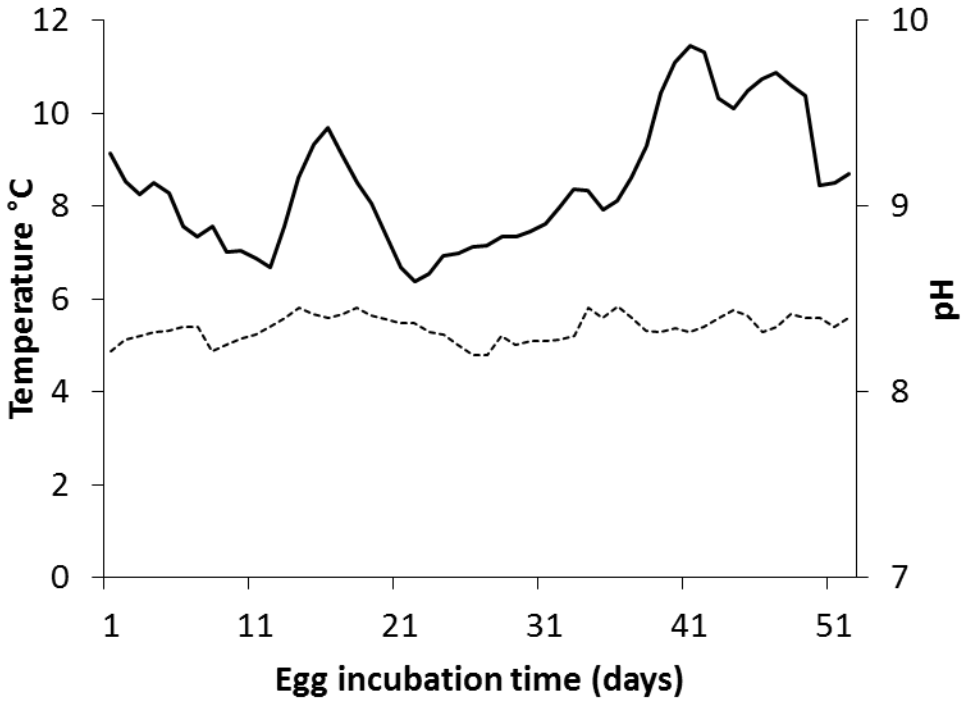

Figure 2.1 Daily temperature (solid line) and pH (dotted line) over the course of the brown trout (*Salmo trutta*) egg and larva incubation experiment.

Table 2.1 Mean ± S.E. (n = 3) element concentrations in the spring water used for brown trout (*Salmo trutta*) egg incubation at weeks 1, 3 and 6. Element concentrations that varied significantly between 14-day water samples are indicated (*) (ANOVA, $P < 0.05$). Concentrations of trace elements in sea water (Brown et al., 1989) are presented for comparison.

Week	Mn* (ppb)	Al (ppb)	Mg (ppb)	Sr (ppb)	Ba* (ppb)	Ca (ppb)
1	0.90 ± 0.02	4.1 ± 0.3	25.8 ± 0.3	362.4 ± 5.6	21.8 ± 0.3	29.3 ± 0.4
2	0.3 ± 0.2	4.4 ± 1.6	25.8 ± 0.1	362.2 ± 1.8	19.7 ± 0.3	29.1 ± 0.1
3	0.20 ± 0.02	6.3 ± 7.2	26.2 ± 0.2	368.2 ± 8.0	22.6 ± 0.3	29.4 ± 0.5
Sea water	0.2	2	1.29×10^6	8000	20	4.12×10^5

Most element concentrations remained stable in the brown trout eggs and then fry over the entire 7-week study period (Figure 2.2), however, a significant difference was found for Mn [Figure 2.2(a) and Table 2.2]. Elemental concentrations measured for duplicate egg and water samples were consistent to within ± 5% of each other.

Table 2.2 ANOVA comparisons of element concentrations in eggs and alevins of brown trout (*Salmo trutta*) over a 7 week study period. Element concentrations that varied significantly between samples are indicated (*) ($P < 0.05$). Values for d.f. are illustrated for between and within groups.

Element	Mn* (ppb)	Al (ppb)	K (ppb)	Mg (ppb)	Sr (ppb)	Ba (ppb)	Ca (ppb)
F	28.577	2.993	0.049	0.090	0.334	0.392	0.232
d.f.	2, 23	2, 23	2, 23	2, 23	2, 23	2, 23	2, 23
P	< 0.001	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

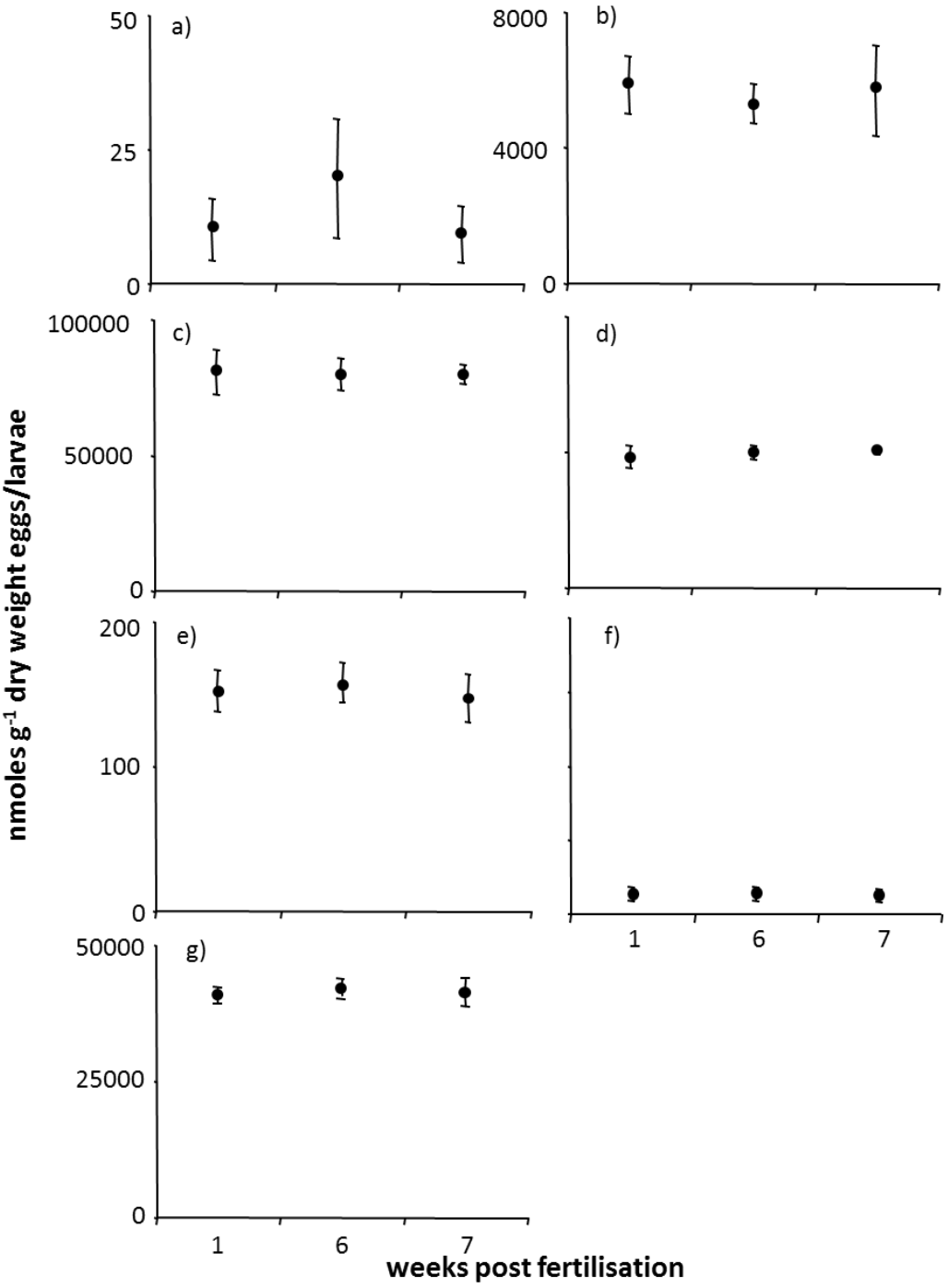

Figure 2.2 Mean ± S.E. (*n* = 3) trace element levels in brown trout (*Salmo trutta*) egg samples (weeks 1 and 6) and larvae (week 7): (a) manganese, (b) aluminium, (c) potassium, (d) magnesium, (e) strontium, (f) barium and (g) calcium. Graphed element averages are the means from all egg tested, per sample period, during the study. Only manganese concentrations varied significantly between sampled periods (ANOVA, $P < 0.05$).

2.5 Discussion

While others have previously demonstrated the transfer of maternally derived trace elements into offspring, none have tested the degree of stability of trace element signatures in eggs during development. The results from this study are therefore the first rigorous confirmation that concentrations of key trace elements, most notably Sr and Ba which are regularly used as indicators of marine or freshwater residency (Zimmerman & Reeves, 2000; Elsdon & Gillanders, 2003), remain constant in eggs and recently hatched larvae derived from anadromous maternal fish that have been subsequently incubated in fresh water. The temporal stability of most other elements tested, including Ca, also confirms that results can be reliably reported as trace element to Ca ratios as well as absolute concentrations for comparisons of egg or roe signatures from different habitat types. Providing suitable elements are monitored it may also be possible to differentiate between fish that have formed eggs in different freshwater habitats with distinct water chemistries, even where those differences may be subtle. The results are consistent with previous work that found larvae hatched from eggs spawned in freshwater environments by anadromous fishes exhibit high levels of marine-derived Sr in their otolith primordia (Kalish, 1990; Rieman et al., 1994; Volk et al., 2000; Zimmerman & Reeves, 2000) further indicating that maternal Sr is conserved in eggs and transferred to developing embryos during development. The variation in Mn concentration observed in this study, however, suggests that it cannot be routinely assumed that all trace elements will remain constant during egg development. For example, Elsdon & Gillanders (2003) observed that Mn incorporation into otoliths can be highly variable in different species and under different conditions. Further, elevated levels of Mn have also previously been observed in the otolith primordia of several fish species suggesting this element is influenced by metabolic processes during embryonic development (Ruttenberg et al., 2005). Thus, both environmental and metabolic processes may influence concentrations of this element in the developing egg and embryo. Nevertheless, despite variation in Mn concentrations, the results presented here support the argument of Waite et al. (2008) that brown trout ova collected from spawning

redds can be used to determine the upstream extent of spawning migrations by anadromous maternal fish.

Unfertilised eggs were not tested in this study, hence direct inferences regarding the impact that water uptake during fertilisation may have on trace element signatures cannot be made. Potts & Rudy (1969) suggested that water uptake during fertilisation can increase egg mass by up to 25%, so it is possible that water uptake could impact the egg trace element signature. For anadromous fishes the uptake of fresh water, which is generally low in Sr, is unlikely to add significant amounts of additional Sr to the total amount of Sr already inside an egg. Hence, final Sr concentrations based on the analysis of whole dried eggs or newly hatched larvae should be relatively unaffected by water uptake. This contention is further supported by a comparison between Sr concentrations of fertilised eggs in this study, and the highest Sr concentrations observed in unfertilised eggs analysed by Waite et al. (2008) and Kristensen et al. (2011), which are all comparable to each other.

Despite the uptake of fresh water by eggs in this study, concentrations of Sr observed in fertilised eggs were higher than the highest Sr concentration (104.2 nmol Sr g^{-1} dry mass of egg) observed in unfertilised eggs by Waite et al. (2008). The eggs analysed by Waite et al. (2008), however, were collected from fish that were likely to have been estuarine rather than marine residents, and had in some cases migrated a considerable distance inland, and hence may have spent some time in fresh water. It is conceivable that for long distance migrants concentrations of Sr may be slightly diluted if egg development is not completed when the female enters fresh water (Volk et al., 2000). In this study, the fish sampled were confirmed as being truly anadromous by analysis of Sr isotopic ratios, and appeared to have spent only a limited time in fresh water given the lack of variation in the $^{87}Sr/^{86}Sr$ close to the otolith edge. This result is not surprising given the small size of the Water of Leith stream and the limited habitat it provides for large brown trout. Thus, the results indicate that fertilised eggs derived from anadromous female brown trout are likely to exhibit a concentration in the region

of 150 nmol Sr g^{-1} dry mass of egg over the incubation period, providing an approximate guideline Sr concentration for distinguishing anadromous from non-anadromous spawning sites using eggs.

This study, together with Waite et al. (2008) and Kristensen et al. (2011), confirms the potential application of trace element analysis of eggs or newly hatched larvae sampled from redds as a tool for tracking the upstream extent and magnitude of spawning migrations by anadromous salmonids. A good example of how this technique could be applied to investigate population dynamics and migration patterns was demonstrated by Kristensen et al. (2011). They observed higher Sr concentrations in eggs sampled from redds in the lower reaches of downstream tributaries of the Taieri River, South Island, New Zealand, suggesting that spawning in the lower Taieri River catchment is dominated by anadromous brown trout. In contrast, Sr concentrations of eggs were consistently lower in eggs sampled in tributaries upstream of the Taieri River gorge, a high gradient section of river with numerous rapids, suggesting that this gorge may act as a partial barrier to the upstream migration of anadromous brown trout.

In conclusion, this study confirms that the analysis of the Sr content of eggs deposited in spawning redds can provide an important tool for determining the upstream extent of spawning migration by anadromous salmonids. Furthermore, the stability of several other elements, and the observation of two distinctly different trace element signatures in eggs collected from multiple redds sampled from a single stream upstream from the Taieri Gorge by Kristensen (2006), indicates the method may have potential to track migration in non-anadromous riverine populations of salmonids. It may also be possible to utilize this approach to differentiate between the spawning areas of lake run and river resident fishes in some freshwater systems, providing such stocks can be adequately separated using the elements proven to be stable over time in this study. This technique thus has the potential to provide fisheries managers and researchers with a cost-effective alternative to the traditional radio tracking, large scale tagging and fish trapping programmes that are currently used to assess patterns of upstream

catchment-scale migration by both anadromous and freshwater resident salmonids.

Chapter 3 : Diadromous prey species can affect otolith chemistry in salmonids

3.1 Abstract

Natural geochemical markers in otoliths are increasingly used to reconstruct the environmental histories of migratory fishes and address questions relating to fish ecology and migration patterns. However, the relationship between water and otolith chemistry has not always been consistent for salmonids. This study tested if, and to what degree, natural element tracers (Ba, Li, Mg, Mn and Sr) and strontium isotopic composition ($^{87}Sr/^{86}Sr$) in salmonid otoliths are influenced by diet. Firstly, wild brown trout (*Salmo trutta*) were reared in freshwater or saltwater environments and fed either a freshwater (*Chironomidae*) or marine diet (diadromous *Galaxiids* and brine shrimp *Artemia*). Secondly, the influence of a marine fish-derived hatchery food source on otolith chemistry was examined using data from brown trout and Chinook salmon (*Oncorhynchus tshawytscha*) reared in hatcheries over a broad range of water chemistry conditions ($^{87}Sr/^{86}Sr$ 0.7045 - 0.7097). Results confirmed that both natural and artificial food sources with contrasting trace element signatures and $^{87}Sr/^{86}Sr$ ratios can significantly influence the otolith chemistry of salmonids. Hence, this study provides strong evidence that seasonally available natural food sources also contribute to the variation in otolith chemistry that may have previously been exclusively attributed to differences in the physio-chemical properties of water. Consequently, researchers using otolith chemistry to reconstruct movement of piscivorous freshwater fishes need to carefully consider the possible effects of diet when reconstructing environmental life histories and migration patterns.

3.2 Introduction

Many resource management agencies routinely conduct studies of fish movement and migration patterns to better understand spatial distributions and connectivity needs of fishes. For instance, knowledge of fish migration and dispersal patterns from natal environments is considered both fundamental to understanding metapopulation dynamics (Hanski and Gilpin, 1997) and critical for conservation of riverine fishes (Phelps et al., 2012; Quiñones et al., 2015). Historically information on fish migration has primarily been derived from mark-recapture studies that use physical (fin clips) or artificial tags (*e.g.* electronically coded or archival tags, and radiotelementry). Although tag technology is increasingly sophisticated (*e.g.* Block et al., 2005; Lacroix, 2013), this approach can only yield information about movements subsequent to tag application after the fish reaches a minimum size threshold (Webster et al., 2002). It is also well recognised that the use of tag and recapture methods for assessing species movement and population structure only provides information on tagged fish, often requires large numbers of fish to be tagged for meaningful returns, and can be expensive (Barnes and Gillanders, 2013). Hence, recent studies of fish population connectivity and metapopulation dynamics increasingly rely on alternative methods such as otolith chemistry (*e.g.* Gillanders, 2002; Chittaro et al., 2006; Bradbury et al., 2011; Veinott et al., 2012; Willmes et al., 2018).

Otoliths are calcium carbonate structures forming part of the inner ear of teleost fishes (Campana, 1999). As individuals age, new material is continuously accreted on the exterior surface of the otolith, and chemical trace elements from the surrounding environment are incorporated with the major constituents (carbon, oxygen, and calcium) of the otolith (Veinott et al., 2014). Natural chemical tags recorded in otoliths have some obvious advantages over applied tags, providing they are unique and distinct at appropriate geographical scales, because all fish are marked from early life (many as embryos), the tags are usually permanent, and tags can be related to fish age (Elsdon et al., 2008; Phillis et al., 2018). Thus, interpreting fish movements through geochemical profiles,

comprising trace element composition and strontium (Sr) isotope signatures (or isoscapes) that are recorded as sequential growth layers in otoliths provides a robust method for reconstructing a fish's environmental history (natal origin, spatial distributions, movement patterns, and spawning locations) within large stream networks (*e.g.* Muhlfeld et al., 2012;) that compliments and extends the utility of conventional tagging and tracking techniques. However, while the chemical composition of otoliths often reflects the ambient water chemistry of the environments occupied by a fish, a growing body of literature indicates that a range of intrinsic (*i.e.*, physiological and genetic) and extrinsic (*i.e.*, environmental: food, temperature and salinity) factors can also potentially affect otolith chemistry (see reviews by Elsdon and Gillanders, 2003; Elsdon et al., 2008). Furthermore, species-specific responses tend to make generalized predictions of environmental effects on otolith chemistry difficult to predict (Barnes and Gillanders, 2013). Thus, for many species knowledge of how these different factors influence otolith chemistry is still limited, making it challenging to translate the observed variability in natural tags into an interpretable pattern of movement (Elsdon et al., 2008).

Environmental interpretations based on otolith chemistry rely on the underlying assumption that fish derive trace elements predominantly from the water and that these trace elements will be faithfully incorporated into the otolith in proportion to their concentrations in the water mass that a fish occupies (Campana, 1999; Buckel et al., 2004; Phillis et al., 2018). Several studies attempting to identify the sources of elements that are ultimately deposited in otoliths have confirmed that at least the elements frequently used in otolith chemistry (Sr and Ba) are indeed predominantly sourced from the water, and moreover, that no resolvable 'elemental fractionation' occurs when these elements are removed from the water and precipitated in the $CaCO_3$ mineral structure of the otolith (Hoff and Fuiman, 1995; Farrell and Campana, 1996; Milton and Chenery, 2001). In addition, studies that have specifically examined the influence of diet on otolith composition, often through food manipulation, on a range of potadromous or diadromous species raised in freshwater have also determined that diet usually only has a minor influence on otolith chemistry

(Gibson-Reinemer et al., 2009; Collingsworth et al., 2010; Doubleday et al., 2013). Nevertheless, a few studies have shown that considerable discrepancies between otolith and water chemistry can exist, suggesting that at least in some species (or instances), elements derived from dietary sources can have substantial influences, potentially confounding otolith - water chemistry relationships and reducing the accuracy of data interpretation. For instance, Limburg (1995) found that otolith chemistry (Sr/Ca) in American shad (*Alosa sapidissima*) raised in freshwater documented a change of diet from natural zooplankton to a marine fish-derived artificial feed. Similarly, otolith strontium isotope ($^{87}Sr/^{86}Sr$) values in hatchery-reared adult Atlantic salmon (*Salmo salar*) predominantly reflected the marine fish-derived origin of their artificial feed rather than the hatchery water suggesting that, averaged over their entire lifetime, approximately 70 % of the Sr in calcified tissues can be derived from diet (Kennedy et al., 2000). Recent simulations by Jaecks et al. (2016) also demonstrate the potential for seasonally abundant and important marine-derived food sources, such as salmon eggs, to produce otolith chemical signatures suggestive of anadromy in freshwater resident fish. Collectively these findings suggest that diet can influence otolith composition and confound, or even limit, the utility of otolith chemistry to track movement patterns for piscivorous freshwater fish that seasonally feed on mobile food sources which transport marine-derived chemical signatures into a freshwater environment *(i.*e. diadromous fish species). Consequently, the effect of diet on otolith composition requires careful consideration. An improved understanding of how diet can influence otolith composition would help better define the limits of, or provide realistic boundaries for, the application of otolith chemistry aiming to track the movements of potadromous fish populations.

Like many other salmonids, freshwater resident brown trout (*Salmo trutta*) have very diverse feeding habits and readily change from relying predominantly on an invertebrate food source and become at least partly piscivorous at a size ranging from 17 – 36 cm, depending on localised growth conditions and the availability of suitable forage fish species (Jonsson and Jonsson, 2011). In New Zealand, juvenile forms of several diadromous (*e.g.*, *Galaxiids, Retropinna* and

Gobiomorphus) and two catadromous (*Anguillidae*) fish species seasonally enter most rivers in order to complete their life cycle, and thereby transport marine-derived nutrients into freshwater environments, sometimes a considerable distance inland (McDowall, 1990). These migrations occur over a six-month period (July - December) each year and are often specifically targeted and utilised as an important seasonal food source by brown trout, possibly to regain condition after spawning (McDowall, 1990). Thus, because of their diverse foraging behaviour, but also as a consequence of their wide global distribution and international reputation as a valued recreational sport fish, brown trout represents a suitable and ecologically relevant species on which to examine to what degree natural migratory prey types may affect otolith chemistry in potadromous piscivorous predators.

This study uses a combination of elemental concentrations and Sr isotope chemical markers to specifically examine the effect of diet on otolith chemistry in brown trout using a combination of a naturally occurring diadromous forage fish (known to transport marine-derived nutrients into a freshwater environment in New Zealand) and two other semi-natural prey sources reflecting a diet in chemical equilibrium with either marine or freshwater environmental conditions. In addition, it also evaluated the influence of marine fish-derived hatchery food on brown trout and chinook salmon (*Oncorhynchus tshawytscha*) reared in hatcheries over a broad range of water chemistry conditions, both higher and lower than the $^{87}Sr/^{86}Sr$ composition of marine waters (0.70918). Based on a previous study of dietary influences on otolith composition in Atlantic salmon, using hatchery food made from a marine fish-derived origin (Kennedy et al., 2000), it was hypothesised that otolith chemistry is not independent of diet in freshwater resident piscivorous salmonids feeding on diadromous forage fish.

3.3 Material and methods

3.3.1 Experimental design

To investigate if diet can influence otolith element concentrations, wild brown trout (n = 57) were collected from several rivers by electrofishing and transferred to a laboratory environment (mean size 190 mm, range 176 – 228 mm). Fish were randomly allocated between two diet treatments, reared in 200L tanks, and fed either a freshwater (freeze dried bloodworms, *Chironomidae*) or a marine diet (a combination of aquatic crustaceans, *Artemia*, and juvenile *Galaxiids*). The latter representing marine-derived nutrients available to piscivorous salmonids feeding on diadromous forage fish. Additionally, half of the fish exposed to each diet treatment were reared in freshwater and the other half in a saltwater environment (33 PSU), using three rearing tanks per water/diet treatment. After being allowed to acclimatise to their respective environment for five days, the applicable food type for each treatment was phased in, and by the end of week two, most fish in all treatments were feeding actively. Fish were fed a surplus of food. Waste was removed via manual siphoning and approximately 1/5 of the tank volume was replaced each day with matching water of the same temperature and salinity. After a six-week acclimation period, the diet experiment was run for a further three weeks, after which all fish were euthanized and stored frozen until otoliths were removed and mounted for analysis. The exact time required for otolith microchemistry to reach equilibrium with ambient element concentrations is species-dependent (Zimmerman, 2005), and differs between elements (Campana, 1999). Nevertheless, based on previous experiments it was assumed that the combined six week acclimation period and 20+ days of full experimental exposure would be sufficient for brown trout to reach a chemical equilibrium with the ambient water chemistry and diet type in each treatment, as well as provide adequate amounts of otolith material for analysis (Zimmerman, 2005; Lowe et al. 2009; Hicks et al. 2010).

In addition to investigating the effect of natural food sources on otolith chemistry in brown trout, the influence of commercial hatchery food was also evaluated separately by collecting water, diet and fish samples (brown trout or chinook salmon) from five hatcheries operated by, or under, the regulation of Fish and Game New Zealand (n = 10 fish per hatchery, mean size 168 mm, range 145 –

186 mm). While the Sr isotope ratio of the rearing water varied between hatcheries, they all use the same brand of commercially available artificial feed (Skretting; www.skretting.com), and similar feeding regimes and rearing practices.

3.3.2 Water and feed chemical analysis

Water samples were collected from each experimental tank at the start, middle and end of the experiment, and once from each hatchery source. Each sample was filtered through 0.45 µm filters into acid-washed 250 ml bottles, acidified with 0.25 ml ultrapure nitric acid (14 M) per 100 ml of sample collected and stored in a fridge (< 4°C) until analysis. Feed samples from each diet type used in the experiment, the hatchery food used by each hatchery and three other commercially available feed types used by the New Zealand salmon farming industry, were either homogenised (wet food types) or ground up (dry food types), and dissolved in ultrapure nitric acid (14 M).

Water and diet samples were analysed for their elemental compositions and Sr isotopic compositions at the Centre for Trace Element Analysis, University of Otago. Trace elements were quantified using an Agilent 7500ce inductively coupled plasma mass spectrometer (ICP-MS) (www.chem.agilent.com) with molar concentrations normalised to calcium submitted for statistical analysis following the approach outlined by Gibson-Reinemer et al. (2009). Prior to analysis each instrument used was calibrated using solutions prepared from The National Institute of Standards and Technology (NIST) traceable standards (www.nist.gov). Elemental concentrations in these solutions spanned several orders of magnitude encompassing the range of concentrations found in the unknown samples. During each analysis, instrument performance was verified with certified reference materials [CRC SLRS-4 (National Research Council – Canada; www.nrc-cnrc.gc.ca/eng/services/inms/referencematerials.html) and USGS T167 (United States Geological Service – Analytical Evaluation Program for Standard Reference Samples; http://bqs.usgs.gov/SRS/)]. All solutions tested were spiked with scandium and indium as reference elements to correct for any instrument drift,

and the assay sequence was randomized across samples to eliminate analytical bias. Analytical consistency was checked by regularly analysing standards, duplicates and blanks throughout each analysis session. All analytical uncertainties for ICP-MS analysis are reported as 1 SD, unless specified otherwise.

To measure the strontium isotope composition, denoted by the $^{87}Sr/^{86}Sr$ ratio, strontium was initially separated and purified from the sample matrix using Sr-SPEC resin (Eichrom Ltd, U.S.A.) in an ISO 4 clean laboratory, using an ion exchange method modified from Pin and Bassin (1992) and Shaw et al. (2010). After purification, the $^{87}Sr/^{86}Sr$ ratios in the water and food samples were determined using a Nu Plasma-HR (Nu Instruments, U.K.) multiple-collector inductively coupled plasma mass spectrometer (MC-ICP-MS). Strontium isotopic data were collected as a single static measurement by simultaneously monitoring ^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr on an array of Faraday collectors. All $^{87}Sr/^{86}Sr$ ratios were corrected for instrumental mass fractionation using the measured $^{86}Sr/^{88}Sr$ normalized to the true value of 0.1194 and the exponential mass fractionation law (Habfast, 1998; Hart and Zindler, 1989), as well as for potential isobaric interferences from krypton (Kr) and rubidium (Rb). In detail, Kr interferences on masses ^{84}Sr and ^{86}Sr were monitored by performing an 'on-peak' baseline prior to each analysis. Any interference on ^{87}Sr from ^{87}Rb was then corrected for by monitoring ^{85}Rb during the course of the measurement, adopting an $^{87}Rb/^{85}Rb$ ratio of 0.3856. The veracity of this approach was evaluated by continuously monitoring $^{84}Sr/^{86}Sr$, which has a generally accepted value of 0.0565 ± 0.0001. Corrected $^{87}Sr/^{86}Sr$ ratios were normalized to $^{87}Sr/^{86}Sr = 0.710251 \pm 0.000009$ for the Sr isotope standard SRM 987 (National Institute of Standards and Technology (NIST), U.S.A.). The accuracy of the Sr isotope measurements was assessed by monitoring the $^{87}Sr/^{86}Sr$ ratios of the secondary in-house standards *Tridacna*, BCR2 and Speights spring water at regular intervals throughout the analysis session, giving rise to respective $^{87}Sr/^{86}Sr$ values of 0.709183 ± 0.000009 (*Tridacna*), 0.705011 ± 0.000024 (BCR2) and 0.704440 ± 0.000016 (Speights). All analytical uncertainties for Sr isotope analysis by MC-ICP-MS are reported as 2 SE, unless specified otherwise.

3.3.3 Otolith sample preparation and chemical analysis

At the conclusion of the diet experiment, all otoliths were extracted, preserved and prepared for analysis according to the methods of Barnett-Johnson et al., (2008) that had been adapted to the requirements of this study. Briefly, to remove external contaminants, otoliths were first cleaned ultrasonically in ultrapure water (18 MΩ), and then soaked in 10% H_2O_2 and rewashed in ultra-pure water before being air dried. All equipment used was soaked in 10% HCl overnight and rinsed with ultrapure water before use and between samples. One otolith from each fish was mounted sulcus side down with Crystal Bond (509) adhesive on microscope slides and rinsed with ultrapure filtered water, air dried, and stored in a dust free environment until analysis.

Otolith elemental composition was determined using an Agilent 7500cs ICP-MS connected to a RESOlution M50 laser ablation system (Australian Scientific Instruments (ASI), Australia) fitted with a HelEx (Laurin Technic and the Australian National University) ablation cell. Prior to ablating each otolith, background counts of masses in the carrier gas were measured for twenty seconds. After an initial pre-ablation to remove potential surface contaminants, signals from the following elements were collected from the outer edge of each otolith: strontium (Sr), barium (Ba), magnesium (Mg), manganese (Mn), calcium (Ca), lithium (Li). Laser parameters comprised a 30 by 60 µm slit size, a repetition rate of 5Hz, and a scan rate of 5 µm s^{-1}.

The Sr isotope ($^{87}Sr/^{86}Sr$) ratio was measured using a Nu Plasma-HR MC-ICP-MS instrument that was connected to the same laser ablation system, using a spot size of 90 µm, a repetition rate of 10 Hz and a scan rate of 5 µm s^{-1}. All measured $^{87}Sr/^{86}Sr$ ratios were acquired and corrected for instrumental mass fractionation as well as Kr and Rb isobaric interferences using the procedures discussed above. Additional molecular interferences across the Sr mass range from Ca argides/dimers were subsequently corrected for by monitoring the $^{42}Ca^{40}Ar$ dimer interference at mass 82, then peak stripping the contributions from

Ca argides/dimers from the peak intensities at strontium masses 88, 86 and 84 accordingly (Woodhead et al., 2005).

Elemental data were processed offline using a purpose built MS Excel spreadsheet and involved a low pass filter, smoothing and a blank subtracting function similar to that used by Barbee and Swearer (2007), and presented as element / Ca ratios, along with Sr isotope ($^{87}Sr/^{86}Sr$) ratios.

3.3.4 Data analyses

Following the approach outlined by Doubleday et al., (2013), significant differences in otolith chemistry between the four diet/water treatments (with 13-15 fish per treatment) were evaluated using a one-way analysis of variance (ANOVA) and then a Tukey's HSD post-hoc test. Prior to the one-way ANOVA analysis, otolith data for each treatment were examined for normality and homogeneity of variance, and Tukey's HSD test was used to examine if significant differences existed between replicate tanks. As no significant difference was observed between replicate tanks for any of the four treatments, all of the otolith data for each treatment were pooled (in total 57 fish), before re-analysing the data to test for differences between treatments using ANOVA.

To compare growth rates of brown trout between diet treatments, the actual growth (mm per day) and the specific growth rates (SGR) were calculated according to the following equation (Cook et al., 2000):

$$\text{SRG (\% body wt. gain/day)} = \left[\frac{(\text{Log Final fish wt.} - \text{Log Inital fish wt.})}{\text{Time interval}} \right] \times 100 \qquad (1)$$

To investigate if differences in growth rate existed between diet treatments, a Welch two sample t-test was used. The relative contribution of diet and water to otolith chemistry was also estimated for each diet type using a simple mixing model detailed in Kennedy et al., (2000) as follows:

$$\% \, Sr_{(\text{from water})} = \left[1 - \left(\frac{\text{Sr water} - \text{Sr otolith}}{\text{Sr water} - \text{Sr diet}}\right)\right] \times 100 \qquad (2)$$

$$\% \, Sr_{(\text{from diet})} = \left[1 - \left(\frac{\text{Sr otolith} - \text{Sr diet}}{\text{Sr water} - \text{Sr diet}}\right)\right] \times 100 \qquad (3)$$

The influence of a marine fish-derived hatchery food source on otolith chemistry was examined using data from brown trout and Chinook salmon reared in hatcheries over a range of water chemistry conditions. The relationship between otolith, hatchery food and water chemistry was demonstrated by a regression analysis. Further comparisons between the chemical composition of experimental diet types, and the other commercially available hatchery food types analysed was restricted to simply reporting the results of each food type analysed. All statistical tests were performed using R Statistical Software (R Development Core Team 2014).

3.4 Results

3.4.1 Water and diet chemistry

Water temperature in the rearing tanks ranged from 12.3 – 18.9 °C, while pH ranged between 7.2 – 8.2 (Figure 3.1). For each diet treatment, elemental water concentrations of either seawater or freshwater remained relatively constant throughout the experiment, having a group mean Sr/Ca (± SD) of 4.5 ± 0.3 µmol mol^{-1} for freshwater and 7.1 ± 0.4 µmol mol^{-1} for seawater (Table 3.1). Mean elemental concentrations differed significantly between diet (prey) types, with lower Sr but higher Ba concentrations in freshwater compared to marine prey (Table 3.2). Duplicate samples analysed were found to consistently be within ± < 5% of each other.

At the Fish and Game hatcheries ambient $^{87}Sr/^{86}Sr$ ratios (± 2SE) reflected the respective local water sources, ranging from 0.70445 ± 0.00002 to 0.70968 ± 0.00003, while the artificial hatchery food used had an $^{87}Sr/^{86}Sr$ ratio of 0.70914 ± 0.00002, reflecting the marine origins of its major ingredients (Table 3.2).

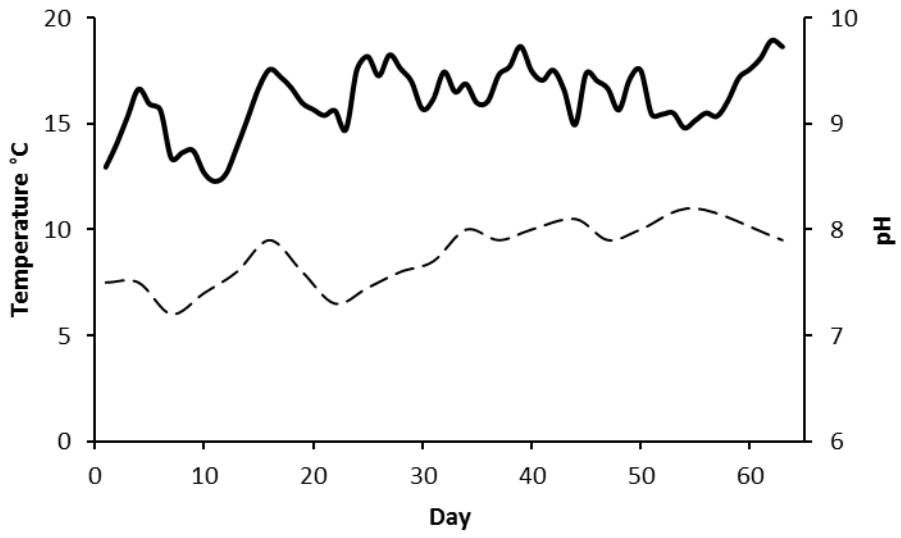

Figure 3.1. Daily temperature (solid line) and pH (dashed line) over the course of the diet experiments.

Table 3.1. Mean (SD) trace element / Ca ratios (µmol mol^{-1}) and Sr isotopic composition (2SE) of water sources for brown trout (*Salmo trutta*) diet experiments, and the hatchery / tank water used to rear Chinook salmon (*Oncorhynchus tshawytscha*).

Experimental treatment / location	Sr/Ca	Ba/Ca	Mg/Ca	Mn/Ca	Li/Ca	$^{87}Sr/^{86}Sr$
Freshwater – Otago Univ.	4.5 (0.3)	0.34 (0.02)	0.9 (0.1)	0.54 (0.01)	*BLD*	0.70445 (0.00002)
Seawater (33 PSU) – Otago Univ.	7.1 (0.4)	< 0.01 (0.001)	2.2 (0.1)	0.08 (0.01)	1.7 (< 0.1)	0.70916 (0.00003)
McKinnon's Creek Salmon Hatchery	3.5 (0.3)	0.07 (< 0.01)	259.4 (7.6)	*BLD*	0.3 (< 0.1)	0.70968 (0.00003)
Spring Creek Salmon Hatchery	2.7 (0.4)	0.06 (< 0.01)	290. 9 (5.3)	*BLD*	0.4 (< 0.1)	0.70885 (0.00004)
Ngongotaha Trout Hatchery	2.9 (0.2)	0.30 (0.02)	879.1 (4.2)	*BLD*	22.8 (< 0.1)	0.70581 (0.00003)
Sawyers Bay Salmon Hatchery	3.8 (0.2)	0.45 (0.02)	611.3 (8.8)	2.84 (0.01)	0.6 (< 0.1)	0.70533 (0.00005)

Note: BDL = below detection limit.

Table 3.2 Mean (SD) trace element / Ca ratios (µmol mol^{-1}) and Sr isotopic composition (2SE) in each food type used or tested.

Diet type	Sr/Ca	Ba/Ca	Mg/Ca	Mn/Ca	Li/Ca	$^{87}Sr/^{86}Sr$
Bloodworms (*Chironomidae*)	2.6 (0.9)	4.1 (0.2)	526.1 (61.4)	14.2 (1.3)	0.285 (0.016)	0.70948 (0.00004)
Brine shrimp (*Artemia*)	15.3 (0.2)	1.1 (< 0.1)	147.1 (3.2)	0.3 (< 0.1)	0.015 (< 0.001)	0.70918 (0.00002)
Galaxids	10.7 (0.4)	< 0.1 (< 0.1)	173.8 (5.9)	0.7 (< 0.1)	0.009 (0.004)	0.70918 (0.00002)
Hatchery feed*	3.7 (0.2)	0.3 (< 0.1)	45.0 (1.2)	0.8 (< 0.1)	0.008 (< 0.001)	0.70914 (0.00002)

*Note: * Hatchery food type used by Fish & Game New Zealand and in diet experiments.*

3.4.2 Otolith chemistry

Mean otolith Sr, Ba and Li concentrations (relative to Ca) were significantly different between most, but not all, treatments (ANOVA: $P < 0.001$, Figure 3.2), while Mg concentrations remained relatively unchanged in response to all treatments. Further analysis of where the significant differences were found indicated that otolith Sr/Ca signatures changed predictably as diet changed from a freshwater to marine-derived food source in both a freshwater and marine environment. However, the difference between mean otolith Sr concentrations of fish fed a marine diet in freshwater, and those fed a freshwater diet in marine water were not statistically significant (ANOVA: $P > 0.05$). In addition, a marine diet significantly increased otolith Li concentrations and decreased Ba and Mn concentrations in a marine environment, but did not seem to influence otolith concentrations of fish reared in a freshwater environment.

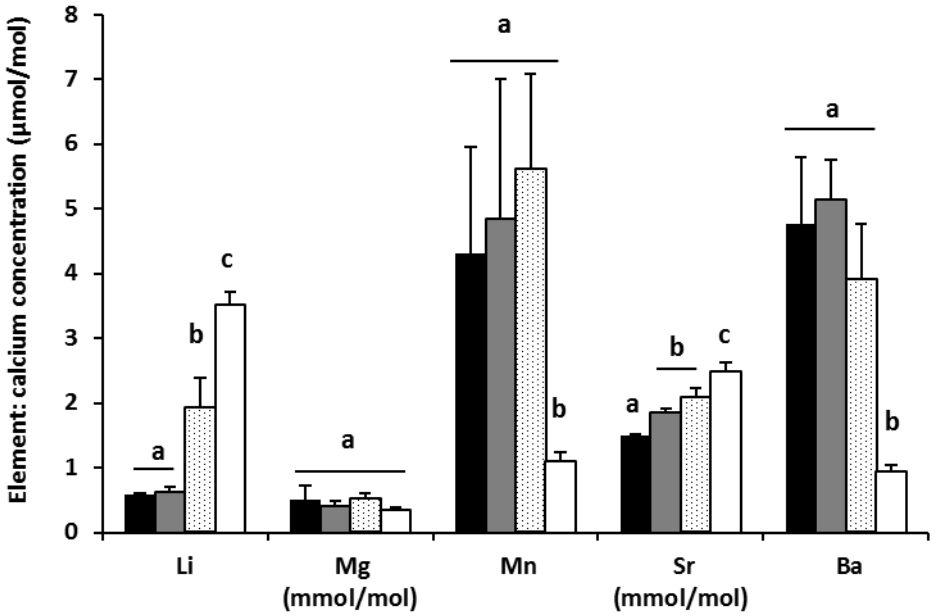

Figure 3.2 Mean otoliths (+SE) trace element / calcium concentration (µmol mol^{-1}) for brown trout (*Salmo trutta*) reared in either a freshwater (FW) or seawater (SW) environments and feed either a freshwater (FW: *Chironomidae*) or marine (SW: a mixture of *Galaxiids* and *Artemia*) diet. ■, FW & FW diet; ▒, FW & SW diet; ⬚, SW & FW diet; □, SW & SW diet. Letters (a-c) denotes where within each element block statistical differences occurred between treatments (ANOVA, $P < 0.05$).

3.4.3 Growth rates

Mean growth rates differed significantly between treatments (one-way ANOVA, $P < 0.05$) with fish fed a marine diet displaying higher growth rates than fish fed a freshwater diet, although the actual magnitude of the difference between treatments was small (< 0.1 mm day^{-1}, Figure 3.3).

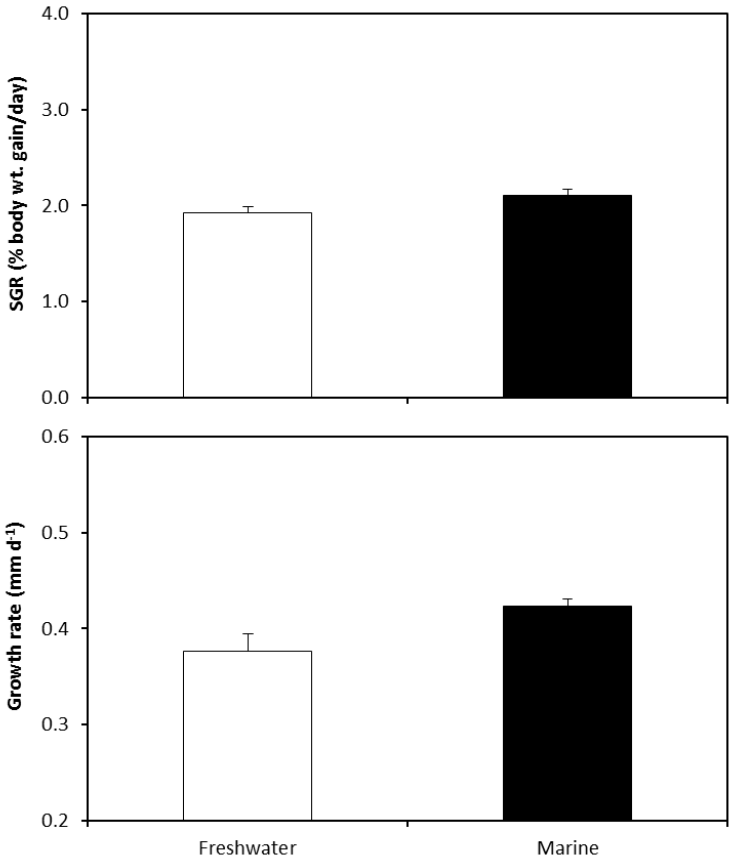

Figure 3.3 Mean (+SE) specific growth rates denoted by SGR (% body weight gain per day) and actual growth rates (mm per day) of brown trout (*Salmo trutta*) reared under similar environmental conditions but fed either freshwater or marine diet types.

3.4.4 Percent contribution of water and diet to otolith chemistry

Mean $^{87}Sr/^{86}Sr$ ratios in brown trout otolith, food and water sources showed that on average 87 % of the Sr in otoliths of fish fed a food source in chemical equilibrium with the marine environment (*i.e.* $^{87}Sr/^{86}Sr = 0.70918$; Hodell et al., 1989) can be derived from diet, and the remainder of the signature presumably comes from the water source (Figure 3.4). Moreover, examining the relative contributions of water and diet to brown trout and chinook salmon otolith chemistry, over a range of aquatic conditions, revealed diet influences change predictably as a function of increasing dissimilarity between water source and food chemistry (Figure 3.5). The relationship between mean water, diet and otolith $^{87}Sr/^{86}Sr$ values was best described by fitting a linear regression which had a R^2 value of 0.7081 (Figure 3.5).

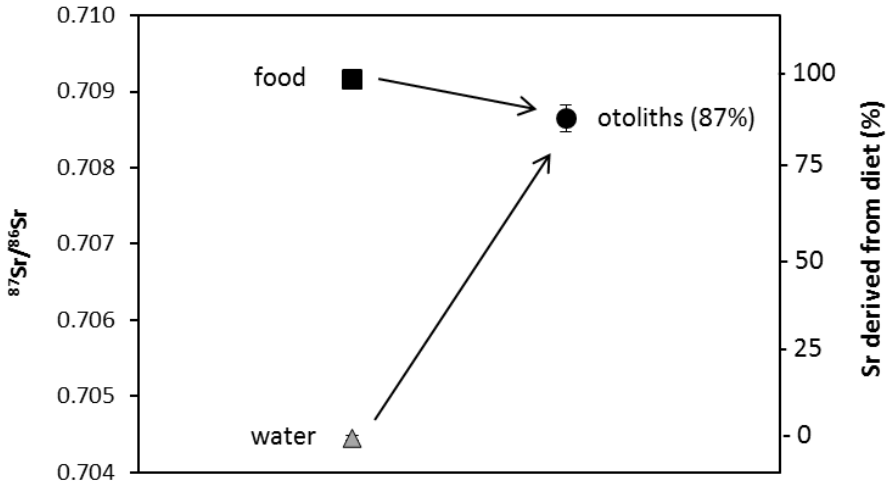

Figure 3.4 Relative influence of food and rearing water chemistry on mean $^{87}Sr/^{86}Sr$ ratios in brown trout (*Salmo trutta*) otoliths, showing on average 87 % of the Sr in otoliths of trout fed food in chemical equilibrium with the marine environment can be derived from diet. Error bars for mean otoliths, food and water source $^{87}Sr/^{86}Sr$ ratios are ± 2SE, but in some cases error bars are smaller than the symbol size.

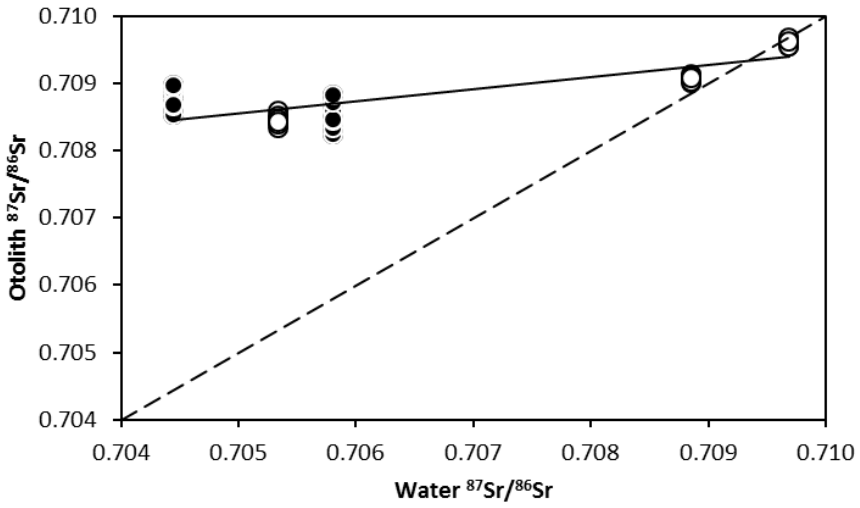

Figure 3.5 Examination of relationship between observed otolith $^{87}Sr/^{86}Sr$ ratios and rearing water $^{87}Sr/^{86}Sr$ ratios for brown trout (*Salmo trutta*)(filled symbol) and Chinook salmon (*Oncorhynchus tshawytscha*)(open symbols) raised across five hatcheries, but feed the same brand of marine based artificial feed in chemical equilibrium with the marine environment. The dashed line indicates the 1:1 relationship between rearing water and otolith $^{87}Sr/^{86}Sr$ values, which would be expected if a marine diet had no influence on otolith chemistry. The solid line shows the fitted linear regression which has an R^2 value of 0.71 and slope of Y = 0.1784x + 0.5828.

3.5 Discussion

The objective of this study was to address discrepancies among previous studies regarding how diet may affect otolith chemistry by specifically examining if natural prey sources containing marine-derived nutrients can influence the elemental concentrations within otoliths. Results confirmed that a diet of natural and semi natural prey types can significantly influence Sr or Ba concentrations in otoliths of salmonids reared in freshwater and marine environments. Otoliths from brown trout reared in freshwater and fed a mixture of marine-derived diadromous fish (*Galaxiids* sp.) and crustaceans (*Artemia* sp.) had significantly higher concentrations of Sr compared to otoliths from brown trout reared in freshwater and fed a freshwater diet (*Chironomidae*). Conversely, otoliths from

fish reared in sea water and fed a freshwater diet had significantly higher concentrations of Ba compared to fish reared in freshwater and fed a mixed diet reflecting marine prey sources. In addition, the otolith $^{87}Sr/^{86}Sr$ ratios in brown trout and chinook salmon raised in hatcheries also reflected the marine fish-derived origin of either diadromous fish or their artificial food source. Taken together, these findings confirm previous suggestive but inconclusive observations and simulations reported by Jaecks et al. (2016) highlighting that dietary influences on otolith chemistry are likely to occur whenever the primary food source is not in chemical equilibrium with the local environment. As such, my results improve our understanding about dietary effects on otolith chemistry.

While these results contradict several previous studies that have concluded water is the predominant source of elements in the otolith (Buckel et al., 2004; Milton and Chenery 2001; Marohn et al., 2009; Doubleday et al., 2013), they do help explain some of the observed discrepancies and variations in how diet may affect otolith chemistry found in previous studies. As such, when considered in conjunction with observations and simulations from previous studies on salmonids (e.g. Atlantic salmon: Kennedy et al., 2000, Rainbow trout: Gibson-Reinemer et al., 2009, or Dolly Varden: Jaecks et al. 2016), and other anadromous fishes (Limburg, 1995) these new results suggest that: (1) the confounding influences of diet on otolith chemistry are likely to increase as the dissimilarity between water and diet chemistry rises, especially when ambient concentrations are low; (2) comparable dietary effects to those observed in this study are anticipated to apply to all salmonids; and (3) in all probability, several other piscivorous freshwater fishes are likely to record mixed food and water signatures as well.

It is important to recognise that dietary influences on otolith chemistry in freshwater environments are unlikely to be limited to piscivorous fishes that seasonally feed on diadromous prey sources for extended periods, or the use of marine-derived artificial feed in hatcheries. In fact, because ecosystems are rarely bounded by the area selected for study, many external factors have the potential

to substantially affect the patterns and dynamics of focal systems (Nakano et al., 1999). Food web dynamics, in particular, are often strongly influenced by trophic linkages and the movements of prey organisms and nutrients across contiguous habitats (Polis et al., 1996, 1997). This implies that opportunistic feeders, like salmonids and several other freshwater fishes, which frequently change their diet according to food availability may, in addition to diadromous fish, also be exposed to other dietary influences. For example, diet studies have shown that eggs from spawning salmon and fragments of flesh from rotting carcasses are frequently eaten and increase growth rates, energy stores, and possibly survival rates of resident salmonids and other fishes (Bilby et al 1998; Wipfli et al., 2003; Heintz et al., 2004). In addition, terrestrial invertebrate subsidies can seasonally equal the importance of aquatic-derived invertebrates as a prey and energy source for drift-feeding salmonids and other riverine fishes alike (Wipfli, 1997; Nakano et al., 1999; Gustafsson et al., 2010). Thus, researchers need to consider that such prey items will not always be in chemical equilibrium with the local environment when interpreting datasets based on otolith chemistry.

Otolith chemistry has considerable potential to reveal patterns of origin and movements in freshwater populations, which greatly benefits fisheries management (Gibson-Reinemer et al., 2009). Nevertheless, given the confirmation presented in this study that natural food sources containing marine-derived nutrients can influence otolith chemistry in salmonids, it is prudent to consider to what degree dietary influences may confound our ability to reconstruct lifetime movement patterns and the natal origin of freshwater fish. For instance, Doubleday et al., (2013) state that in regards to measuring movements of fish across heterogeneous environments with differing chemical compositions (*e.g.* marine to freshwater, upper to lower reaches of a river, and among tributaries with distinct bedrock geochemistry) even a relatively minor contribution from diet may have a significant effect on how otolith data, and thus life time movement patterns of fish are interpreted. Strontium, and to a lesser extent Ba, are the most commonly used elements to distinguish between residency in freshwater, estuarine and marine environments (Elsdon et al., 2008).

Researchers aiming to utilise Sr isoscapes as their primary marker to track movements and understand the life history of fishes in river networks (see work by Muhlfeld et al., 2012) should therefore routinely consider the potential for dietary influences to have confounding effects. If not, there is a risk that high Sr concentrations in otoliths may be mistakenly interpreted as evidence for anadromy or estuarine residency instead of merely being a product of foraging behaviour. Such misclassifications could potentially be serious as processes occurring in freshwater, estuarine and marine habitats strongly influence the growth, survival and reproductive success of salmonids (Barnett-Johnson et al., 2010). In contrast, dietary influences are less likely to result in a noteworthy influence when investigating and reconstructing the natal origin of fishes. Natal signatures in unknown origin adult fish are normally measured close to the otolith core (c. 250 µm from primordia, Olley et al., 2011; Martin et al., 2013) in a region just distal to the dark otolith growth band indicative of the onset of exogenous feeding (Barnett-Johnson et al., 2008), and compared against a chemical baseline of water and/or otolith signatures collected from resident juveniles (0+) in the study catchment. Potentially confounding food sources for juvenile salmonids, such as salmon eggs or flesh, are only available during the spawning season, which normally occurs during the fall and winter periods for most anadromous salmonids (Quinn, 2005). Salmonids also need to reach a minimum size before their gape size is sufficient to enable them to eat other fish. However, if natal stream signatures were based on samples collected in the winter from streams known to support spawning anadromous fishes, and/or uses the most recently accreted otolith surface material of larger resident juveniles (age 1+), it is prudent to consider the likelihood of dietary influences.

While dietary influences from natural food sources require careful consideration by researchers using otolith chemistry to reconstruct life time movement patterns, they may also present a unique opportunity to advance our understanding of fish ecology. For instance, by identifying suitable markers for establishing the dietary influences of specific prey species, like juvenile galaxids, it may be possible to detect when a shift from drift feeding on aquatic invertebrate

to a piscivorous diet occurs. The onset of piscivory is an important event in the life history of salmonids, because it represents a significant increase in growth potential (Elliott and Hurley, 2000). Hence, the ability to determine to what degree seasonal high value food sources contribute to salmonid growth rates in upriver fisheries will help fishery managers and researchers better understand the role these food sources, and the habitats they inhabit, play at a population level.

Buckel et al., (2004) raised the possibility that indirect effects of diet on otolith chemistry, such as growth rates, gonad development and stress may potentially be responsible for some of the differences in how elements are incorporated into the otolith instead of diet. In my study, brown trout fed a mixture of diadromous fish and crustaceans did indeed grow slightly faster than those fed freshwater bloodworms. However, while these differences were statistically significant the actual imbalance in observed growth rates between diet types was relatively minor. Moreover, none of the fish used in this study underwent significant gonad development during the study period, and while not measured specifically, stress levels did not appear to differ between treatments (R. Gabrielsson, personal observation). In addition, neither Bath et al., (2000) or Buckel et al., (2004) found any evidence that growth rate significantly influenced the incorporation of Sr and Ba in otoliths in comparable studies. Hence, while piscivory can increase the growth rate of salmonids by up to approximately 50% compared to an invertebrate diet (Elliott and Hurley, 2000) the marginal differences in actual growth rates between diet types reported in my study are unlikely to have caused any confounding effect on otolith chemistry. Moreover, the $^{87}Sr/\ ^{86}Sr$ ratio, based on two isotopes of the same element, rather than different elements, is not influenced by external factors, such as growth rate and gonad development. This is because any isotope fractionation that might occur during these processes is reliably corrected for during analysis. Consequently, any changes in $^{87}Sr/\ ^{86}Sr$ signature can be attributed entirely to changes in source composition, namely the food consumed and the water inhabited by the fishes of this experimental study.

3.4.1 Conclusion

The chemical records contained in otoliths have undoubtedly provided many new insights into the movements and life history events of numerous fish species and populations (*e.g.*, see reviews by Thresher, 1999; Elsdon and Gillanders, 2003; Elsdon et al., 2008; Secor, 2010). While the possibility that diet may confound otolith-water chemistry relationships has received some attention from fish ecologists (*e.g.* Buckel et al., 2004; Gibson-Reinemer et al., 2009; Doubleday et al., 2013), most studies have concluded water is the dominant source of elements in the otoliths. However, although it would be ideal if trace element uptake into otoliths was completely passive and directly proportional to ambient water concentrations, this does not appear to be true for any element (Hicks et al., 2010; this study). The present study has illustrated the potential for prey sources that differ chemically from the local environments occupied by a fish to significantly impact salmonid otolith chemistry. Results also show that these influences appear to become more pronounced as the divergence between water and food chemistry increases away from a chemical equilibrium. As such, these findings confirm previous simulations made by Jaecks et al. (2016) advancing our understanding for how and when dietary influences on otolith chemistry may have ecologically relevant influences on otolith microchemistry, and thus will in some cases need to be considered. In light of my results I recommend that researchers attempting to use otolith chemistry to reconstruct the movements of freshwater fishes, whose foraging behaviour and diet seasonally include high quantities of prey that are not in chemical equilibrium with the local environments, carefully consider the possible effects of diet on the observed trace element and Sr isotope signatures. Providing the potential impacts and likelihood of dietary influences is considered appropriately, it is concluded that otolith microchemical analysis remains an extremely valuable tool for studying movement and life-history characteristics of freshwater fish.

Chapter 4 : Over-abstraction turns a nursery stream into a recruitment sink for a migratory brown trout population

4.1 Abstract

Balancing escalating demands for human use of water resources with conservation efforts aimed at protecting migratory stream fishes represents a major challenge for resource and fishery managers alike. Satisfactory solutions require both an understanding of life-history specific habitat needs and consideration of the role of dispersal and migration in the persistence of local and regional populations. This study investigated the natal origin of Lake Dunstan's migratory brown trout (*Salmo trutta*), population using a combination of natural chemical markers in eggs, otoliths and water samples. Using growth modelling it also examined some of the likely drivers behind observed movement patterns. Finally the observed recruitment contribution from spawning streams was compared against data on actual spawning efforts from redd count surveys. Results identified the main spawning areas used, but also revealed that not all of these contribute as expected to recruitment. While approximately 30% of the annual spawning effort is located in the Lindis catchment, this river contributed only 11% of the adult population in the lake. Examination associated this discrepancy with fragmentation and loss of connectivity during low flows, implicating water extraction practices for causing the Lindis River to act as a recruitment sink. Thus, taken together, this study highlights the need for holistic instream-effects assessments of water extraction practices at the catchment rather than sub-catchment scale, in order to minimise negative effects of anthropogenic impacts on recruitment dynamics of migratory fish.

4.2 Introduction

Migratory species that rely on multiple habitats to complete their life cycle can be particularly susceptible to population declines if one or more of their habitats are degraded, or if the connectivity between habitats is fragmented. Numerous studies show that the ecology of salmonids can be greatly affected by human activities. For example, mining, logging, agricultural practices, and urban development frequently affect the quality of freshwater habitats, and survival of embryos and fry (Quinn, 2005; Jonsson and Jonsson, 2011; Lennox et al., 2019). Dam construction, flow-regulation and water extraction are capable of interrupting the ecological connectivity in riverine landscapes, which might in turn affect the ecology and distributions of migratory salmonids (Greenberg and Calles, 2010; Young et al., 2011; Lange et al., 2014). Many of these anthropogenic impacts on the aquatic environment also have the potential to create ecological traps, which can cause source-sink population dynamics to develop (Hickford and Schiel, 2011; Jeffres and Moyle, 2012). Consequently, protecting and maintaining connectivity between high value habitats has long been the principal conservation strategy of freshwater fishery managers worldwide. Resource managers tasked with balancing increasing demands for anthropogenic use of water resources with stream fish conservation efforts therefore require an understanding of habitat use at different life history stages in order to consider the role of dispersal and migration in the persistence of local and regional populations. In this study, I examine if source-sink dynamics occurs in a lake migratory brown trout population.

Brown trout (*Salmo trutta*) populations are often partially migratory, where one part of the population leaves and feeds elsewhere, while another part stays as residents (see reviews by Klemetsen et al., 2003; Jonsson and Jonsson, 2011). The tendency to migrate is influenced by both environment and inheritance, but at a fundamental level is thought to largely be a conditional tactic dependent on growth rate, density and expected mortality (Olsson et al., 2006; Jonsson and Jonsson, 2011). However, O'Neal and Stanford (2011) pointed out

that the “decision” to migrate is undoubtedly a complex interaction involving genetic control of phenotypic plasticity and physiological (energetic) and environmental (food supply, physical habitat) correlates. Nevertheless, the ability to migrate is widely considered to be important in many catchments for both individual fitness and at a population level (Klemetsen et al., 2003; Carlsson et al., 2004; Jonsson and Jonsson, 2011).

In New Zealand, the majority of all salmonid angling occurs in lakes (Deans et al., 2004; Unwin, 2009). While these lakes often provide excellent adult feeding habitat and recreational angling opportunities, the location and relative importance of the different spawning and juvenile rearing areas that support them are often unknown. One such fishery is Lake Dunstan in the upper Clutha River basin in Central Otago where a semi-arid climate and ongoing agricultural intensification, facilitated by the use of historical water rights for irrigation purposes, dewater and fragment many of the streams that feed the lake. This has prompted fishery managers to raise concerns that water extraction practices might have detrimental effects on some of the lakes major spawning areas, like the Lindis River. In New Zealand the progeny of migratory brown trout tend to stay in their nursery stream for at least the first summer (Hayes, 1988; Kristensen and Closs, 2008; Hayes et al., 2010), but sometimes for up to 1-2 years before emigrating in response to flows, density related competition, or to access better growth opportunities (Graynoth, 1999; Kristensen and Closs, 2008; Holmes et al., 2014). Still, adult lake-feeding and river resident brown trout in New Zealand often attain similar sizes (up to 60 cm or more)(Graynoth, 1996; Hayes et al., 2000), and both normally spawn between April-July. As a result, it is generally not possible to visually distinguish between them or their spawning effort based on size, morphology, or temporal differences. Thus, establishing new techniques that accurately distinguish between spawning efforts of the two forms would provide a valuable tool for identifying the location and importance of spawning habitats that support lake migratory (adfluvial) populations.

Geochemical markers in otoliths (fish ear stones) are a powerful tool to address several fundamental questions in fish ecology, conservation, and fishery management (Kennedy et al., 1997; Campana and Thorrold, 2001; Elsdon et al., 2008; Walther and Limburg, 2012). The approach capitalises on detectable differences in otolith chemistry among juvenile fish raised in different natal locations. These differences can function as a permanent source tag, making it possible to determine the natal origin of individual adult fish, and quantify the recruitment contribution of a particular natal source to a population (Kennedy et al., 2000, 2002; Barnett-Johnson, 2005). In freshwater systems, radiogenic strontium (Sr) isotope ratios (reported, by convention, as the $^{87}Sr/^{86}Sr$ ratio) have proven to be a particular useful marker because they, unlike elemental concentrations, show no evidence of biological fractionation, which ensures $^{87}Sr/^{86}Sr$ values in otoliths are typically very similar to ambient stream waters (Kennedy et al., 2000, 2002; Walther and Thorrold, 2008). Furthermore, the chemical composition of other salmonid body parts (eggs) has also been found to match the maternal rearing habitat (Kalish, 1990; Waite et al., 2008; Gabrielsson et al., 2012), making it possible accurately to separate the spatial distribution of anadromous and resident salmonid spawning efforts in a catchment by sampling eggs in spawning redds (nests) (Kristensen et al., 2011). Strontium isotope ratios in eggs from lake rearing and stream resident salmonids can therefore provide a powerful marker for separating lake and river resident spawning effort in a spawning stream, while the $^{87}Sr/^{86}Sr$ of otoliths could be used to identify the proportional recruitment contribution that spawning stream makes to the adult trout population in a lake. Consequently, it appears possible to clarify if a particular river is drawing in migratory spawners but not returning many recruits to a lake fishery by combining the use of Sr isotope analysis in otoliths and eggs. This offers an opportunity to improve our understanding of the relationship between human activities such as water extraction and sink habitats. However, neither of these methods provides any detailed insight into the potential driver(s) behind the observed movement and recruitment patterns.

Bioenergetic growth models, on the other hand, are well suited to investigate mechanisms controlling growth, predict habitat selection and distribution, and explore growth-limiting factors (Hughes, 1998; Hayes et al., 2000; 2016; 2019). A description and test of several brown trout growth models, based on experimental research undertaken by Elliot et al. (1995), was given in Hayes et al. (2010). Because these growth models are based on the functional responses of fish to physical and prey variables, and factor out the effect of temperature on growth, food limitation can be identified whenever the observed growth falls short of the predicted growth (Hayes et al., 2010). This makes it possible to determine if and when metabolic demands and food constraints make it advantageous for juvenile brown trout to initiate outmigration from their nursery habitat. Thus, the combined use of these three techniques (Sr isotope analysis in otoliths, eggs and growth modelling) might both aid efforts to identify spawning areas used by lake migratory salmonids and identify some of the potential drivers behind such movements. Such clarification also has the potential to improve our understanding of fundamental ecological processes such as the presence of source-sink dynamics, which have been proposed to be abundant in nature (Krkošek and Lewis, 2010) but have seldom been demonstrated by empirical studies on salmonids (Johnson et al., 2012).

The objectives of this study were to (1) investigate the natal origin of adult brown trout in Lake Dunstan; (2) compare the recruitment contribution from the Lindis River, which has traditionally been considered the primary recruitment source, with other potential sources; (3) evaluate if the spatial distribution of freshwater migratory (adfluvial) and river resident brown trout spawning effort can be separated using egg chemistry; and (4) examine some of the underlying drivers and potential growth advantages of a migratory versus resident life history strategy for juvenile trout from the Lindis River.

4.3 Methods

4.3.1 Study area

Lake Dunstan (26 km^2) is an artificial lake created by the construction of the Clyde Dam in 1992 (Figure 4.1), and supports a highly valued recreational brown trout fishery. Estimated angler use has ranged from 17,000–26,000 angler days per year, ranking Lake Dunstan the second to fifth most used lake/reservoir fishery in the South Island of New Zealand (Unwin, 2016). The Lindis River is a 6^{th} order tributary that flows into the Clutha River approximately 9 km upstream of Lake Dunstan (Figure 4.1). It is considered to be an important recruitment source (Jellyman and Bonnett, 1992; Jellyman and Graynoth, 1993; Otago Regional Council, 2008), and spawning grounds for circa 30% of Lake Dunstan's brown trout population (this study). The Lindis drains a catchment area of 1055 km^2 and has a measured mean annual flow of 5.21 m^3/s, a median flow of 3.61 m^3/s and estimated naturalised 7-day mean annual low flow (MALF) of 1.75 m^3/s near its confluence with the Clutha River (Otago Regional Council, 2014). However, because the lower sections are one of the driest catchments in New Zealand (Pain and McConchie, 2015) historical water rights originally issued for mining purposes (a.k.a. deemed permits) currently allow for up to 4.14 m^3 to legally be taken from the Lindis River for agricultural irrigation purposes (Horrell, 2014). This results in an actual measured 7-day MALF of 0.26 m^3/s, which is < 15 % of the estimated naturalised MALF (Otago Regional Council, 2014). Consequently, large sections of the lower Lindis River are severely dewatered for much of the irrigation season (Dec-April) each year, even when only a small proportion of the legally available water is utilised for out-of-stream use. This results in a loss of connectivity with other waterways and degrades ecological conditions and fish rearing habitat in > 25 kilometres of the lower river, for up to 100 days each season. Large scale mortalities of juvenile trout and native fish (*Gobiomorphus cotidianus*) are common each summer (Otago Regional Council, 2008; Otago Fish & Game, unpublished data). While views differ as to what degree water extraction practices actually impact on the recruitment contribution the Lindis River makes, or could make, to the Lake Dunstan recreational fishery the termination of deemed permits

in 2021 obligate local authorities to reconsider minimum flow-levels and water allocation limits.

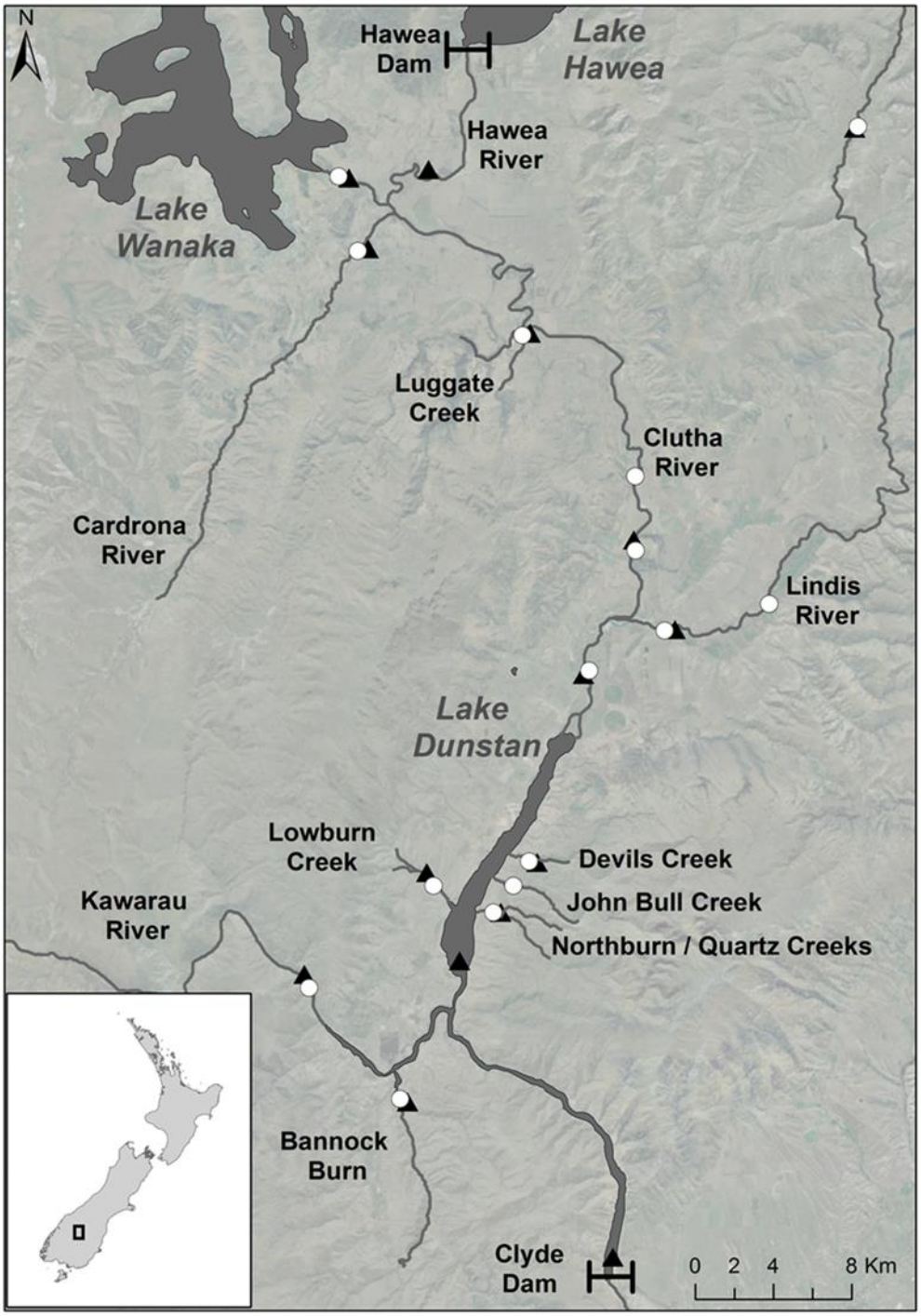

Figure 4.1 Map of the study area showing the location of fish migration barriers (dams), fish sampling (white circles) and water (chemistry and temperature) sampling sites (black triangles).

4.3.2 Distribution of brown trout spawning effort

Data from spawning surveys (redd counts) conducted by local fishery managers between 2009 – 2011 were used to examine the proportional distribution of spawning effort by Lake Dunstan brown trout population across known and probable spawning sites in the upper Clutha catchment. All surveys were conducted by experienced observers, mostly from a helicopter, in June and July each year once most females had spawned. Additionally, some smaller streams and side braids were surveyed on foot. Each spawning area was surveyed at least twice but sometimes as many as four times per season, except for the Cardrona River. The Cardrona River was surveyed only in 2011 because it is not recognised by fishery managers as an important spawning area for the Lake Dunstan brown trout population, based on previous surveys and available tagging data (pers. comm. C. Halford, Otago Fish & Game). The validity of this assumption was verified indirectly by comparing the amount of observed spawning effort there in 2011 to other spawning streams of a similar size, which confirmed it could be excluded from the overall assessment.

Once summarised, the available observational data on brown trout spawning effort (i.e. the % of all redds counted per season observed in a particular stream) was used as a coarse prediction of the potential recruitment contribution each spawning stream might make to Lake Dunstan's brown trout population, and compared with the actual observed recruitment contribution, quantified via chemical otolith analysis, in order to explore how observed spawning effort corresponded to the quantified recruitment contribution (see below).

4.3.3 Otolith collection

To identify the natal sources of the adult brown trout population in Lake Dunstan it was first necessary to establish a baseline of chemical habitat signatures (*i.e.* $^{87}Sr/^{86}Sr$ ratios) in juvenile trout otoliths and water samples collected from a range of likely natal sources. Juvenile brown trout were collected from spawning streams in summer (December — February) over three years, using an electric fishing machine (Figure 4.1). To ensure juveniles were indeed collected from their

natal rivers only young-of-the-year trout (maximum length < 80 mm) were collected and all sampling sites were located > 2km upstream of the confluence with other waterways, except for smaller tributaries that drain directly into Lake Dunstan. In these (Bannockburn, Devils, John Bull and Northburn/Quartz Creeks), access was only practical to sites located > 0.5 but < 1 km upstream from the Lake confluence, due to the steep stream gradient beyond that point. After recording wet weight and length of fish captured, a subsample of young-of-the-year brown trout (N > 10) from each site (N = 15) were selected for analysis. These were all euthanized and stored frozen whole, before the otoliths were removed. Finally, to quantify the recruitment contribution from each spawning stream to the Lake Dunstan brown trout population, a subsample of adult brown trout (N = 53 in total) captured in Lake Dunstan during the 2009 summer season were also selected for otolith analysis. Subsampled fish represented the size distribution and sex-ratio seen in angler harvest surveys (Otago Fish and Game, unpublished data).

4.3.4 Chemical preparation and strontium isotope analysis

1. Otoliths

To determine the natal origin of unknown origin adult trout captured in Lake Dunstan otoliths were prepared for chemical analysis according to methods outlined in Gabrielsson et al., (2012). Biological tissues were removed from otoliths by cleaning them ultrasonically in ultrapure water (18.2 MΩcm). Otoliths were then air dried under a laminar flow hood, before sectioning in a transverse plane, mounted on glass slides with thermoplastic resin (Crystal Bond 509) and sanded to or near to the otolith core with 1200-2000 grit wet-dry sandpaper. As a final preparation stage, each otolith section was gently polished using a slurry of ultrapure water and alumina powder (5 μm). Once finished, polished otolith sections were rinsed in ultrapure water, remounted on two glass slides for analysis, and again rinsed in ultrapure water and air dried before analysis. Juvenile trout otoliths were cleaned in the same manner but mounted whole with the sulcus side up in a randomly assigned order on glass slides.

The Sr isotopic ratios in otoliths were determined using methods modified from Gabrielsson et al., (2012) using a Nu-Plasma multiple-collector inductively-coupled-plasma mass-spectrometer (MC-ICP-MS, Nu Instruments Ltd, U.K.; www.nu-ins.com) coupled to a RESOlution M-50-LR excimer (193 nm) laser ablation system (Resonetics, U.S.A.) fitted with a HelEx laser ablation cell (Laurin Technic, Australia and the Australian National University) at the University of Otago Centre for Trace Element Analysis, New Zealand. Potential surface contaminants were removed during a pre-ablation step using a spot size of 130 µm, a repetition rate of 10 Hz and a scan rate of 10 µm s^{-1}. This was followed by sample ablation under a pure helium atmosphere whilst the laser was operated in continuous scan mode using a spot size of 90 µm, a repetition rate of 10 Hz and a scan rate of 5 µm s^{-1}. A data reduction procedure was conducted off-line using purpose built Excel spreadsheets, in order to correct for instrumental mass fractionation (using $^{88}Sr/^{86}Sr$ normalization), as well as interferences across the Sr mass range, such as ^{86}Kr on ^{86}Sr and ^{87}Rb on ^{87}Sr. The corrected $^{87}Sr/^{86}Sr$ ratios were then plotted against ablation distance (µm). The natal $^{87}Sr/^{86}Sr$ values in adult trout were taken as the average chemical signature occurring between 250-300 µm from the otolith core, hereafter referred to as the natal region. This distance represents the otolith material derived shortly after emergence and exogenous feeding has begun (Olley et al., 2011), and represents an average of >40 data points. The performance and accuracy of analytical protocols and $^{87}Sr/^{86}Sr$ measurement was monitored using an in-house *Tridacna* carbonate standard (Australian National University), measured at regular intervals throughout each analytical session, which allows correction for any instrumental drift during analysis. Strontium isotopic data acquired for this standard yield a mean (± 2 SD) $^{87}Sr/^{86}Sr$ of 0.709193 (± 0.000063).

2. Stream water and egg samples

To generate a chemical profile across the study region, water samples (>250 mL/site) were collected at base flow in the fall - winter of 2010 and 2012 using acid washed equipment and filtered (0.45 µm) directly into high density polyethylene (HDPE) sample bottles (Nalgene Ltd, USA) (see Figure 4.1 for sampled

locations). Samples were then kept on ice (< 40 h), and acidified with 0.25 ml ultrapure nitric acid (14 M) per 100 ml of sample to prevent adsorption of trace metals onto the walls of the bottle during storage, and stored in a fridge (< 8°C) until analysis.

To determine if egg chemistry can separate river resident and freshwater migratory (adfluvial) brown trout eggs, reference samples were collected from ripe female trout captured in their adult rearing habitats, in the upper Lindis River and Lake Dunstan, at the onset of the annual spawning migration (late May). Eggs from recently formed spawning redds were also collected along the Lindis River (June – July, 2012) for subsequent evaluation of the spatial distribution of freshwater migratory (adfluvial) and river resident brown trout spawning. All egg samples were initially chilled on ice before being stored frozen at -20°C until analysis. Otolith analysis was used to confirm that Lindis River fish were true river residents, and when considered together with chemical measurements of water samples, provide a reliable $^{87}Sr/^{86}Sr$ reference signature for resident spawning effort. In total samples were collected from 31 spawning redds along the Lindis River, along with the eggs from eight ripe females captured in Lake Dunstan and three ripe resident females captured in the upper Lindis River.

The strontium isotopic composition ($^{87}Sr/^{86}Sr$) of water and eggs samples were determined at the Centre for Trace Element Analysis, University of Otago following the approach outlined by Gabrielsson et al., (2012) and Waite et al., (2008). Strontium was eluded from each sample and purified through Eichrom Sr-SPEC resin using a slightly modified version of an established method (Pin and Bassin, 1992; Shaw et al. 2010). After elution with 0.05 M HNO_3 and appropriate dilution, the $^{87}Sr/^{86}Sr$ ratios were determined using a Nu Plasma MC- ICP-MS. Instrument performance was verified with certified reference materials [CRC SLRS-4 (National Research Council – Canada; www.nrc-cnrc.gc.ca/eng/services/inms/referencematerials.html) and USGS T167 (United States Geological Service – Analytical Evaluation Program for Standard Reference Samples; http://bqs.usgs.gov/SRS/)]. Analytical performance was also assessed by

analysing duplicate samples and blanks containing ultrapure water (collected using the same sampling protocol as adopted for the water samples). In-house internal standards processed in parallel with water and egg samples for verification of $^{87}Sr/^{86}Sr$ analysis resulted in a mean (± 2 SD) $^{87}Sr/^{86}Sr$ ratio of 0.709183 ± 0.000005 for *Tridacna*, while $^{87}Sr/^{86}Sr$ = 0.704440 (± 0.000016) for Speights spring water, and $^{87}Sr/^{86}Sr$ = 0.705011 (± 0.000024) for BCR2.

4.3.5 Classification analysis

Any interannual variability in juvenile otolith signatures and water samples was accounted for following the approach of Walther et al. (2008), pooling all ground-truthed chemical signatures from juvenile otoliths and water samples across the entire sampling period according to their source habitats. To determine if the mean $^{87}Sr/^{86}Sr$ isotopic signatures were significantly different between natal sources, Sr isotope ratios in juvenile trout otoliths and water samples were compared using analysis of variance (ANOVA). Natal sources that could not be statistically separated were aggregated. Such groupings tended to combine nearby locations into clusters. Hence, pooling of sites did reduce the overall spatial resolution for the middle parts of the catchment (see results section).

To determine the accuracy of juvenile trout classification according to their known natal origin (or cluster), a single-factor linear discriminant analysis (LDA), with jackknife resampling, was used. Prior probability of group membership was assumed to be equal between all groups, and included the possibility of an "unknown" source. The validated LDA model was subsequently used to predict the natal origin of unknown origin adult fish, captured in Lake Dunstan, as determined by the $^{87}Sr/^{86}Sr$ ratio in the natal region of their otoliths. Finally, to assess the relative reproductive contribution of each spawning area to the mixed-stock recreational fishery in Lake Dunstan the numbers of adult trout predicted to have originated in each spawning area were summarised as a percentage. All statistical analysis was carried out using R Statistical Software (R Development Core Team 2014).

To separate freshwater migratory and resident brown trout spawning effort using egg chemistry measurements of Sr isotope composition in reference water, otolith and roe samples collected from the upper Lindis River and Lake Dunstan were first compared with each other, and then against those observed in egg samples collected from spawning redds along the Lindis River. Instead of using a predetermined cut-off value to distinguish the maternal life-history of each spawning redd (e.g., Waite 2008; Kristensen et al., 2011), the cut-off value was estimated using univariate *K*-mean clustering. This procedure separated egg Sr signatures into two groups, whereby redds with egg Sr isotope composition less than the estimated cut-off value were assigned a resident life history, and those greater than the estimated cut-off value were assigned a migratory life history.

4.3.6 Growth modelling

To evaluate differences in growth potential between rearing habitats and determine if metabolic demands and food constraints could trigger migration, a whole-lifetime brown trout growth model, developed by Hayes et al. (2000) and based on energy balance equations from Elliott et al. (1995), was used in this study. The growth model predicts the wet weight of drift feeding brown trout over time based on observed average daily water temperature, by accounting for diurnal drift-foraging and reproductive costs while assuming that the availability of invertebrate food is not limiting growth (Hayes et al., 2000; 2010; Hayes 2013). Because the growth model factors out the effect of temperature on growth, and assumes that trout achieve maximum 24-h energy intake, food limitation can be identified if observed growth falls short of the predicted growth (Hayes et al., 2010). This convenient property allowed us to determine if metabolic demands and food constraints could influence initiation of juvenile trout outmigration during low flow summer conditions.

To run the growth model the average daily water temperature for each nursery stream was calculated from continuously logged data (Onset HOBO Data Loggers, recorded at 30 minute intervals) over the period June 2008 – April 2013 (refer to Figure 4.1 for locations). Minor temporal gaps in the temperature records

were addressed by first fitting a sine curve model to the recorded data from each stream (Mosley, 1982), and subsequently using predicted data as the input required to conduct growth modelling. This allowed life time growth trajectories for a resident life history strategy to be modelled in the five largest natal habitats in the upper Clutha basin (i.e. Lake Dunstan; the Clutha River; the Lindis River; the Cardrona River and Luggate Creek).

In a second step, to specifically determine if food constraints occur during summer low flow conditions, growth rates over two summer seasons (Dec – March) in the lower Lindis River for juvenile fish (age 0+) were modelled separately. Again the growth model was run on a daily time-step to predict growth based on the average daily water temperature, assuming maximum food consumption. While the primary model output is weight (g) at age, it also calculates an estimated length (mm) from predicted weights, using length-weight relationships developed for brown trout in New Zealand Rivers by Hayes et al. (2000).

4.4 Results

4.4.1 Distribution of brown trout spawning effort

The proportion of spawning redds counted (expressed as a percentage of all redds observed that year) did not vary greatly over time for the two most frequently used spawning areas, the Lindis and Clutha rivers (Table 4.1). On average, the Lindis River accounted for 32% of all redds observed each year (range: 26-36%), while the Clutha River accounted for 50% (range: 43-54%; Table 4.1). Remaining streams on average individually represented < 10%, and combined < 19%, of the total annual spawning effort observed each year. Examination of the coefficient of variation (CV) showed that the interannual variability in observed spawning effort was small for the Lindis and Clutha rivers, but greater in smaller streams and lake tributaries (Table 4.1).

Table 4.1 Observed spawning effort (expressed as % of redds observed) across natal streams in the Upper Clutha catchment during the 2009 – 2011 spawning seasons, used to predict the potential recruitment contribution to Lake Dunstan's brown trout (*Salmo trutta*) population.

Natal source	Percentage of all redds observed (± SD)		
	Mean	Range	CV
Lindis River	31.7% (± 4.2)	26 – 36%	0.16
Clutha River	49.7% (± 4.8)	43 – 54%	0.12
Luggate Creek	5.0% (± 2.2)	3 – 8%	0.53
Lake Dunstan tributaries	9.7% (± 3.3)	6 – 14%	0.42
Kawarau River & tributaries	4.0% (± 0.8)	3 – 5%	0.25
In total	*100%*		

Source: Spawning surveys conducted with assistance from Otago Fish & Game and the Clutha Fisheries Trust.

4.4.2 Classification analysis to determine the natal origin of trout

Mean $^{87}Sr/^{86}Sr$ values at the edge of juvenile otoliths, and in water samples, varied considerably among natal stream sites across the Upper Clutha basin. Mean $^{87}Sr/^{86}Sr$ values were highest in the Lindis River (ca. 0.7096), and lowest in the Kawarau River sub-catchments (ca. 0.7070), but similar for several natal sources located in the middle parts of the catchment, and (or) draining into Lake Dunstan. The similarity of chemical signatures (ANOVA, $P > 0.05$) made the construction of a detailed LDA model based on Sr ratios sensitive to misclassifications. Subsequent aggregation of sites with overlapping chemical signatures isolated four geochemically-distinct clusters (Figure 4.2, Table 4.1), and resulted in an LDA classification success of juvenile trout and water samples back to their known capture group (or location), as determined by a jack-knife cross-validation procedure, ranging from 91% to 100% (Table 4.2). Given that the principal purpose of this study was to determine the recruitment contribution from the Lindis River, these four groups provided the best achievable and

ecologically most relevant spatial resolution on the natal origin of adult trout captured in Lake Dunstan.

After using juvenile otolith signatures and water samples to validate the LDA model, the natal origin of adult brown trout captured in Lake Dunstan, as determined by their otolith $^{87}Sr/^{86}Sr$ values in the natal zone, was predicted to establish the proportion originating from the Lindis River (Figure 4.2). The LDA trout predicted 15% percent of adult brown trout captured in Lake Dunstan originated from the Kawarau River system (Group 1), 38% from the upper Clutha River or eastern tributaries of Lake Dunstan (Group 2), 36% from the lower Clutha River or western tributaries (Group 3), and 11% from the Lindis River (Group 4)(Figure 4.2).

Table 4.1 Classification success matrix showing the proportion of correctly assigned juvenile brown trout (*Salmo trutta*) and water samples to their known-origin sampling locations (in bold), using $^{87}Sr/^{86}Sr$ values. Percent correct classification for each cluster group are shown on the diagonal (in bold), blank spaces indicate no classification.

	Cluster Group 1	**Cluster Group 2**	**Cluster Group 3**	**Cluster Group 4**
Cluster group 1	**100**			
Cluster group 2		**91**	9	
Cluster group 3		5	**95**	
Cluster group 4				**100**

Natal cluster grouping codes are: 1 = Kawarau River and tributaries; 2 = Eastern tributaries of Lake Dunstan and upper section of the Clutha River; 3 = lower sections of the Clutha River, Cardrona River, Bannockburn, Lowburn and Luggate Creeks; 4 = Lindis River and tributaries.

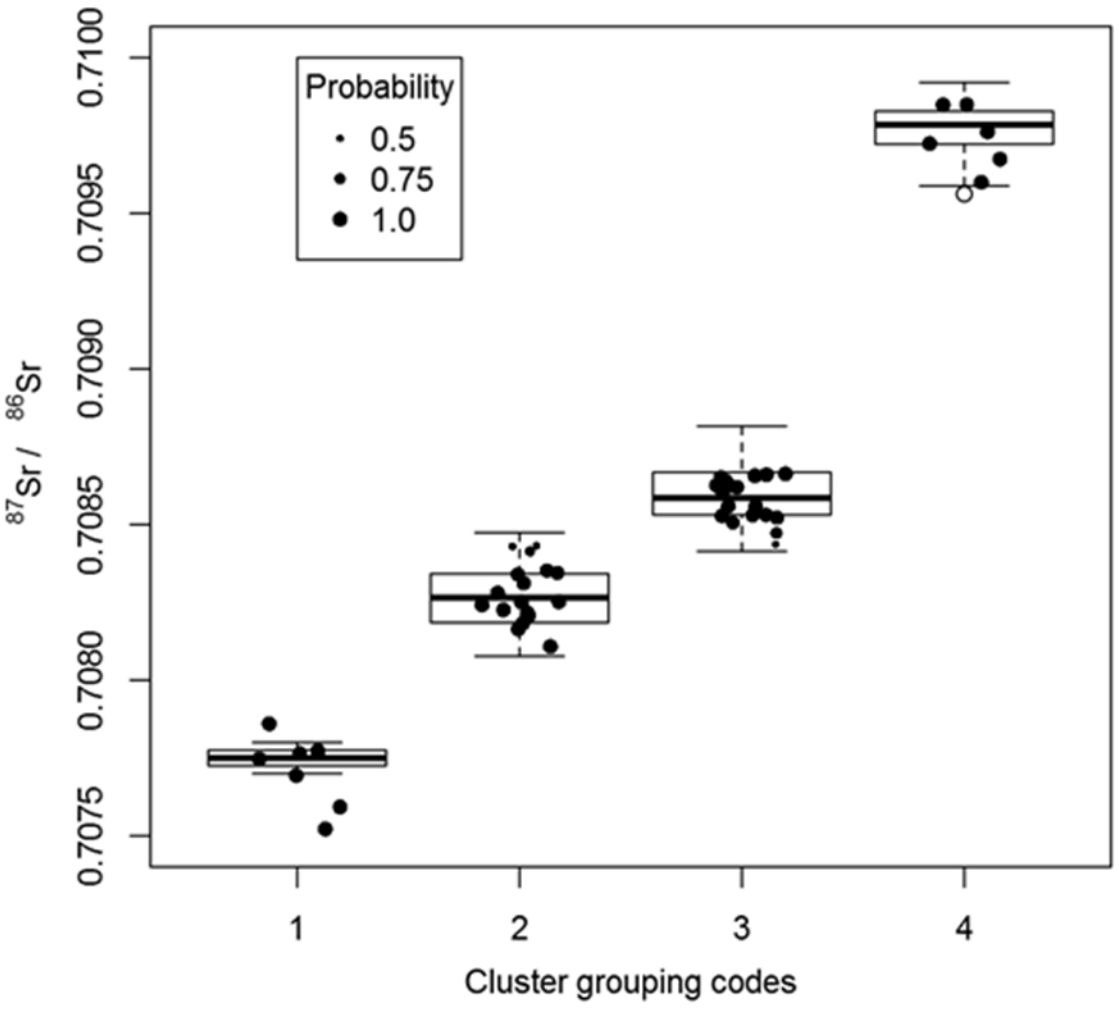

Figure 4.2 Box and whisker plots of $^{87}Sr/^{86}Sr$ ratios from juvenile brown trout (*Salmo trutta*) otoliths and water samples collected from natal stream groups across the upper Clutha catchment. The lower and upper limits of the boxes represent the 25th and 75th percentiles, respectively; while whiskers extend to the furthers data point within 1.5 times the interquartile range; data beyond this extent are represented by open dots. Black circles illustrate the predicted natal origin of adult brown trout captured in Lake Dunstan (N = 53), based on otolith $^{87}Sr/^{86}Sr$ ratios in their natal zone. The probability associated with each prediction is shown by the location and size of the circles. Natal cluster grouping codes are: 1 = Kawarau River and tributaries; 2 = Eastern tributaries of Lake Dunstan and upper section of the Clutha River; 3 = lower sections of the Clutha River, Cardrona River, Bannockburn, Lowburn and Luggate Creeks; 4 = Lindis River and tributaries.

4.4.3 Separating freshwater migratory and resident brown trout spawning effort using egg chemistry

Strontium isotope values ($^{87}Sr/^{86}Sr$ ratios) in reference samples (waters, otoliths and roe) from both the upper Lindis River and Lake Dunstan respectively were in excellent agreement to one another, and provided strong evidence for a distinct chemical signature between these locations (Figure 4.3). Eggs collected from resident female brown trout in the upper Lindis River had significantly higher $^{87}Sr/^{86}Sr$ ratios than those collected from female brown trout in Lake Dunstan (mean ± 2 SD $^{87}Sr/^{86}Sr$ ratio: Lindis River = 0.7096 ± 0.0001; Lake Dunstan = 0.7083 ± 0.0002, $P < 0.001$, Figure 4.3). *K*-means cluster analysis estimated a cut-off $^{87}Sr/^{86}Sr$ value of circa 0.7090, and designated spawning redds with egg $^{87}Sr/^{86}Sr$ values > 0.7090 to be classified as the product of a resident maternal life-history, while those with $^{87}Sr/^{86}Sr$ values < 0.7090 were classified as migratory maternal life-history. Egg samples collected from spawning redds along the full length of the Lindis River exhibited a range of $^{87}Sr/^{86}Sr$ values, extending from 0.7082 to 0.7097 (Figure 4.3), however $^{87}Sr/^{86}Sr$ values > 0.7090 (indicative of resident spawning effort) were only collected from the upstream end of the river (Figure 4.4). Overall *K*-means cluster analysis assigned 83.9% of all spawning redds sampled in the Lindis River to the migratory maternal life-history, and 16.1% to the resident maternal life-history.

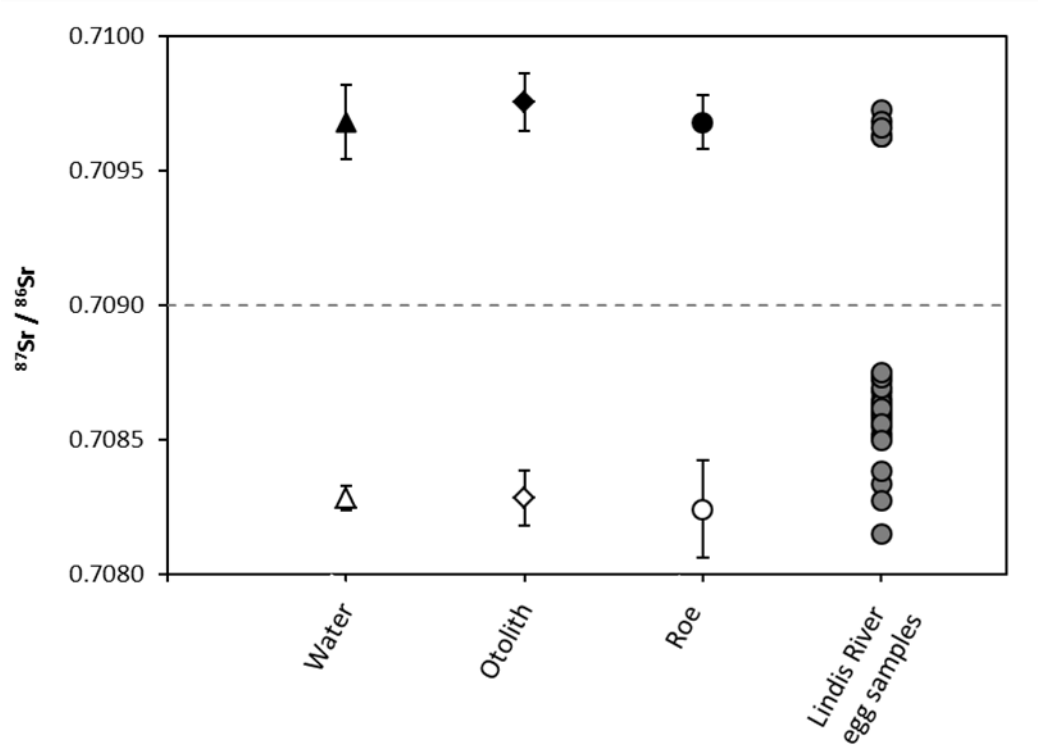

Figure 4.3 Scatter plot showing mean $^{87}Sr/^{86}Sr$ ratios in brown trout (*Salmo trutta*) egg samples (N = 31) collected from spawning redds in the Lindis River (grey circles), along with reference signatures from water samples, brown trout otoliths and roe collected from ripe fish captured in Lake Dunstan (open symbols) or the upper Lindis River (closed symbols). Error bars are ± 2 SD, while the dashed line illustrates the estimated threshold between resident and migratory trout egg Sr signatures, based on *K*-means cluster analysis.

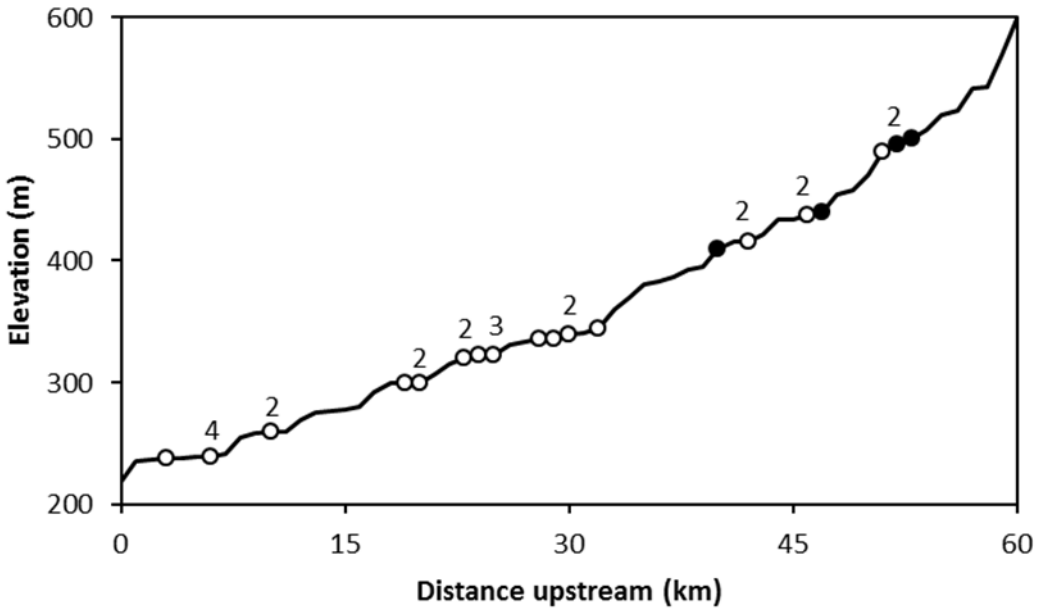

Figure 4.4 Elevation profile for the Lindis River, as it leaves the Clutha River, illustrating the distribution of spawning redds (N = 31) from migratory (open circles, $^{87}Sr/^{86}Sr < 0.7090$) and river resident (black circles, $^{87}Sr/^{86}Sr > 0.7090$) brown trout (*Salmo trutta*). Numbers indicate locations where > 1 redds were sampled at a single location.

4.4.4 Growth modelling

Modelling of lifetime growth trajectories, based on daily water temperature records, for larger brown trout spawning streams in the Upper Clutha River catchment highlighted large differences in lifetime growth potential between different nursery habitats (Figure 4.5). Anticipated growth trajectories for fish rearing in all tributaries deviate noticeably from those in Lake Dunstan or the mainstem Clutha River after fish reach one year of age. The growth model predictions demonstrate that female brown trout rearing in the Clutha River or Lake Dunstan can reach the observed minimum size at maturity in three years (Length > 36 cm, Weight > 550 g; R. Gabrielsson unpublished data) while those in the Lindis River would require > four years, and from Luggate Creek or the Cardrona River > five years to reach a similar size. Furthermore, comparisons of observed versus predicted growth rates of juvenile brown trout from two sections of the lower Lindis River demonstrated notable differences across both seasons

surveyed (Figure 4.6). Comparison of observed and predicted growth rates indicate juvenile brown trout achieve about 70 % of maximum consumption rate, i.e. demonstrating growth rates are likely food limited.

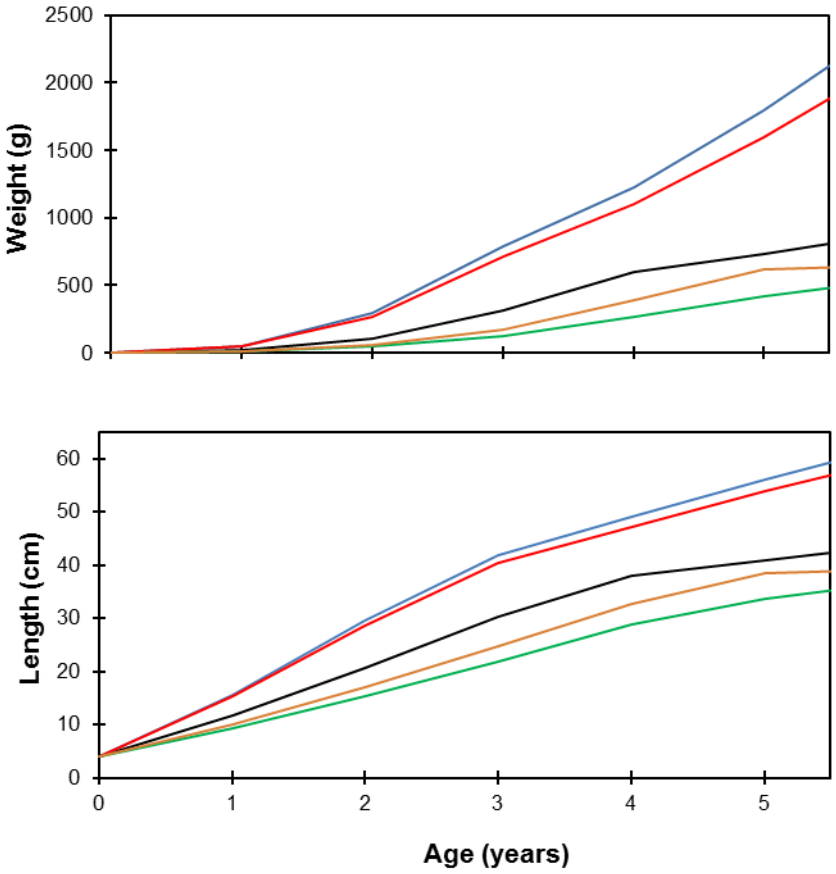

Figure 4.5 Comparison of predicted lifetime growth trajectories from bioenergetics modelling based on measured water temperature across the Upper Clutha Catchment. Panels illustrated predicted wet weight (g); and estimated length (cm) over time in each location, assuming maximum daily rations of invertebrate prey, while accounting for diurnal drift-foraging costs and reproductive costs. Lake Dunstan (**blue**), Clutha River (**red**), Lindis River (**black**), Cardrona River (**orange**) and Luggate Creek (**green**).

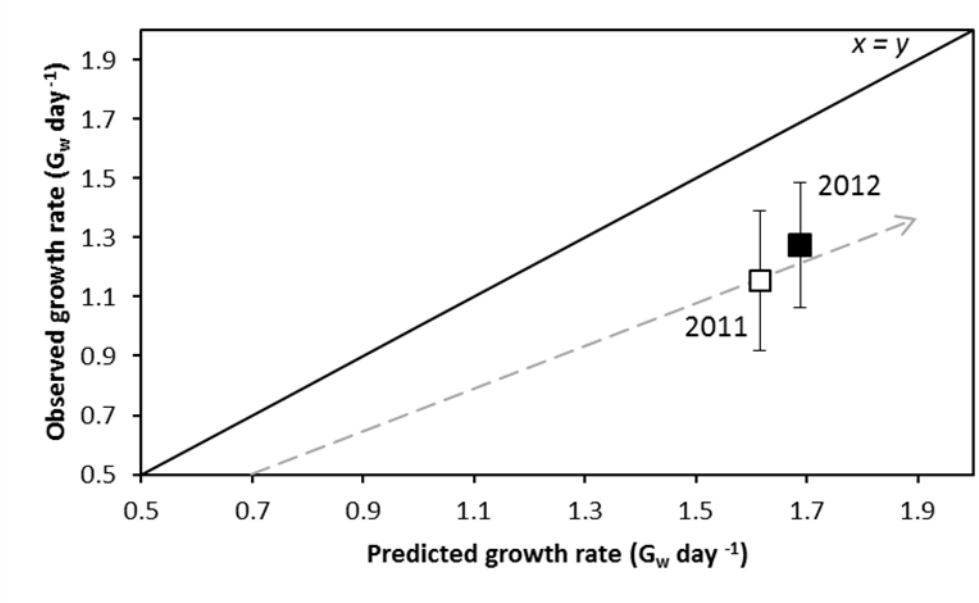

Figure 4.6 Comparison between mean observed instantaneous (G_{obs}) growth rate (g/day) of age-0 brown trout in the Lindis River at the end of each growing season (Dec – April) and the predicted maximum (G_{max}) growth rate for brown trout (i.e. expected growth of fish feeding on maximum rations). Lines represent a 1:1 relationship (solid line) and 70 % of the predicted maximum growth rate (dashed line), while error bars show ± 1 SD.

4.5 Discussion

Salmonids have complex life cycles involving major changes in habitat requirements at different stages in their life history (Nislow and Armstrong, 2012). As a result, they often display complicated migration patterns that present challenges for those seeking to understand relationships between movements and the population dynamics of local and regional populations. My study examined recruitment sources and movement patterns of a lake-migratory brown trout population using a combination of chemical markers in eggs, otoliths and water samples taking advantage of the excellent discriminatory power of $^{87}Sr/^{86}Sr$ isotope ratios. It also explored some of the likely drivers behind observed movement patterns using growth modelling. Results revealed several interesting points that provide resource managers with an improved understanding of where

the key recruitment areas for Lake Dunstan's brown trout population are located, as well as the likely drivers behind observed movement patters. Moreover, the integrated analysis of data from spawning surveys, chemical markers in eggs and otoliths, and growth modelling indicates that when water extraction practices result in prolonged loss of connectivity within and between waterways through fragmentation of the migration corridors, it has the potential to cause nursery streams to act as recruitment sinks for migratory trout populations.

Habitat protection actions targeted at protecting spawning streams and nursery areas are often prioritised based on some form of confirmation of their recruitment contribution to a lake migratory trout population of interest. For example, in the last decades otolith elemental fingerprints are increasingly being employed as effective natural tags. However, the lack of an established significant recruitment input from catchments where large amounts of spawning activity is observed can also provide a warning that human use of water resources may currently be causing negative effects. For example, of the 53 fish harvested in Lake Dunstan only 11 % were classified as having originated in the Lindis River. This was lower than expected and aligns poorly with predictions made from observed spawning efforts which estimated that on average about 30% of the Lake Dunstan brown trout population annually spawn in the Lindis catchment. It also contradicts predictions by Jellyman and Bonnett (1992) that the Lindis River was likely to become a major future source of recruits for the Lake Dunstan brown trout fishery. Possible explanations for this discrepancy between the observations and predictions include extreme flow events (floods or droughts), which can cause a large natural variability in recruitment success and survival (Hayes, 1995; Fausch et al., 2001; Elliott and Elliott, 2006). Alternatively a large component of the observed brown trout spawning redds in the Lindis River could be the product of resident and not migratory trout.

Floods are a dominant natural feature driving variation in juvenile trout abundance (Allen, 1951; Hayes, 1995; Fausch et al., 2001). Studies show mortality of juvenile salmonids increases significantly in years with high river flows during

the alevin stage as well as the first week after emergence, while high flows during early egg incubation and more than one week after emergence appear to be less important (see reviews by Fausch et al., 2001; Jonsson and Jonsson, 2011). Specifically, Hayes (1995) found that in one New Zealand river large floods ≥13 times the mean flow with a return period of 4 years occurring during late incubation and early fry rearing stages (i.e. August – November) can have a detrimental effect, and be associated with impaired recruitment linked to a weak year-class of juvenile trout. By contrast, a larger flood (c. 5 year return period) during later life stages (December and March) had no apparent effect on the survival of age 0+ trout (Hayes, 1995). As flows >10 times the mean flow regularly move a substantial portion of the river bed (Clausen and Plew 2004), these findings are consistent with Quinn (2005) who suggested that movement of streambed gravel is one of the main physical factors affecting embryo and fry survival. However, hydrological records during late incubation and early fry rearing (i.e. up to 6 weeks after mean emergence) in the years preceding my study show no large floods occurred. Thus, recruitment failure due to large floods during early life stages is an unlikely explanation for the discrepancy between the observed and predicted recruitment from the Lindis River.

A close proximity to Lake Dunstan strongly suggests, but does not confirm, that the majority of the spawning activity recorded in the Lindis River is likely to be the product of migratory rather than resident fish. However, the close alignment between Sr isotope ratios ($^{87}Sr/^{86}Sr$) in water samples, eggs and otoliths collected from ripe female brown trout in Lake Dunstan and the upper Lindis River show that egg chemistry can be a valuable tool for distinguishing between spawning efforts of lake migratory and river resident salmonids. Application of this method confirmed the majority of the brown trout spawning effort observed in the Lindis River is the product of migratory females (100% in the first 30km, and > 83% of all samples tested). Modelling of lifetime growth trajectories (Figure 4.4) also illustrated that trout from the Lindis River can realise a measurable fitness (size) advantage by adopting a migratory life history strategy. These discoveries demonstrate the importance of the Lindis River as a nursery stream for Lake

Dunstan's migratory trout population and confirm Kristensen et al (2011) findings that it is possible to accurately separate the spatial distribution of resident and migratory salmonid spawning efforts in a catchment by sampling eggs in spawning redds. While egg samples indicative of resident spawning effort ($^{87}Sr/^{86}Sr$ values > 0.7090) varied little, and were only collected from the upstream end of the Lindis River, egg samples from migratory redds ($^{87}Sr/^{86}Sr$ values < 0.7090) collected along the full length of the Lindis River exhibited a greater range of $^{87}Sr/^{86}Sr$ values (Figure 4.3). A similar pattern of more variable chemical signatures downstream was also observed by Kristensen et al. (2010). Volk et al., (2000) showed that if egg development is not completed when the female leaves the adult feeding habitat egg Sr concentrations may potentially be altered during the spawning migration. The observed range of Sr isotope values could therefore be interpreted as confirmation that ongoing egg development during the spawning migration modifies egg Sr isotope composition, due to admixing of additional Sr with a different Sr isotope signature, in at least some of the migratory female brown trout spawning in the Lindis River. While this did not confound the ability to separate resident and migratory spawning effort in this study, it suggests chemical analysis of eggs deposited in spawning redds is mostly suitable for systems with comparably large chemical differences. Accordingly it is advisable to consider the likelihood and potential implications of ongoing egg development during the spawning migration, on a case by case basis, before attempting to utilise chemical analysis of eggs from spawning redds to separate the spawning effort or progeny of resident and migratory female fish in other systems.

The confirmation of widespread spawning by migratory brown trout in the Lindis River, and that large floods during early life stages are an unlikely explanation of recruitment failure (see earlier paragraphs), implicate water extraction practices for causing the Lindis River to contribute fewer recruits than expected to Lake Dunstan. Droughts, regardless of whether they are natural or as a result of water withdrawals, can have catastrophic effects on stream-living salmonids by reducing the water-covered area available for fish, resulting in increased fish mortality due to low oxygen concentrations and high temperatures,

and decreased invertebrate (food) production and drift rates (see review by Jonsson and Jonsson, 2011). Summer droughts were the main factor explaining outliers in the stock-recruitment relationships for juvenile brown trout in the Black Brows Beck (Elliott and Elliott, 2006). Water extraction for agriculture annually creates conditions resembling catastrophic drought events in the lower 20 km of the Lindis River (i.e. low flows alter habitat quality and connectivity, increase water temperature and reduced food production). Trotter (2016) established that these conditions result in high mortality rates of juvenile trout, although it is unknown to what degree juvenile trout in the middle and upper sections of the Lindis River experience similarly mortality rates.

The migration of young fish from nursery streams to a lake is a common way of trying to escape harmful environmental conditions, such as droughts, that constrain growth and survival (Jonsson and Jonsson, 2011). However, extreme low flows often cause extensive heating of shallow areas and reduce streams to a series of isolated pools (Stanley et al., 1997), effectively stopping salmonids from leaving nursery streams to avoid hostile conditions. Thus, while the adverse effects of extreme droughts on juvenile salmonids are complex, and most likely further complicated by possible sublethal effects induced by thermal stress, the combined effects of extended periods of predation pressure, reduced growth rates due to food limitation and (or) density dependent competition should be detectable. Data presented in this study show that observed growth rates of young-of-the-year brown trout in the Lindis River are lower than predicted during the irrigation season (Figure 4.6). Higher than optimum summer stream temperatures can reduce growth rates of fish by increasing metabolic rates (Nicola and Almodovar, 2004), but variations in stream temperature are accounted for in the predictive growth model (Elliott et al., 1995). Invertebrates are considered to be the primary food base for young salmonids and other stream fishes (Allen, 1951; Chapman, 1966; Wipfli, 1997; Allan et al., 2003). Factors or processes that restrict the production, or delivery, of food items (invertebrates) in streams were not accounted for in the growth model, but can be expected to dramatically influence their ability to support salmonid populations (Wipfli, 2009). Thus, limited food

availability either through reductions in wetted area, drift rates or as a consequence of increased competition between fish provides a likely explanation for the lower than predicted growth rates observed. None of these factors are accounted for in the growth model but has been reported for other populations (Lagarrigue et al., 2001; Jensen et al., 2000; Nicola and Almodovar, 2004). While the abundance of invertebrates in the stream benthos or drift was not directly measured in this study, the observed reduction in growth rates do parallel those from a study of how reduced stream flow lowers summer growth of rainbow trout (Harvey et al., 2006), and a 10 year study relating growth of Artic grayling to annual flow variation and other physical variables (Deegan et al., 1999). As such, the detected inconsistency between the predicted and observed growth rates of juvenile trout in the Lindis River suggests food limitation could initiate juvenile brown trout outmigration during summer, presumably as a result of low flows, drift rates and invertebrate production.

As emigration from the Lindis River is not possible during the low flow period, due to habitat fragmentation and loss of connectivity with other waterways as a result of water extraction practices, juvenile trout are subjected to extended periods of high predation pressure and food limitation. For instance, both Nicola et al. (2009) and Trotter (2016) showed that the magnitude and duration of low flows during summer drought appears to be a critical factor for survival of young brown trout. Food restriction can also lead to increased predation risks associated with elevated foraging activity (Lima and Dill, 1990; Jönsson et al., 1996; Gotthard 2000) and decrease starvation endurance (Stockhoff 1991; Gotthard et al., 1994), which might have a large impact on winter survival. It has been suggested fish may try to escape high mortality rates and growth constraints in the lower Lindis River by entering unscreened irrigation races feeding flood irrigation schemes (Jellyman and Bonnett, 1992). While diversion of out-migrants into unscreened irrigation canals was not studied in detail in this study, or by Trotter 2016, it has previously been found to contribute significantly to declining populations of migratory salmonids in New Zealand (Unwin et al., 2005). Reduced growth rates therefore appear to directly, or

indirectly, translate into reduced survival which results in a lower than expected recruitment contribution from the Lindis River. Thus taken together, this study along with Trotter (2016) demonstrate that the negative effects associated with water extraction provide a plausible explanation for the mismatch between observed spawning effort and quantified recruitment contribution from the Lindis River. Collectively they suggest extended periods of lost connectivity with other waterways is likely to be the primary reasons for why the river currently appears to be acting as an ecological trap and recruitment sink rather than a primary source.

4.5.1 Conclusions and management implications

The present study demonstrates the usefulness of a combination of methods to reveal landscape patterns of trout movement and source-sink populations dynamics. It also predicted used growth modelling to examine some of the potential drivers behind migration patterns inferred from otolith chemistry, and to determine if juvenile fish experience growth constraints during the low flow period. As such, it provides a novel example for how natural chemical markers in soft (eggs) and hard (otoliths) body parts of fishes can be integrated with growth modelling and field surveys to improve our understanding of population dynamics in migratory fishes. Taken together, results from this study and Trotter 2016 show combined pressure from high levels of water extraction causing habitat reduction, fragmentation, reduced growth rates and loss of connectivity with other waterways make it difficult for juvenile trout in the Lindis River to realise the potential growth benefits provided by a migratory life history. Thus, the combined use of field surveys and chemical markers in eggs and otoliths used in this study provides compelling evidence that the Lindis River, a spawning tributary previously thought to be the primary source of recruits to a valued recreational lake fishery, may in fact currently be acting as sink habitat for Lake Dunstan's adfluvial brown trout population. These findings highlight the need for holistic instream effects assessments of water extraction practices at the catchment rather than sub-catchment scale, in order to minimise potential negative effects on recruitment and population dynamics of migratory fish. Future studies should

aim to clarify if exposure to harmful environmental conditions, losses of out-migrants via unscreened water races, or competition for food, space and predation at low flows are primarily responsible for the observed mismatch between spawning effort and recruitment contribution. Along with informing efforts aimed at establishing an environmental flow regime to alleviate these factors.

Chapter 5 : Progeny from self-sustaining Chinook salmon populations landlocked above hydropower dams increases the resilience of anadromous Chinook salmon in a regulated river system without fish-passage structures through source-sink dynamics.

5.1 Abstract

Metapopulation dynamics can play a critical role in the persistence and recovery of vulnerable salmonid populations. I investigated the natal origin of Chinook salmon in the Clutha River, New Zealand, where a hydropower dam has blocked access to traditional spawning areas since 1956. Despite an absence of fish passage facilities and high-quality spawning areas below the dam, a small residual run of anadromous Chinook salmon has persisted. Analysis of otolith microchemistry ($^{87}Sr/^{86}Sr$, Sr/Ca and Ba/Ca ratios) assigned harvested adult salmon from the lower river to natal sources located both above (61%) and below (39%) the hydropower dam. Above-barrier recruitment contribution was dominated by progeny from one of three self-sustaining landlocked salmon populations, which on average represented 55% of all recruitment across the study period. Reconstructed length at ocean entry, based on otolith chemistry, revealed 82% had adopted a stream-type life history strategy, typically emigrating at a mean size of 138 mm, regardless of natal origin. Results demonstrate that progeny of landlocked populations can be important resources for the resilience, and possible recovery, of vulnerable anadromous populations. As such my findings may have implications for conservation plans aimed at restoring or enhancing other remnant anadromous salmonid populations in other regulated catchments.

5.2 Introduction

Chinook salmon (*Oncorhynchus tshawytscha*) display great variation in life history traits, morphology, behaviour, and other characteristics (Quinn and Unwin, 1993). Individual populations are often classified into two distinctly different behavioural forms: stream-type, spending one or more years in freshwater before migrating to sea; and ocean-type, migrating to the sea before their first winter as a subyearlings (following Healey, 1991). These differences in juvenile life history are linked to genetically controlled growth tendencies, environmental influences, and size-biased juvenile survival. In general stream-type behaviour tends to arise in slow-growth environments, whereas fast-growth environments favour ocean-type behaviour (Unwin et al., 2000). Thus, the diversity partly reflects differences in rearing conditions, but also genetic adaptations to local environmental conditions, a consequence of strong natal stream homing behaviour (Quinn, 2005). However, chinook salmon can also display a high degree of phenotypic plasticity and are capable of undergoing rapid evolution (within 90 years) in response to changes in environmental conditions (Quinn et al., 2001; Hendry and Stearns, 2004). Hence, it appears that life-history diversity and phenotypic plasticity are key components that create population resilience, by spreading the risk of mortality in fluctuating environments (Waples et al., 2009, Riva-Rossi et al., 2012). Moreover, if a metapopulation contains sub-populations exhibiting a range of life history traits overall abundance is thought to be further buffered against disturbance, and population resilience and viability are increased (Hilborn et al., 2003, Koski et al., 2009). Thus, the variety and phenotypic plasticity in life history tactics across a metapopulation and dispersal of recruits between sub-populations could explain why some populations have been able to persist in the face of anthropogenic disturbance.

The effects of river impoundments on anadromous salmonids are well documented (see reviews by Myers et al., 1998; Budy et al., 2002; Young et al., 2004; and Young et al., 2011). Adverse effects include: (1) blocking or disrupting fish migration, (2) reduced habitat availability, (3) decreased spawning success and

juvenile survival (due to hydropeaking and through direct and delayed mortality during outmigration), and (4) reduced invertebrate (food) production. Programmes that aim to mitigate the effects of impoundments on anadromous salmonid populations often rely on hatchery releases (Fleming and Petersson, 2001; Molony et al., 2003; Morita et al., 2006), although the various positive and negative effects of such releases are the subject of ongoing debate (Mann and Plummer, 2000; Aprahamin et al., 2003; Brannon et al., 2004; Chilcote et al., 2011). Commonly raised concerns include the potential for released fish to interbreed with wild populations and alter their genetic composition; reduced population viability; and altered age at maturity (McMeel and Ferguson, 1997; Unwin and Glova, 1997; Laikre et al., 1999). Ultimately a long-term reliance on large-scale hatchery supplementation programmes may hinder the recovery and even contribute to accelerating decline of locally adapted wild anadromous salmonid populations, eventually leading to a situation where hatchery fish effectively replace wild stocks (Quiñones et al., 2013; Willmes et al., 2018). However, there are many salmonid populations in impounded and regulated river systems that are self-sustaining without the aid of hatchery releases (Young et al., 2004). Improving our understanding of changes in the ability of affected populations to sustain themselves in the face of environmental perturbations appears to be important (Hutchings, 2002), regardless of whether these changes are linked to habitat loss, climate change or interactions with hatchery stocks. One of the critical issues for population resilience is whether a population's store of phenotypic and behavioural plasticity can keep pace with ecosystem change (Secor, 2015). Thus, it is useful to study the features of residual populations that display an unexpected resilience to the effects of hydropower dams, particularly in systems where no or very limited hatchery supplementation has occurred, as they may provide valuable insights into management strategies that can lead to effective recovery efforts.

Chinook salmon from North America were introduced to New Zealand as a commercial venture in the 1900s, and quickly established self-sustaining anadromous runs in several South Island river systems (Deans et al., 2004). The rapid establishment of salmon populations in New Zealand might partly be due to

the highly diverse source stock from the Sacramento River in California, having four runs of Chinook salmon with unique life histories named after the season when adults return to spawn (i.e. spring, summer, fall, and winter runs) (Phillis et al. 2018). Post introduction the spawning run in the Clutha River, believed to have been one of the largest in the country (McDowall, 1994), was severely reduced by the construction in 1956 of a hydropower dam that lacked fish-passage facilities (Figure 5.1). Since then further dams have been built on both the mainstem (Clyde Dam 1993) and several side tributaries. However, despite losing access to all traditional spawning waters, and a lack of suitable spawning habitats below the dam, a residual anadromous population has persisted in the lower river. Releases of hatchery fish were never required as mitigation, although attempts at commercial ocean ranching did occur between 1977 and 1985. While this briefly boosted the population the endeavour was deemed uneconomic, and the residual spawning run quickly returned to previous levels of ≤ 1000 fish per year (Otago Fish and Game, unpublished data). It has since been proposed that the residual run of adults downstream of the Roxburgh Dam maybe largely maintained by downstream movement of juveniles from self-sustaining landlocked Chinook salmon populations in the three source lakes upstream of the dam (McDowall, 1990; 1994). This view was based on evidence from scales of adult salmon taken in the lower river which showed an extended period of freshwater rearing which is associated with slower growth rates than those post ocean entry (Flain, 1980), and reports that most mainstem spawning areas below the Roxburgh Dam were subject to daily dewatering, which can cause high rates of mortality amongst pre-emergent alevins (McMichael et al., 2005). No current information is available about how the landlocked and anadromous populations may interact via migration and dispersal. However, most anadromous adults still seem to congregate below the Roxburgh Dam wall for extended periods before eventually attempting to spawn in the mainstem Clutha River rather than in side tributaries below the dam (Otago Fish and Game, unpublished data). Improved understanding of how the residual anadromous chinook salmon population is maintained would provide a useful guide for future conservation efforts.

Chemical analysis of otoliths is now widely regarded as an integral fisheries tool for understanding patterns of fish movement, recruitment, and metapopulation dynamics (Elsdon et al., 2008; Veinott et al., 2014; Barnes and Gillanders, 2013). The approach exploits chemical differences recorded by fish from different habitats because otoliths: *(i)* are chemically stable; *(ii)* have an elemental composition that reflects the physical and chemical environment in which the fish has resided in the past; *(iii)* continue to grow incrementally throughout the life of a fish (Campana, 1999; Campana and Thorrold, 2001; Outridge et al., 2002). A common method of otolith analysis is to assign older fish to probable natal sites in order to establish the relative recruitment contribution made by different nursery locations. This can be done by relating otolith chemistry in the natal zone (*i.e.* near the otolith core) of adults to baseline signatures from juveniles collected from likely nursery habitats (Olley et al., 2011; Muhlfeld et al., 2012; Veinott et al., 2012), or directly to the water chemistry of nurseries (Barnett-Johnson et al., 2008; Courter et al., 2013), or using a combination of both (Crook et al., 2013).

The primary objective of my study was to quantify to what degree progeny from landlocked lake rearing populations above the hydropower dams are a source of recruitment to the anadromous Chinook salmon population in the lower Clutha River. Based on previous studies (Flain, 1980; McDowall, 1994; Hayes et al., 2012) it was hypothesized that despite 60 years of strong selection against anadromy, progeny from resident salmon populations above barriers would still make a significant contribution to the residual anadromous population. To test this I investigated the natal origin and size at ocean entry of anadromous salmon over two years using otolith microchemistry analysis. I also examined the natal origin of landlocked salmon to further determine the consistency of the relative contribution of the three possible source populations to recruitment over time.

5.3 Methods

5.3.1 Study site

The Clutha River is the second longest and highest volume (mean flow 614 m^3s^{-1}) river in New Zealand. Three large natural lakes (Wanaka, Hawea and Wakatipu) feed the river, which has two hydropower dams located on the mainstem and several smaller hydropower dams, weirs or control structures on tributaries that block fish access (Figure 5.1). The three source lakes are all classified as oligotrophic, but there are appreciable differences in nitrogen concentration and trophic status and considerable variation in annual and seasonal discharge (Table 5.1; Figure 5.2). All three lakes have self-sustaining populations of landlocked Chinook salmon, which reproduce in smaller tributaries at the head of each lake. However, in the two hydropower reservoirs, Lake Roxburgh and Lake Dunstan (Figure 5.1), landlocked salmon are generally transient and mostly caught only during the spring – summer period in close proximity to the dams.

Table 5.1 Characteristics of lakes that support self-sustaining populations of landlocked Chinook salmon (*Oncorhynchus tshawytscha*) in the Clutha River catchment for the period 2004–2013*.

Location	Lake area (km^2)	Catchment area (km^2)	Elevation (masl)	Mean annual discharge / outflow (m^3s^{-1})	Mean annual temp. (°C)	Mean Trophic Lake Index	Mean TP (mg/m^3)	Mean TN (mg/m^3)	Max Depth (m)	Hydraulic residence time (years)
Lake Wakatipu	289	2674	310	162	7.0	1.94	6.32	118	380	11.8
Lake Wanaka	192	2590	300	194	8.5	2.15	6.09	159	311	5.5
Lake Hawea	141	1394	348	59	7.5	1.65	6.83	35	384	2.3

*Data sources: Otago Regional Council, the National Institute for Water and Atmospheric Research (NIWA), and Verburg et al. (2010).

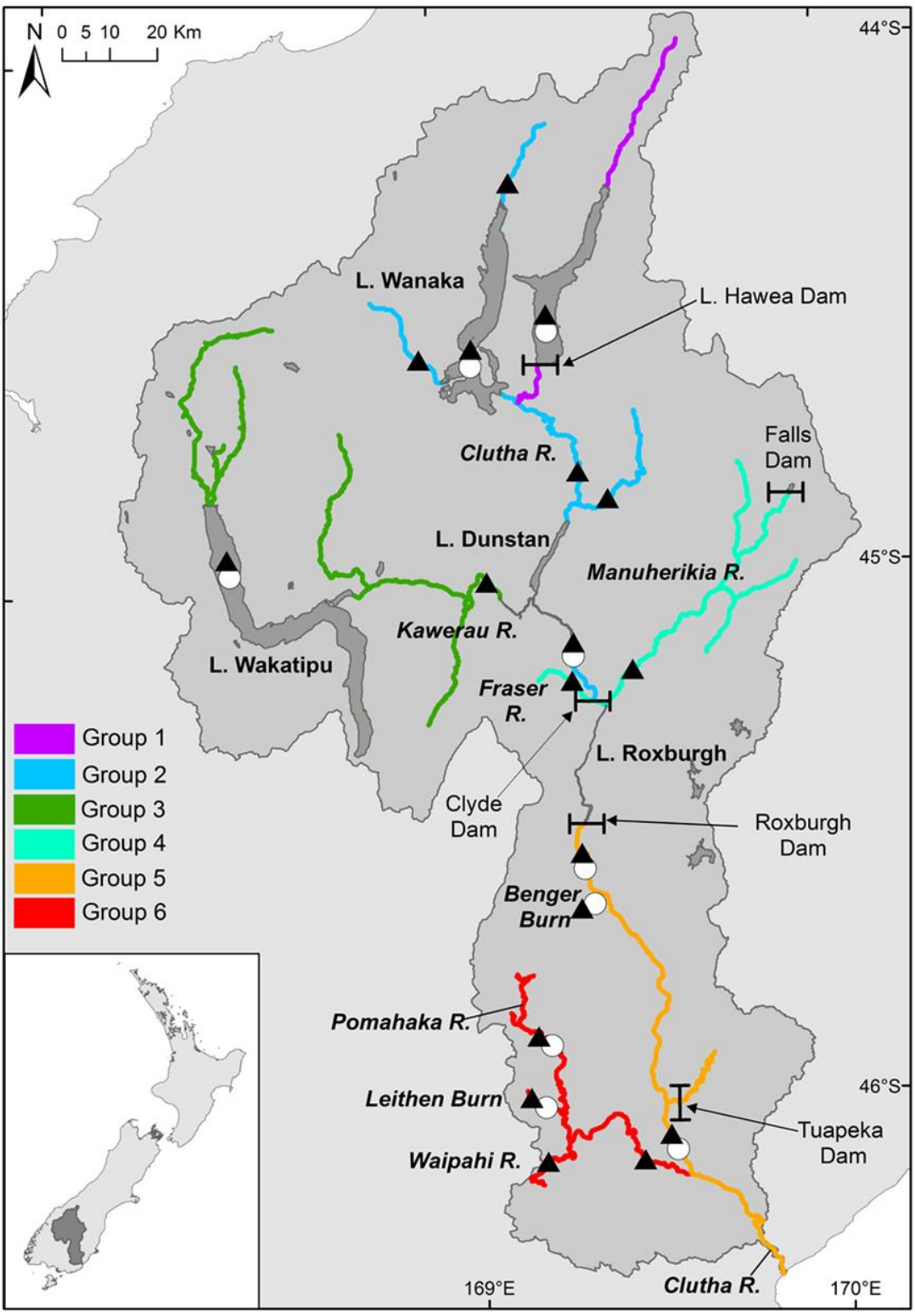

Figure 5.1 Map of the Clutha River catchment showing the six water chemistry groups (each containing several (3-8) water sampling sites), locations of fish migration barriers, fish sampling sites (white circles), and water sampling sites (black triangles).

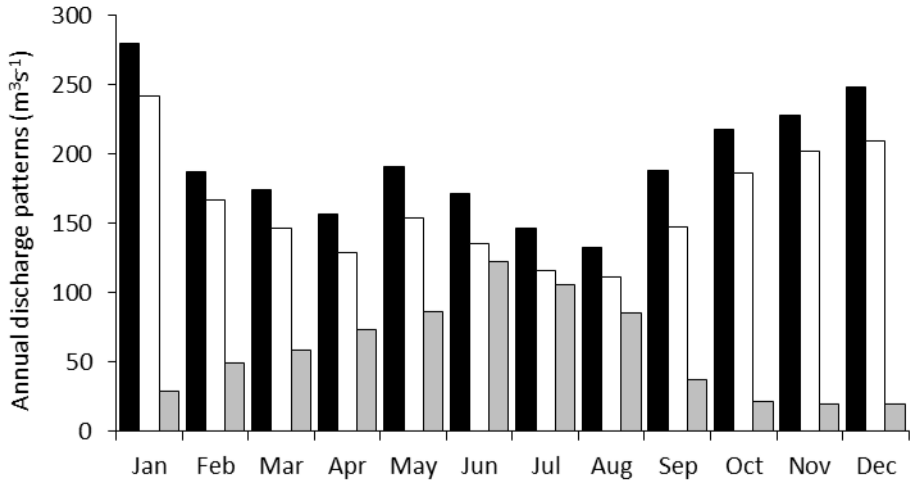

Figure 5.2 Average monthly lake discharge / outflow (m^3s^{-1}) for Lakes Wanaka ■, Wakatipu □, and Hawea ■ during 2004 – 2014, calculated from flow data supplied by Otago Regional Council, the National Institute for Water and Atmospheric Research (NIWA) and Contact Energy Ltd.

5.3.2 Fish and water sample collection

To develop a baseline map of $^{87}Sr/^{86}Sr$ and trace element ratios (Sr/Ca and Ba/Ca) across natal sources, landlocked salmon (age 2+) were sampled from all three source lakes along with juvenile salmon (age 0) from five spawning areas below the Roxburgh Dam during 2010 and 2011 (Figure 5.1). These locations were selected to encompass all sources of landlocked salmon and a broad range of possible spawning areas below the Roxburgh Dam. Ten to 20 individuals were collected from each location. Otoliths were extracted and cleaned ultrasonically in ultrapure water (18MΩ) and then sectioned in a transverse plane following methods described by Gabrielsson et al., (2012). In addition, to characterise water $^{87}Sr/^{86}Sr$ values across the Clutha River basin a 200 mL water sample was collected at each sampling site during base flow conditions in 2010 and 2011 (Figure 5.1). Additional water samples were collected during 2012 to further investigate temporal changes in water chemistry and compliment the catchment baseline data with water signatures from three other possible natal sources within the Clutha River basin where no juvenile salmon were captured (*i.e.* the Manuherikia, Fraser and Waipahi rivers).

Harvested Chinook salmon (*n* = 64) collected by fishery managers and recreational anglers fishing the lower Clutha River in 2009 and 2010 were made available for chemical analysis. In order to investigate the natal origin of out-migrants, landlocked salmon (*n* = 18) were also collected immediately above the Clyde Dam, presumably on their way towards the ocean, during the 2010 spring migration period (October – December).

5.3.2 Geochemical analysis

Otolith and water chemistry were measured at the Centre of Trace Element Analysis, Otago University. Strontium isotope ratios ($^{87}Sr/^{86}Sr$) in otoliths were measured using a Nu Plasma-HR laser ablation multi collector inductively coupled plasma mass spectrometer (LA-MC-ICP-MS) fitted with a HelEx (Laurin Technic and the Australian National University) laser ablation system, while otolith trace element concentrations were determined using a Agilent 7500cs quadrupole mass spectrometer (Q-ICP-MS) and HelEx LA system. In both cases any potential surface contaminants were first removed during a pre-ablation step whereby the surface was ablated down about 5 µm using a spot size of 130 µm. After pre-ablation a laser transect was run from the otolith core to the edge under a pure helium atmosphere whilst the laser was operated in continuous scan mode using a spot size of 90 µm, a repetition rate of 10 Hz and a scan rate of 5 µm s^{-1}. Data reduction was conducted off-line using purpose-built Excel spreadsheets, and presented either as $^{87}Sr/^{86}Sr$ or element/Ca ratios and plotted against ablation distance (µm). The natal $^{87}Sr/^{86}Sr$ values were taken as the average of values occurring between 250-300 µm from the otolith core, resulting in an average of >40 data points. Instrument accuracy was monitored using an in-house *Tridacna* carbonate standard (Australian National University) to assess the performance of the analytical protocols for $^{87}Sr/^{86}Sr$ or element/Ca measurements, and correct for any machine drift. Strontium isotopic data acquired for this standard yields a mean (± 2SD) $^{87}Sr/^{86}Sr$ value of 0.709193 (± 0.000063), which is in agreement with the $^{87}Sr/^{86}Sr$ composition of ocean water (~ 0.70918; Hodell et al., 1989).

Water chemistry was measured using a modified version of an established method (Pin and Bassin, 1992; Shaw et al. 2010). Strontium was first eluded and purified through Eichrom Sr-SPEC resin, and after elution, the $^{87}Sr/^{86}Sr$ ratios in each water samples was determined by using a Nu Instruments Nu Plasma-HR multi collector inductively coupled plasma mass spectrometry (MC- ICP-MS). Instrument performance was verified with certified reference materials [CRC SLRS-4 (National Research Council – Canada; www.nrc-cnrc.gc.ca/eng/services/inms/referencematerials.html) and USGS T167 (United States Geological Service – Analytical Evaluation Program for Standard Reference Samples; http://bqs.usgs.gov/SRS/)]. Instrument drift was monitored, and if required corrected for, by prior to analysis spiking water sample solutions with an internal standard of indium and scandium, as reference elements. Analytical consistency was inspected by analysing duplicate samples and blanks containing ultrapure water, collected using the same sampling protocol as field samples.

5.3.3 Natal origin

Following the same approach as Barnett-Johnson et al., (2008) Sr isotope ratios among natal sources were first compared using Analysis of Variance (ANOVA) with post-hoc pairwise comparisons using Tukey's Honestly Significant Difference (HSD). The same process was repeated for trace element signatures. After examining isotope and trace element ratios for normality and homogeneity of variance (Miller and Kent, 2009) the latter were log transformed. Natal sources that could not be separated were clustered, and a linear discriminate analysis (LDA) with jack-knife resampling was used to determine the accuracy with which individual Chinook salmon and water samples could be assigned to their known capture or sampling location. A two-step process was used to predict the natal origin of harvested adults. Firstly, $^{87}Sr/^{86}Sr$ values within the natal zone of adult anadromous salmon otoliths were compared with known $^{87}Sr/^{86}Sr$ values from a baseline of possible natal sources. Using LDA, fish were separated from most natal areas, except those captured in the Clutha River below hydropower Dams and the Lake Wanaka catchment. In a second stage Sr/Ca and Ba/Ca ratios for fish from these two locations were therefore used in a trace element LDA to further

differentiate among natal sources and create a predictive recruitment model for classifying unknown adults. In order to balance the sample size of the two remaining groups pseudo-sampling, with replacement, was used. Analyses were carried out using the R Statistical Software (R Development Core Team 2014) and the LDA function used was drawn from the MASS package (Venables & Ripley, 2002). Prior probability of group membership was assumed to be equal for all groups in both LDAs.

5.3.4 Estimating size at ocean entry and life history classification

An estimate of the individual fork length (FL) at ocean entry was reconstructed from chemical profiles of adult salmon using a back-calculation model. Following the approach described by Miller et al. (2010) I first confirmed that the transverse otolith radius (OR) reliably predicts FL by collecting juvenile and sub-adult Chinook salmon from several wild and hatchery sources to quantify the relationship (Table 5.2). I measured juvenile FL (1 mm) prior to preservation, removed the otolith, and measured OR along the same axis as chemical profiles would be generated from in adults (*i.e.* the OR was measured to the nearest µm along the dorsal – ventral growth axis at the widest point of otoliths that had been sectioned in a transverse plane). Life history (stream or ocean type) and size at ocean entry was determined by combining three types of information: (i) the distance (µm) from the otolith core to the first annual check (determined from juveniles), (ii) the $^{87}Sr/^{86}Sr$ ratio in the otoliths of the adults at the first annual check, and (iii) the distance from the otolith core at which the $^{87}Sr/^{86}Sr$ ratio indicated a marine signature.

Table 5.2 Source, year of collection, and size range of Chinook salmon (*Oncorhynchus tshawytscha*) used to develop the relationship between otolith and fish size in New Zealand.

Source	Year of collection	Fork length (mm)	Sample size (n)
Clutha River catchment	2009–2012	39–385	86
McKinnon Hatchery	2013-2014	140–166	22
Spring Creek Hatchery	2013-2014	110–194	20
Isaacs Hatchery	2009–2012	38–239	39
Rangitata River catchment	2013-2014	58–83	11
Portobello Aquarium	2010	210–280	49

5.4 Results

5.4.1 Spatial and temporal variation in water $^{87}Sr/^{86}Sr$

Water sample $^{87}Sr/^{86}Sr$ values revealed little temporal variation but considerable spatial variation between most, but not all, sites. For example, initially $^{87}Sr/^{86}Sr$ values could only differentiate five sub-catchments each containing several (3-8) water sampling sites (ANOVA, $P < 0.05$). There was relatively little spatial variation in $^{87}Sr/^{86}Sr$ values collected from sampling sites located along the Clutha River and its primary source (Lake Wanaka) (see Table 5.3). To overcome the lack of spatial variation along the Clutha River a hierarchical cluster analysis of $^{87}Sr/^{86}Sr$ values, followed by a multidimensional scaling (MDS) of trace elements values, was adopted. Using this combination of methods samples collected from Lake Wanaka and the upper Clutha River could be distinguished from samples collected along the lower Clutha River (see Figure 5.3). The final model contained six separate water chemistry groups, as shown in Figure 5.1.

Table 5.3 Site details, $^{87}Sr/^{86}Sr$ ratios and range for water samples, and measured otolith $^{87}Sr/^{86}Sr$ ratios from Chinook salmon (*Oncorhynchus tshawytscha*) collected from the Clutha catchment over the period 2010–2012.

Site	**Median water $^{87}Sr/^{86}Sr$**	**Range $^{87}Sr/^{86}Sr$**	**Median otolith $^{87}Sr/^{86}Sr$**
Lake Wakatipu	0.70726 (*n* = 2)	0.70720–0.70732	0.70726
Lake Wanaka	0.70809 (*n* = 3)	0.70804–0.70817	0.70814
Lake Hawea	0.70917 (*n* = 3)	0.70915–0.70919	0.70923
Upper Clutha River	0.70812 (*n* = 5)	0.70806–0.70819	0.70814
Middle Clutha River	0.70809 (*n* = 3)	0.70807–0.70811	
Lower Clutha River	0.70805 (*n* = 4)	0.70798–0.70808	0.70809
Fraser River	0.70854 (*n* = 2)	0.70842–0.70867	0.70876
Manuherikia River	0.70879 (*n* = 1)		
Benger burn	0.70661 (*n* = 1)		
Upper Pomahaka River	0.70660 (*n* = 3)	0.70625–0.70707	0.70645
Lower Pomahaka River	0.70587 (*n* = 2)	0.70541–0.70632	

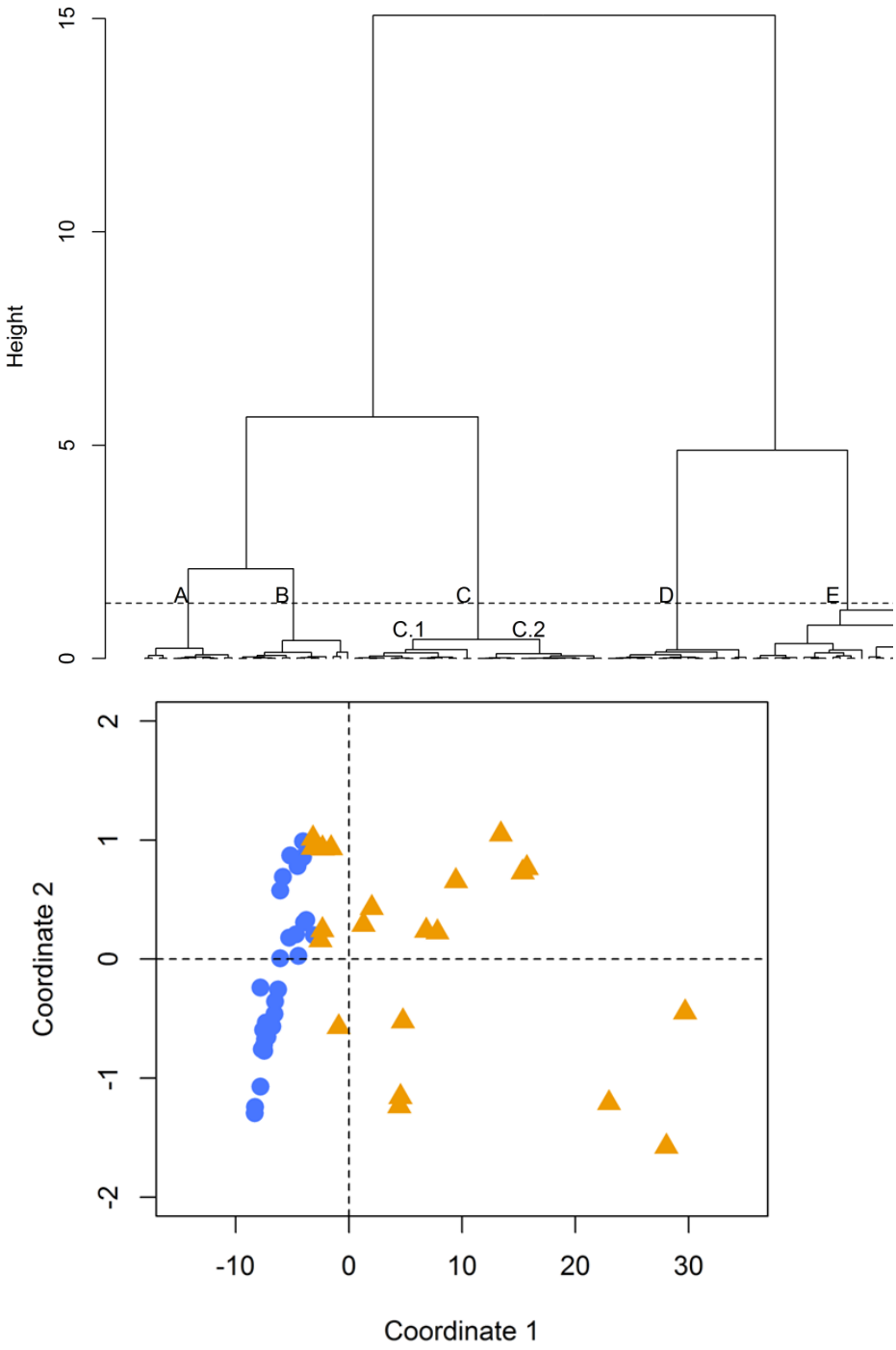

Figure 5.3 Cluster Analysis preformed on $^{87}Sr/^{86}Sr$ values in otolith and water samples, and multidimensional scaling (MDS) of trace elements with otoliths: (a) Dendrogram illustrating the five main clusters (A-E) and two sub clusters (C.1, C.2). (b) Nonmetric MDS scores for otoliths samples belonging to sub-cluster C, plotted in multivariate space, based on otolith Sr/Ca and Ba/Ca ratios in samples collected from the upper (**blue**) and lower (**orange**) sections of the Clutha River.

5.4.2 Relationships between water and otolith $^{87}Sr/^{86}Sr$ values

Linear regression analysis confirmed that water $^{87}Sr/^{86}Sr$ values were a good predictor of otolith $^{87}Sr/^{86}Sr$ values (R^2 = 0.95, *n* = 58, *P* < 0.01), indicating that it was possible to clearly identify and separate some of the sites where no juvenile salmon had been captured (*i.e.* the Manuherikia, Fraser and Waipahi rivers, Figure 5.1) from known natal sources.

5.4.3 Spatial and temporal variation in multi-elemental otolith signatures

Fish originating from Lake Wanaka or the lower Clutha River were discriminated with an LDA model based on Sr/Ca and Ba/Ca ratios. Pairwise comparison revealed that Sr/Ca and Ba/Ca values in Chinook salmon otoliths were able to discriminate between salmon captured in Lake Wanaka and the lower Clutha River (below the Roxburgh Dam, Figure 5.1, HSD, *P* < 0.05). There was a high degree of discrimination in the multi-element LDA plot, and jack-knife resampling resulted in the correct classification of 88 – 100% of cross-validated grouped cases. Thus, taken together otoliths and water samples were considered to characterise a comprehensive baseline of chemical multi-element signatures across all known major, and most of the probable, spawning and rearing areas in the study area.

5.4.4 Estimating size at ocean entry and life history classification

There was a strong positive correlation between fish length and otolith radius (R^2 = 0.95, n = 207, *P* < 0.01). Therefore, we could use a linear relationship (Equation 1) to estimate fish size at the time of ocean entry.

FL = 0.1499 * OR – 14.603; where FL = fork length (mm) and OR = otolith radius (μm) (Equation 1)

Fish were grouped into 10 mm bins based on back-calculated size when entering the ocean. Overall, estimated lengths at ocean entry ranged from 48 mm – 366 mm, mean (± SD) = 138 mm (± 53 mm) (Figure 5.4). There was no evidence of a significant difference in size distribution at ocean entry between fish hatched above or below the hydro dams (Mann-Whitney' test: W = 423; d.f. = 1; *P* > 0.05)

or between sexes (chi-squared test: $\chi^2 = 1.087$; d.f. = 1; $P > 0.05$); although, two individuals hatched above the Clyde Dam were estimated to have exceeded 300 mm FL (*i.e*. c. 2 years old). Based on age at ocean entry 82% of all adults analysed were classified as having a stream-type life history strategy (Table 5.4).

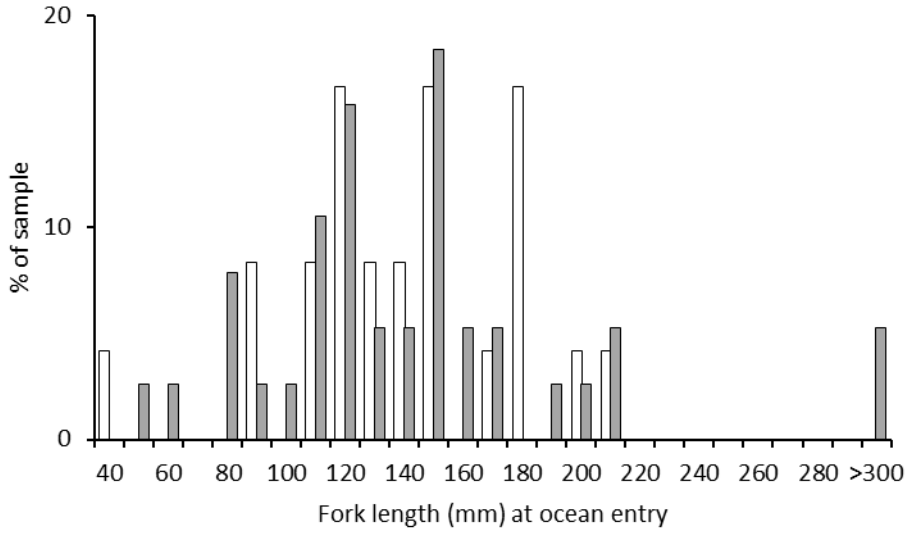

Figure 5.4 Frequency distribution of back-calculated size at ocean entry for adult Chinook salmon (*Oncorhynchus tshawytscha*) based on otolith chemistry. Solid bars represent the progeny of landlocked salmon while open bars represent recruitment from natal sources below hydro dams.

Table 5.4 Variation in freshwater residency period (% stream- vs. ocean-type) for five New Zealand anadromous Chinook salmon (*Oncorhynchus tshawytscha*) populations.

Population	% stream-type	% ocean-type
Poulter River (1979 - 84)	75.6	24.4
Rakaia River (1965 - 81)	37.8	62.2
Rangitata River (1960 - 84)	29.3	70.7
Waitaki River (1959 - 86)	51.1	48.9
Clutha River (1980)	73.7	26.3
Clutha River (2009 - 2010)*	82.0	18.0

*Denotes brood year for the present study, while comparative data was drawn from Flain (1980) and Quinn and Unwin (1993).

5.4.5 Predicting natal origin of anadromous and landlocked Chinook salmon using multi-elemental signatures

When entered into the LDA 38 of the 62 adult anadromous Chinook salmon captured in the lower Clutha River were classified as having originated above the hydropower dams, based on their most likely group membership. The majority of all fish analysed were predicted to have come from Lake Wanaka (53 %). Only one fish matched the signature from the Manuherikia/Fraser catchments, and no fish was matched to Lake Hawea. Of the 24 (38 %) fish predicted to have come from the lower Clutha river basin, the LDA estimated 25 % came from the Pomahaka catchment and 13 % from the lower Clutha River (Figure 5.5). The proportional recruitment contribution from each natal area did not differ significantly over the two years studied (Figure 5.5; Chi-square tests: $P > 0.05$). There was also no significant difference in the gender ratio of fish born above or below the hydro dams (Chi-square test: $\chi^2 = 1.087$; d.f. = 1; $P > 0.05$). Comparisons between the predicted natal origin of anadromous Chinook salmon classified as having originated above hydropower dams and landlocked Chinook salmon captured above the Clyde Dam revealed Lake Wanaka to be the central recruitment source (Figure 5.6).

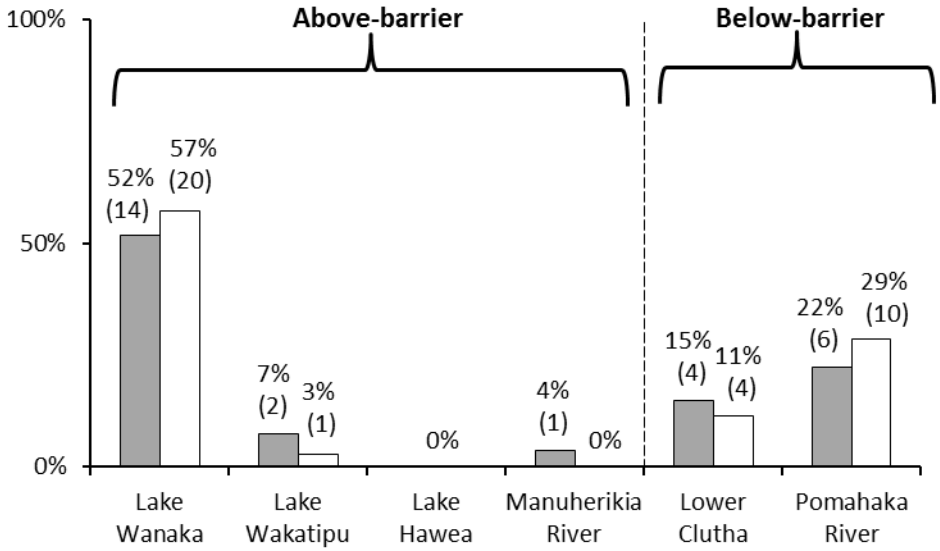

Figure 5.5 Recruitment contribution (% and number) from natal areas located above and below barriers to upstream migration (*i.e.* hydropower dams) of the spawning run of anadromous Chinook salmon (*Oncorhynchus tshawytscha*) in the lower Clutha River. Solid bars represent fish captured in 2009 and open bars 2010.

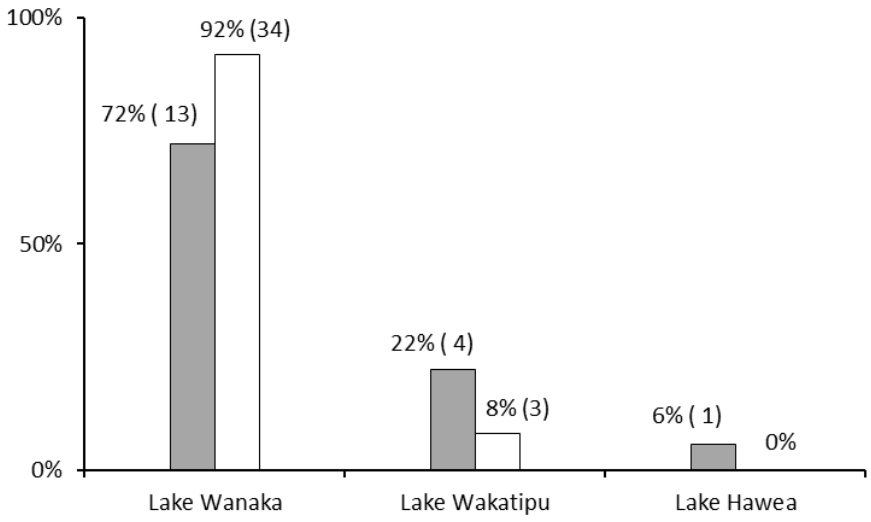

Figure 5.6 Comparison of the percentage and number (in brackets) of landlocked Chinook salmon (*Oncorhynchus tshawytscha*)(*n* = 18) captured above the Clyde Dam in 2010 (solid bars), and anadromous Chinook salmon captured below hydropower dams (open bars)(*n* = 38) during 2009 and 2010 predicted to have come from each lake.

5.5 Discussion

Quantifying the recruitment contribution from natal sources located both above and below migration barriers is an important step in understanding how the residual anadromous Chinook salmon populations in regulated rivers, like the lower Clutha River, are maintained. Results from recruitment modelling, using otolith microchemistry data, predicted that on average over 61 % of the salmon captured in the lower Clutha River were the progeny of landlocked (adfluvial) populations. This demonstrate that sustained dispersal of progeny from adfluvial salmon populations above hydropower dams can have positive effects on the resilience and persistence of anadromous populations in regulated river systems, through source-sink dynamics. Partly buffering the adverse effects associated with hydropower development by supplementing recruitment and reducing the risk of extinction. These findings support suggestions made by Hayes et al., (2012) that resident salmonid populations above migration-barriers can potentially be an important resource in the recovery of vulnerable anadromous populations impacted by dams.

Recruitment of anadromous salmon in the lower Clutha appears to be dominated by two sources, one located above migration-barriers (Lake Wanaka, 55 %) and the other below (the Pomahaka River, 26 %). Recruitment modelling predicts these sources, on average, account for > 80 %, while mainstem spawning below the dam only contributes about 13 %. These findings highlight that adult anadromous salmon returns to the Clutha River fishery is largely dictated by the outmigration success of progeny from landlocked populations in previous years. This makes the population vulnerable to changes in spawning success and habitat quality at key spawning and rearing areas around Lake Wanaka. For example, from floods, droughts or enduring negative impacts on salmon, or the life supporting capacity of the waterways they depend upon, from intensification of agricultural land use practices. Moreover, under the current flow management regime it appears mainstem spawning efforts contributes a proportionally small number of recruits to the anadromous Chinook salmon population. As a result, it is doubtful

whether proposals to release large numbers of hatchery produced smolt, aimed at increasing spawning effort in the lower mainstem river, would contribute meaningfully to the long-term recovery of a self-sustaining anadromous population. However, increasing the outmigration success of salmon smolt past control structures and hydropower dams, along with riparian and instream habitat protection and restoration efforts around key nursery stream, may offer more enduring and cost-effective ways to increase recruitment success and resilience of the Clutha salmon population.

Self-sustaining landlocked populations in the three source lakes did not contribute equal or proportional amounts of recruits (based on lake / catchment size) to the anadromous population in the lower river. The differences in recruitment contribution are somewhat puzzling, but appear persistent as they were stable across both years studied, and reinforced by a separate analysis (Figure 5.6). A range of factors and differences between the lakes may be contributing to the observed variation in recruitment contribution. Firstly, large differences in seasonal discharge patterns exist between Lake Hawea and the other lakes (Figure 5.2). Lake Hawea is also the only lake that has a control structure blocking its outlet. Thus, the combined effect of both a reduced discharge regime and a control structure, which could be causing high mortality in out-migrants, provide a plausible explanation for the low recruitment contribution from this source. Secondly, salmon may be less likely to emigrate from Lake Wakatipu compared to Lake Wanaka because of a larger lake area and smaller discharge decreases the likelihood of them finding the outlet, combined with and slightly cooler temperature. Large, fast-growing individuals are more likely to develop migratory behaviour than slow-growing individuals, particularly when food resources are low (Metcalfe et al. 1995; Forseth et al. 1999; Morinville and Rasmussen 2003). Only limited information is available on the status of the landlocked salmon populations in each lake, but creel surveys indicate recreational anglers capture fewer salmon in Lake Wanaka (13% of all fish captured) compared with lakes Wakatipu (32%) and Hawea (29%) (Otago Fish &

Game Council, unpublished data), which supports the notion that proportionally more salmon remain in lakes Wakatipu and Hawea.

An important consideration when employing otolith chemistry analysis is the degree of temporal variability in the chemical parameters measured (Crook et al. 2013). For instance, while temporal variability in Sr^{87}/ Sr^{86} ratio of otolith and water samples collected from smaller catchments is often found to be minor (Kennedy et al. 1997; 2000; Barnett-Johnson et al. 2008), variability can increase in response to localised rain events and managed flow releases in larger river networks (Walther and Thorrold 2009; Crook et al. 2013). Significant temporal variability in otolith Sr/Ca and Ba/Ca ratios has also been reported for a variety of estuarine or marine species (see review by Gillanders, 2002), and some freshwater systems (Walther and Thorrold 2009), although the degree of variability is not always ecologically significant (Thorrold et al 1998; Walther and Thorrold 2009; Olley et al. 2011; Veinott et al. 2012). Nevertheless, the degree of temporal variation in natal geochemical otolith signatures should be considered as it could potentially confound spatial differences between natal sites, and limit the ability to correctly assign fish to their natal origin. In addition, identification of features explaining why some catchments may be less sensitive to interannual variability in rainfall and weathering processes than most rain-fed rivers is helpful for other studies of this type. In my study I found low temporal variation in Sr^{87}/ Sr^{86} ratios in both water and otolith samples collected over two years, making it possible to accurately differentiate most but not all natal areas using these signatures (Table 5.3). The complementary Sr/Ca and Ba/Ca ratios were able to discriminate between juvenile salmon that were sourced from Lake Wanaka and those from the lower Clutha River, below migration barriers. The low temporal variability in these ratios in water and otoliths, which allowed confident matching of juvenile salmon to their known capture location regardless of which year they were born despite relatively small spatial differences in multi-elemental otolith signatures, suggests large lakes may buffer temporal variation in ambient water chemistry. Moreover, a parallel study on brown trout (*Salmo trutta*) also found that otolith Sr isotope and trace element ratios (Sr/Ca and Ba/Ca) in otoliths were not

significantly different in samples taken 25 years apart from Lake Wakatipu resident trout (i.e. the habitat signature remained stable) (Gabrielsson 2011).

Many river systems throughout the world have been fragmented by human activities, and there is now widespread interest in reconnecting them by modifying or even removing barriers to fish migration (Press et al. 2012, Quiñones et al., 2015). However, conservation biologists often emphasise that scientifically based management principles should be used to improve the efficacy of such efforts (Seddon et al. 2007; Armstrong and Seddon 2008). The outcomes of my study highlight the importance of considering source-sink population dynamics when undertaking conservation planning and recovery efforts in regulated catchments. It confirms that the enduring population of anadromous Chinook salmon in the Clutha River is largely maintained by out-migrating smolt from landlocked populations above migration barriers, demonstrating that metapopulation dynamics can be an important component of the persistence and recovery of anadromous salmonid populations in regulated river systems where self-sustaining populations exists above migration barriers.

My study confirms earlier suspicions that the residual population of anadromous Chinook salmon in the Clutha River is largely maintained by recruitment from the progeny of landlocked populations above migration barriers. It is therefore concluded landlocked and anadromous Chinook salmon population's needs to be considered as a metapopulation in the Clutha River system. Results also highlight the importance of considering sink and source population dynamics when undertaking conservation planning and recovery efforts for river population in regulated catchments. This confirms that metapopulation dynamics can be an important component of the persistence and partial recovery of anadromous salmonid populations in regulated river systems where self-sustaining populations exists above migration barriers. I therefore submit it may be worth investigating the feasibility of establishing landlocked Chinook salmon populations in regulated river systems that have large lakes, as a component of longer-term conservation efforts aimed at either increasing the

abundance of anadromous Chinook salmon below migration barriers. Or even when attempting to reintroduce the species to catchments where local extirpations have already occurred.

In the context of Chinook salmon metapopulation dynamics specifically the concept of progeny from self-sustaining lake populations supporting anadromous populations appear to be unusual. I am not aware of any published records that link self-sustaining adfluvial populations of Chinook salmon to the persistence of anadromous populations through such source-sink dynamics outside of New Zealand. Although enquiries suggest similar population dynamics might be occurring in North America, there is currently no evidence per se (T. Quinn, pers. comm.). If the phenomenon is a unique feature of the Clutha populations it raises the question why it does not occur in North America, and conversely, do similar source-sink population dynamics present occur in other introduced populations of Chinook salmon? For instance, Chinook salmon are currently undergoing a rapid expansion of their range in Argentinian and Chilean Patagonia (see Araya et al., 2014 and references therein), where many river systems contain large lakes. Many researchers have previously speculated about which factors may promote successful invasion of salmonids in southern Chile (Soto et al., 2001), and New Zealand (McDowall, 2003) and southern Argentina (Pascual and Ciancio 2007). Yet the specific features that increase resilience and have permitted Chinook salmon to repeatedly be a more effective invader than other introduced, or escaped, salmons has largely remained a mystery. I submit the ability to rapidly adopt an adfluvial life history and establish metapopulation structures linking landlocked (or voluntarily residual) and anadromous sub-populations could be the central attributes that increase resilience and invasion success.

Chapter 6 : General discussion

The objective of this thesis was to advance understanding of salmonid population dynamics by examining migration and recruitment patterns in a large, regulated, New Zealand river system. In the preceding chapters, I first described experimental studies designed to examine the reliability of using geochemical markers in eggs and otoliths as natural tags to track fish movement and recruitment patterns at across-catchment scales, in order to define limits and provide realistic boundaries for their application to wild populations. I then applied the knowledge gained from these controlled experiments to field studies investigating source-sink dynamics, to evaluate the effects of dispersal, migratory behaviour, and human use of water, on local and regional salmonid populations. These studies used combinations of natural trace elements (Sr, Ba and Ca) and isotopic markers ($^{87}Sr/^{86}Sr$) to reconstruct the natal origin, habitat use and recruitment contribution of spawning areas at the river basin level in two wild populations that displayed a lake migratory and anadromous life history strategy. In this final chapter I discuss how findings from preceding chapters help expand the utility of natural chemical markers to track salmonid migration and recruitment patterns, and advance our understanding of salmonid population dynamics in New Zealand. Drawing on case studies, I illustrate the relevance of my findings for conservation efforts aimed at better understanding salmonid ecology, along with adverse effects of human water use on salmonid populations, and submit that they will help generate new hypotheses for salmonid population dynamics, resilience, and range expansion.

6.1 Synthesis of research findings

Collectively, my research has demonstrated that:

(i) the chemical composition of developing eggs and newly hatched larvae are reflective of their maternal origin, and can therefore be used to separate the spawning locations of migratory and resident salmonids (Chapters 2 and 4);

(ii) a diet of diadromous forage fish and commercially available marine-fish-derived artificial feed types used in hatcheries have the potential to influence salmonid otolith chemistry (Chapter 3), which could cause errors when reconstructing the movement and habitat use of freshwater migratory salmonids;

(iii) case studies using a combination of chemical makers in water, eggs and otoliths can help identify sink habitats (Chapter 4); and

(iv) chemical markers show how metapopulation dynamics link partially migratory (lake-rearing) Chinook salmon populations with a residual anadromous Chinook population (Chapter 5), increasing the resilience of the anadromous population to the negative effects of hydropower development and flow regulation.

6.2 Fisheries monitoring using natural trace element and isotope markers

While fish dispersal and movement patterns can be studied using a variety of traditional and contemporary fish tagging tools and techniques, it is often difficult adequately to mark a sufficiently large proportion of a wild population to ensure appropriate rates of recapture for robust analyses. Justifiably, Schtickzelle and Quinn (2007) stressed that several important aspects concerning dispersal, including the spatial scale of dispersal movements and the reproductive success of dispersers, remain poorly understood. Interpreting fish movements using natural chemical tags recorded in hard (otoliths) or soft (eggs) body parts has become an established tool as, in theory, it is an effective method for reconstructing the movements of individual fish sampled from wild populations inhabiting large stream networks. However, relationships between water and otolith or egg chemistry published in previous studies are seldom directly proportional to ambient water concentrations (Volk et al. 2000; Elsdon & Gillanders 2003; Wait et al. 2008). Dietary contributions to otolith chemistry have also been found to vary (see Kennedy et al. 2000; Gibson-Reinemer et al. 2009;

Doubleday et al. 2013; Jaecks et al. 2016). These inconsistencies can make it challenging to translate observed variations in natural markers into interpretable movement patterns without at times oversimplifying the potential influences of different factors on otolith chemistry for several species, including salmonids.

6.2.1 Egg chemistry

Experimental results (Chapter 2) confirmed that the majority of natural chemical markers tested (Sr, Ba, Ca, Mg, Mn, K, and Al) in eggs and recently hatched fry from anadromous maternal fish reared in freshwater remained stable over the seven week study period. This discovery, together with earlier observations made by Kristensen (2006), indicate it might be possible to use this technique to track migration in non-anadromous populations, and separate freshwater migratory and river resident spawning redds. As experimental studies lack the complexity of natural ecosystems, it is important also to test predictions derived from experiments in natural environments and on wild populations. Limitations of my experiment include only sourcing fish from one stream and having a small sample size.

In Chapter 4, I tested the utility of chemical analysis of eggs collected from spawning redds as a tool for separating freshwater (lake) migratory and river resident brown trout spawning sites, and found a close alignment between strontium isotope values ($^{87}Sr/^{86}Sr$ ratios) in reference samples (waters, otoliths and eggs) from the upper Lindis River and Lake Dunstan. While not tested in the first experiment, these findings, along with outcomes from a subsequent field survey (Chapter 4), provide strong evidence that $^{87}Sr/^{86}Sr$ ratios can be a particularly useful natural marker for separating redds of river resident and lake migratory (adfluvial) salmonids in catchments where distinct differences in trace element:Ca ratios (e.g. Sr:Ca and Ba:Ca) are subtle or temporally unstable, such as in parts of the upper Clutha catchment.

While egg chemistry has been previously used to separate anadromous and freshwater migratory brown trout spawning efforts (Kristensen 2011),

attempts to use $^{87}Sr/^{86}Sr$ ratios to separate river resident and lake migratory salmonid spawning efforts and locations have not been reported. As such this study builds on earlier studies by Kristensen (2006), Waite et al. (2008) and Kristensen et al. (2011) by (1) establishing that maternal egg chemistry is a reliable method for separating freshwater migratory and river resident spawning effort; and (2) demonstrating the link between $^{87}Sr/^{86}Sr$ ratios in stream/lake water, eggs and otolith chemistry. The latter demonstrates that water samples or predictive models (e.g. see Hegg et al. 2013 and Hegg 2017) can be used to identify catchments where the resolution of geochemical markers make it possible to determine the choice of spawning sites at the microhabitat scale for multiple life history forms (e.g. river resident, lake migratory and anadromous fishes). These findings are supported by Sawada et al., (2019), who in a recent study discriminated between lake and river spawning fish using stable isotope analysis, based on different $\delta^{15}N$ and $\delta^{13}C$ values of prey organisms between the lake and its tributaries.

6.2.2 Diet influences

Controlled diet experiments demonstrate the potential for marine-derived prey sources to have ecologically relevant influences on otolith chemistry within a three week period (Chapter 3). This confirms suggestions by Jaecks et al. (2016), who cautioned that freshwater fish reliant on seasonally abundant marine-derived food sources, such as salmon eggs (or diadromous forage fish: this study), can produce otolith chemical signatures suggestive of anadromy. While it is recognised that diet can affect otolith chemistry, most studies have concluded that water is the dominant source of elements in otoliths (Gibson-Reinemer et al. 2009; Collingsworth et al. 2010; Doubleday et al. 2013). While this might be the norm for fish populations whose food sources are in chemical equilibrium with local conditions, this study, along with the observations and simulations reported by Jaecks et al. (2016), demonstrate that recognising the importance of context for understanding diet influences helps us explain and predict why seasonally abundant food sources that chemically differ significantly from local environments occupied by fish may significantly affect otolith chemistry. While such seasonal

foraging behaviours may periodically influence or constrain the utility of otolith chemistry, predictable dietary influences on trace element and isotope markers also have the potential to improve our understanding of fish ecology, for example, by highlighting the seasonal use or importance of marine-derived food sources to a fish population.

Taken together, results from controlled experiments and field surveys show that combined analysis of natural trace element and isotopic markers in eggs from spawning redds, otoliths, and water samples, is a robust and powerful approach for examining the reproductive success of dispersers, and can reveal landscape patterns of trout movement and source-sink population dynamics. When coupled with predictive growth models it also allows researchers to evaluate some of the potential advantages and drivers of observed migration patterns inferred from otolith chemistry.

6.3 Salmonid population dynamics, resilience and diversity of life history strategies

Salmonids display diverse and complex migration and recruitment patterns to exploit habitats within catchments that are disproportionately valuable to growth and reproduction during various life history stages (Jonsson and Jonsson 2011). As a result, maintaining ecological connectivity across riverscapes is considered important to safeguard population resilience. However, in regulated catchments where water resources are managed in order to deliver a balance between ecological, cultural, and economic objectives, it remains challenging to identify at what point a temporary loss of 'critical connectivity' is likely to adversely affect a population. A common approach for assessing connectivity uses otolith chemistry to identify parts of catchments that produce a high proportion of a migratory fish population, helping to prioritise conservation efforts. In chapter 4, I advanced the use of a new approach integrating traditional (spawning surveys) and contemporary monitoring techniques (natural chemical markers) to identify

where nursery streams act as ecological traps and recruitment sinks for migratory trout populations, in this case linked to water abstraction practices that seasonally fragment fish migration routes. I conclude that this multidisciplinary approach offers considerable potential for determining the reproductive success of migratory salmonids. Results also reveal that detecting a lack of significant recruitment contribution from catchments where extensive spawning occurs provides a powerful indicator for determining whether human use of water resources is causing adverse effects. Collectively these findings highlight the benefits of holistically evaluating the consequences of human water use at the catchment / river network rather than sub-catchment scale when aiming to minimise the potential for undesirable negative effects on migratory fish populations.

The roles of life history diversity, metapopulation dynamics, and whole-life-history production between locally adapted life histories (i.e. to what extent resident and migratory, either anadromous or adfluvial, life-history forms are reproductively mixed) are being recognised as important components for increasing the abundance, resilience, and viability of wild salmonid populations (Jonsson and Jonsson 2011; Courter et al., 2013; Kendall et al., 2014, Moore et al. 2014 and Bourret et al. 2016). In Chapter 5, I used otolith chemistry to quantify source-sink dynamics between landlocked and anadromous Chinook salmon populations. Results illustrate that supplementary recruitment input from an upstream land-locked population increases overall population resilience, and allows Chinook salmon to persist despite the negative impacts of hydropower development by buffering against environmental variability in freshwater. For example, hydropeaking [3] adversely affects freshwater habitat conditions for example by increasing turbidity, dewatering spawning sites and scouring redds, all of which increase egg mortality (see Schmutz et al. 2015 and references therein). Rapid and frequent changes in water levels can also be detrimental for juvenile life stages that favour shallow riparian habitats as it can cause strandings, increase

[3] Hydropeaking refers to frequent and rapid changes to the operation of a hydropower dam resulting in large flow fluctuations for the downstream river.

predation risk, and reduce food availability (Young et al. 2011). Moreover, while adfluvial and anadromous maternal origin salmon offspring were found to enter the ocean at similar sizes (Chapter 5), they likely do so at different times because environmental conditions (e.g. temperature, nutrient and mineral content, food availability) and juvenile growth rates differ between rearing habitats. As the time of ocean entry is a critical life history event that influences smolt survival and cohort strength, a mix of migration times can be expected to further increase population resiliency of anadromous fishes to variable marine conditions (Quinn 2005, Jonsson and Jonsson 2011). Thus, sustained dispersal of progeny from adfluvial populations above dams provides a critical mechanism that increases the resilience and long-term persistence of anadromous Chinook salmon populations in the lower Clutha catchment, buffering the adverse effects associated with hydropower development by supplementing recruitment and reducing the risk of extinction. A detailed investigation of the degree to which similar dynamics also occur in other New Zealand river systems, or among brown trout populations in the Clutha catchment, was beyond the scope of this study. However, based on my findings, I conclude similar source-sink dynamics appear likely.

6.4 Attributes that increase resilience and invasion success

The ability to rapidly adopt an adfluvial life history and establish metapopulation structures linking voluntarily residual or landlocked and anadromous sub-populations are important attributes that increase resilience. In addition to helping maintain a small anadromous population of Chinook salmon in the Clutha River, despite the adverse effects of hydropower development, these qualities also define a successful invader. While countless numbers of successful introductions of non-anadromous salmonids have occurred throughout the world, attempts to create anadromous salmon populations have nearly always failed - but not for a lack of trying as hundreds of millions of salmon eggs, fry, or smolt from various species have been released globally over the last 150 years (Carwford and Muir 2008, Jonsson and Jonsson 2011 Lobón-Cerviá and Sanz 2017). Initially

these releases were aimed at creating new salmon runs, and more recently they occur as unintentional but significant leaks from commercial aquaculture ventures (McDowall 1994, Soto et al. 2001, Mo et al. 2018). The establishment of naturalised self-sustaining sea-run populations of Chinook salmon first in New Zealand (1901-1907), and later South America (1980s onwards), represent rare and notable exceptions, although it is still unclear why. Potential explanations typically fall into three broad categories, namely that their invasion success was facilitated by: A) similarities to native habitats providing a pre-adaptation to new freshwater and marine conditions; with B) low ecological resistance and partially vacant ecological niches (e.g. Soto et al. 2001, 2006; Correa and Gross 2008); or C) a high diversity of life history modes and capability to advance or delay emigration of juveniles from freshwater (e.g. Soto et al. 2007 and Arya et al. 2014). All of these explanations are ecologically sensible, largely complement each other, and align with a synthesis by Healey (2009) listing fundamental attributes that contribute to making salmon a highly resilient species, but none satisfactorily clarify why Chinook differ from other salmon, having virtually uniquely been able to repeatedly establish rapidly expanding anadromous populations outside its native range.

I submit that Chinook salmon in New Zealand display many generalist qualities, such as broader variation in life history traits and metapopulation dynamics, which exceed migration and recruitment patterns described for native populations. This thesis along with a growing number of management reports (e.g. Stevenson 2010, Hicks and Tana 2013, Gabrielsson 2015, Unwin and Gabrielsson 2018), and unpublished otolith data sets (R. Gabrielsson 2010, 2015, 2016), has begun to reveal that many smaller, less-studied New Zealand Chinook salmon populations show a much wider variety of life history strategies, including novel behaviours and metapopulation dynamics not previously reported. These include detection of adult sexually mature females (size range circa 40-55 cm) displaying extensive freshwater rearing with only brief periods (maximum a few months) at sea, as well as smaller mature females (< 50 cm) that display an exclusive freshwater lake resident life history strategy, along with the full range of

commonly observed life history patterns in their native range. As proposed in Chapter 5, it is possible this might partly be a founder effect as the source stock from the Sacramento River in California Central Valley is highly diverse, having four runs of Chinook salmon with unique life histories named after the season when adults return to spawn (i.e. spring, summer, fall, and winter runs) (Phillis et al. 2018). However, it might also be partly or predominantly related to environmental freshwater or ocean conditions present in New Zealand. Some or all of these attributes likely contributed to the successful establishment and high diversity of migration and recruitment patterns displayed by Chinook salmon populations in New Zealand as they can be an effective 'bet hedging' strategy.

Taken together this suggests observed characteristics of some New Zealand Chinook salmon populations appear to align more closely to the range of flexible migratory behaviours and cross-life history recruitment patterns commonly associated with brown trout than has previously been appreciated. Contrary to salmon, introduced brown trout have successfully established anadromous populations in all parts of the world where suitable spawning habitat exists. Brown trout exhibit a continuum of life history strategies within and between populations, and there is evidence that migratory behaviours can switch rapidly in response to environmental or demographic factors (Jonsson and Jonsson 2011). These abilities have repeatedly facilitated rapid natural colonisation of nearby river systems and regions through straying post-introduction, which together with a strong propensity for piscivory has firmly established brown trout as one of the most widely distributed invasive fish species in the world.

6.5 Management implications

Understanding how catchments contribute recruits to adult fish populations, and tracking the extent of migratory movements away from nursery areas, are important resource management tools, partly because they enable resource managers to evaluate the effects of anthropogenic developments on fish

populations and recreational fishery values. They can also help address knowledge gaps which at present pose challenges for fishery managers and water resource decision makers.

My thesis adds to a growing body of research that demonstrates that the use of natural chemical markers in water, otoliths, and eggs to track fish movements and recruitment patterns is a reliable and effective technique for answering ecological questions. My work illustrates the value of quantifying the relative reproductive contributions of different natal sources and life history types, and to determine if local fish populations are primarily replenished via self-recruitment, dispersal or a combination of multiple sources and pathways. Collectively my investigations show natural chemical markers provide researchers, fishery managers, and resource management agencies with a powerful tool for better understanding salmonid population dynamics. For example, these markers can help evaluate the effectiveness of conservation plans and resource management decisions, such as ecological flow regimes, or identify the influences of dispersal and metapopulation dynamics on recruitment patterns and population resilience to those responsible for managing migratory sports and native fish populations.

Although freshwater fishery and resource management agencies in New Zealand (i.e. Fish & Game, Department of Conservation, and regional authorities) are beginning to recognise otolith microchemistry as an effective technique for reconstructing the natal origin and movement history of migratory fishes, practitioners remain largely unfamiliar with how to incorporate this tool into fisheries monitoring and management planning. Providing practical examples of ways to incorporate the power of this approach for gathering population statistics, conducting stock assessment and trend analysis, or answering yet unexplored applied or theoretical questions remains a persistent challenge for researchers. A useful start will be to create regional or national geographical information systems, or similar databases and depositary libraries, for collating and storing Sr isotope results from studies that have analysed water and otolith samples. At a

minimum these would provide an overview of where such studies have taken place and who to contact regarding data sharing when scoping future studies or conducting meta-analysis.

6.6 Further research and summary

The research presented here suggests that we would benefit from focussing future work in three areas. The first is to evaluate whether dispersal of progeny from lake migratory populations contributes to downstream anadromous salmonid populations in other regulated and open river systems. If similar source-sink dynamics are found to also significantly contribute to downstream anadromous trout and Chinook salmon populations outside of the Clutha catchment, a broader consideration as to how this recruitment mechanism influences population resilience may be warranted.

Second, there is a need for further examination of adfluvial Chinook salmon behaviour and populations displaying either delayed outmigration to marine environments, landlocking or voluntarily residual life history strategies. This is particularly needed in regions where effort is already being directed to better understand the mechanism behind rapidly expanding invasive populations of Chinook Salmon, and other invasive or non-native anadromous salmonid populations, but also in locations where adfluvial populations have emerged within their native range (i.e. the Pacific North West).

Thirdly, we need to enhance our understanding of the potential drivers behind observed dispersal movements. The results of this study show that despite fascinating migration patterns at the individual and population level, on its own, otolith microchemistry offers little insight into why such migrations are undertaken. Thus, combining the predictive power of growth models with information on detected movement patterns will enable researchers to better explore some of the likely drivers behind commonly observed migration patterns.

Applied broadly this will further improve our understanding of salmonid ecology, particularly if the capability to model potential marine-derived dietary influences on otolith chemistry is also improved. All of this would aid efforts to quantify the importance of marine nutrients, such as diadromous forage fish consumed seasonally by salmonids or other predatory fishes in New Zealand. For example, longfin eels, a highly valued freshwater fish species in New Zealand, are known to seasonally target adult Chinook salmon pre- and post-spawning in headwater spawning areas yet it is still unclear to what degree this constitutes an important seasonal energy source.

Ecologists seek to identify patterns based on observational data and studies in order to better understand the key processes and mechanisms that appear to drive variability. In relation to fisheries ecology, McKenzie et al. (2012, p. 3) emphasised that "fish marking and tracking is a fundamental tool for fisheries management and research". The research undertaken in this study has aided efforts to expand available tools by performing controlled experiments examining the reliability of using natural chemical markers in eggs and otolith to track catchment-wide fish movement and recruitment patterns, helping to better define suitable applications to wild populations. It has also extended knowledge about salmonid population dynamics in New Zealand by presenting case studies that identify recruitment patterns and proposing factors and mechanisms that explain them.

Chapter 7 : References

Acolas, M. L., Roussel, J. M. and Baglini`ere, J. L. 2008. Linking migratory patterns and diet to reproductive traits in female brown trout (*Salmo trutta* L.) by means of stable isotope analysis on ova. *Ecology of Freshwater Fish* **17:** 382–393.

Allan, J. D., Wipfli, M. S., Caouette, J. P., Prussian, A. and Rodgers, J. 2003. Influence of streamside vegetation on terrestrial invertebrate inputs to salmonid food webs. *Canadian Journal of Fisheries and Aquatic Sciences* **60**: 309–320.

Allen, K. R. 1951. The Horokiwi Stream: a study of a trout population. *New Zealand Marine Department Fisheries Bulletin* **10**: 1–238.

Aprahamin, M. W., Smith, K. M., McGinnity, P. M., McKelvey, S. and Taylor, J. 2003. Restocking of salmonids – opportunities and limitations. *Fisheries Research* **62**: 211–227.

Araya, M., Niklitschek, E. J., Secor, D. H. and Piccoli, P. M. 2014. Partial migration in introduced wild chinook salmon (*Oncorhynchus tshawytscha*) of southern Chile. *Estuarine, Coastal and Shelf Science* **149**: 87–95.

Armstrong, D. P. and Seddon, P. J. 2008. Directions in reintroduction biology. *Trends in Ecology and Evolution* **23**: 20–25.

Barbee, N. C. and Swearer, S. E. 2007. Characterizing natal source population signatures in the diadromous fish *Galaxias maculatus*, using embryonic otolith chemistry. *Marine Ecology Series* **343**: 273–282.

Barnes, T. C. and Gillanders, B. M. 2013. Combined effects of extrinsic and intrinsic factors on otolith chemistry: implications for environmental reconstructions. *Canadian Journal of Fisheries and Aquatic Sciences* **70**: 1159–1166.

Barnett-Johnson, R. 2005. Validation of Sr isotopes in otoliths by laser ablation multicollector inductively coupled plasma mass spectrometry (LA-MC-ICPMS): Opening avenues in fisheries science applications. *Canadian Journal of Aquatic Sciences* **62**: 2425–2430.

Barnett-Johnson, R., Pearson, T., Ramos, F., Grimes, C. and MacFarlane, B. 2008. Tracking natal origins of salmon using isotopes, otoliths, and landscape geology. *Limnology and Oceanography* **53**: 1633–1642.

Bath, G. E., Thorrold, S. R., Jones, C. M., Campana, S. E., McLaren, J. W. and Lam, J. W. H. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta* **64:** 1705–1714.

Bilby, R. E., Fransen, B. R., Bisson, P. A. and Walter, J. K. 1998. Response of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead (*Oncorhynchus mykiss*) to the addition of salmon carcasses to two streams in southwestern Washington, USA. *Canadian Journal of Fisheries and Aquatic Sciences* **55**: 1909–1918.

Block, B. A., Teo, S. L. H., Walli, A., Boustany, A., Stokesbury, M. J. W., Farwell, C. J., Weng, K. C., Dewar, H. and Williams, T. D. 2005. Electronic tagging and population structure of Atlantic bluefin tuna. *Nature* **434**: 1121–1127.

Bourret, S., Coudill, C. C. and Keefer, M. L. 2016. Diversity of juvenile chinook salmon life history pathways. *Reviews in Fish Biology and Fisheries* **26**: 375–403.

Bradbury, I. R., DiBacco, C., Thorrold, S. R., Snelgrove, P. V. R. and Campana, S. E. 2011. Resolving natal tags using otolith geochemistry in an estuarine fish, rainbow smelt *Osmerus mordax*. *Marine Ecology Progress Series* **433**: 195–204.

Brannon, E. L., Amend, D. F., Cronin, M. A., Lannan, J. E., LaPatra, S., McNeil, W. J., Noble, R. E., Smith, C. E., Talbot, A. J., Wedemeyer, G. A. and Wester, H. 2004. The controversy about salmon hatcheries. *Fisheries* **29**: 12–31.

Brazner, J. C., Campana, S. E. and Tanner, D. K. 2004. Habitat fingerprints for Lake Superior coastal wetlands derived from elemental analysis of yellow perch otoliths. *Transactions of the American Fisheries Society* **133:** 692–704.

Brown, J., Colling, A., Park, D., Phillips, J., Rothery, D. and Wright, J. 1989. Seawater: Its composition, properties and behaviour. Oxford: Pergamon Press.

Brown, R. J. and Severin, K. P. 2009. Otolith chemistry analysis indicates that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. *Canadian Journal of Fisheries and Aquatic Sciences* **66:** 1790–1808.

Buckel, J. A., Sharack, B. L. and Zdanowicz, V. S. 2004. Effect of diet on otolith composition in *Pomatomus saltatrix*, an estuarine piscivore. *Journal of Fish Biology* **64**: 1469–1484.

Budy, P., Thiede, G. P., Bouwes, N., Petrosky, C. E. and Schaller, H. 2002. Evidence linking delayed mortality of Snake River salmon to their earlier hydrosystem experience. *North American Journal of Fisheries Management* **22**: 35–51.

Cadrin, S. X. and Secor, D. H. 2009. Accounting for spatial population structure in stock assessment: past, present and future. *In:* B.J. Rothschild and R. Beamish (Editors) 2009. The future of fishery science in North America. Springer Verlag. pp. 405–426.

Campana, S. E. 1990. How reliable are growth back-calculations based on otoliths? *Canadian Journal of Fisheries and Aquatic Sciences* **47**: 2219–2227.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms, and applications. *Marine Ecology Progress Series* **188**: 263–297.

Campana S. E. and Thorrold, S. R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Canadian Journal of Aquatic Sciences* **58**: 30−38.

Carlsson, J., Aarestrup, K., Nordwall, F., Näslund, I., Eriksson, T. and Carlsson, J. E. 2004. Migration of landlocked brown trout in two Scandinavian streams as revealed from trap data. *Ecology of Freshwater Fish* **13**: 161–167.

Cederholm, C. J., Kunze, M. D., Murota, T. and Sibatani, A. 1999. Pacific salmon carcasses: essential contributions of nutrients and energy for terrestrial and aquatic ecosystems. *Fisheries* **24**: 6–15.

Chapman, D. W. 1966. Food and space as a regulator of salmonid populations in streams. *American Naturalist* **100**: 345–357.

Chapman, B. B., Hulthen, K., Brodersen, J., Nilsson, P. A., Skov, C., Hansson, L. A. and Brönmark, C. 2012. Partial migration in fishes: causes and consequences. *Journal of Fish Biology* **81:** 456–478.

Chilcote, M. W., Goodson, K. W. and Falcy, M. R. 2011. Reduced recruitment performance in natural populations of anadromous salmonids associated with hatchery-reared fish. *Canadian Journal of Aquatic Sciences* **68**: 511–522.

Chittaro, P. M., Hogan, J. D., Gagnon, J., Fryer, B. J. and Sale, P. F. 2006. In situ experiment of ontogenetic variability in the otolith chemistry of *Stegastes partitus*. *Marine Biology* **149**: 1227–1235.

Clausen, B. and Plew, D. 2004. How high are bed-moving flows in New Zealand Rivers? *Journal of Hydrology (New Zealand)* **43**: 19–37.

Collingsworth, P. D., Van Tassell, J. J., Olesik, J. W. and Marschall, E. A. 2010. Effects of temperature and elemental concentration on the chemical composition of juvenile yellow perch (*Perca flavescens*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **67**: 1187–1196.

Cook, J. T., McNiven, M. A., Richardson, G. F. and Sutterlin, A. M. 2000. Growth rate, body composition and feed digestibility/conversion of growth-enhanced transgenic Atlantic salmon (*Salmo salar*). *Aquaculture* **188**: 15–32.

Correa, C. and Gross, M. R. 2008. Chinook salmon invade southern South America. *Biological Invasions* **10**: 615–639.

Courter, I. I., Child, D. B., Hobbs, J. A., Garrison, T. M., Glessner, J. J. G. and Duery, S. 2013. Resident rainbow trout produce anadromous offspring in a large interior catchment. *Canadian Journal of Aquatic Sciences* **70**: 701–710.

Crook, D. A., Macdonald, J. I., McNeil, D. G., Gilligan, D. M., Asmus, M., Maas, R. and Woodhead, J. 2013. Recruitment sources and dispersal of an invasive fish in a large river system as revealed by otolith chemistry analysis. *Canadian Journal of Aquatic Sciences* **70**: 953–963.

Deans, N., Unwin, M. and Rodway, M. 2004. Sport fishery management. In: Harding J. S.; Mosely, M. P.; Pearson, C. P.; and Sorrell, B. K. (Editors) 2004. Freshwaters of New Zealand. New Zealand Hydrological Society Inc. and New Zealand Limnological Society Inc., Christchurch, New Zealand. 764 pp.

Deegan, L. A., Golden, H. E., Harvey, C. J. and Peterson, B. J. 1999. Influence of environmental variability on the growth of age-0 and adult Arctic grayling. *Transactions of the American Fisheries Society* **128**: 1163–1175.

Doubleday, Z. A., Izzo, C., Woodcock, S. H. and Gillanders, B. M. 2013. Relative contribution of water and diet to otolith chemistry in freshwater fish. *Aquatic Biology* **18**: 271–280.

Elliott, J. M. 1993. A 25-year study of production of juvenile sea-trout, *Salmo trutta*, in an English Lake District stream. *Canadian Special Publication of Fisheries and Aquatic Sciences* **118:** 109–122.

Elliott, J. M. 1994. Quantitative ecology and the brown trout. Oxford University Press, Oxford, UK.

Elliott, J. M. and Elliott, J. A. 2006. A 35-year study of stock-recruitment relationships in a small population of sea trout: assumptions, implications and limitations for predicting targets. *In*: Harris G, Elliott JM, Hurley MA (1998) Population regulation in adult, but not juvenile, resident trout in a Lake District stream. *Journal of Animal Ecology* **67**: 280–286.

Elliott, J. M., Hurley, M. A. and Fryer, R. J. 1995. A new, improved growth model for brown trout, *Salmo trutta*. *Functional Ecology* **9**: 290–298.

Elliott, J. M. and Hurley, M. A. 2000. Daily energy intake and growth of piscivorous brown trout, *Salmo trutta*. *Freshwater Biology* **44**: 237–245.

Elsdon, T. S. and Gillanders, B. M. 2003. Reconstructing migratory patterns of fish based on environmental influences in otolith chemistry. *Reviews in Fish Biology and Fisheries* **13:** 219–235.

Elsdon, T. S., Wells, B. K., Campana, S. E., Gillanders, B. M., Jones, C. M., Limburg, K. E., Secor D. H., Thorrold, S. R. and Walther, B. D. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. *Oceanography and Marine Biology*: *An Annual Review* **46**: 297–330.

Farrell, J. and Campana, S. E. 1996. Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **115**: 103–109.

Fausch, K. D., Taniguchi, Y., Nakano, S., Grossman, G. D. and Townsend, C. R. 2001. Flood disturbance regimes influence rainbow trout invasion success among five holarctic regions. *Ecological Applications* **11**: 1438–1455.

Flain, M. 1980. Those dammed Clutha salmon. *Freshwater Catch* **7**: 7.

Fleming, I. A. and Petersson, E. 2001. The ability of released, hatchery salmonids to breed and contribute to the natural productivity of wild populations. *Nordic Journal of Freshwater Research* **75**: 71–98.

Forseth, T., Næsje, T. F., Jonsson, B. and Hårsaker, K. 1999. Juvenile migration in brown trout: a consequence of energetic state. *Journal of Animal Ecology* **68**: 783–793.

Gabrielsson, R., Seddon, P. and Closs, G. P. 2011. Validation of Relationships between Ambient Trace Element concentrations and otolith Microchemistry in Trout. A research review report prepared for the New Zealand Fish & Game Council's Research Committee.

Gabrielsson, R. 2015. Review of methods for monitoring chinook salmon in the Lower Clutha River. Prepared for Contact Energy Limited. Cawthron Report No. 2674. 44 p. plus appendix.

Gabrielsson, R. M., Kim, J., Reid, M. R., Stirling, C. H., Numata, M. and Closs, G. P. 2012. Does the trace element composition of brown trout *Salmo trutta* eggs remain unchanged in spawning redds? *Journal of Fish Biology* **81**: 1871–1879.

Gibson-Reinemer, D. K., Johnson, B. M., Martinez, P. J., Winkelman, D. L., Koenig, A. E. and Woodhead, J. D. 2009. Elemental signatures in otoliths of hatchery rainbow trout (*Oncorhynchus mykiss*): distinctiveness and utility for detecting origins and movements. *Canadian Journal of Aquatic Scien*ces **66**: 513–524.

Gillanders, B. M. 2002. Connectivity between juvenile and adult fish populations: do adults remain near their recruitment estuaries? *Marine Ecology Progress Series* **240**: 215–223.

Gotthard, K., Nylin, S., and Wiklund, C. 1994. Adaptive variation in growth rate: life history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. *Oeclogia* **99**: 281–289.

Graynoth, E. 1996. Determination of the age of brown and rainbow trout in a range of New Zealand lakes. *Marine and Freshwater Research* **47**: 749–756.

Graynoth, E. 1999. Recruitment and distribution of juvenile salmonids in Lake Coleridge, New Zealand. *New Zealand Journal of Marine and Freshwater Research* **33**: 205–219.

Greenberg, L. and Calles, O. 2010. Restoring ecological connectivity in rivers to improve conditions for anadromous brown trout (*Salmo trutta*). *In:* P. Kemp (Editor) 2010. Salmonid Fisheries Freshwater Habitat Management. West Sussex: Wiley-Blackwell, pp. 148–163.

Habfast, K. 1998. Fractionation correction and multiple collectors in thermal ionization isotope ratio mass spectrometry. *International Journal of Mass Spectrometry* **176:** 133–148.

Hanski, I. 1989. Metapopulation dynamics: does it help to have more of the same? *Trends in Ecology and Evolution* **4:** 113–114.

Hanski, I. and Gilpin, M. E. 1991. Metapopulation dynamics: brief history and conceptual domain. *Biological Journal of the Linnean Society* **42**: 3–16.

Hanski, I. and Gilpin, M. E. (Editors) 1997. Metapopulation biology, ecology, genetics and evolution. Academic Press, San Diego.

Hanski, I. and Simberloff, D.S. 1997. The metapopulation approach, its history, conceptual domain, and application to conservation. *In*: I. Hanski and M. E. Gilpin (Editors) 1997. Metapopulation biology, ecology, genetics and evolution. Academic Press, San Diego, pp. 5–26.

Hanski, I. 2002. Metapopulations of animals in highly fragmented landscapes and population viability analysis. *In:* S. R. Beissinger and D. R. McCullough (Editors) 1997. Population viability analysis. The University of Chicago Press, Chicago, pp. 86–108.

Hart, S. R. and Zindler, A. 1989. Isotope fractionation laws – a test using calcium. *International Journal of Mass Spectrometry* **89:** 287–301.

Harvey, B. C., Nakamoto, R. J. and White, J. L. 2006. Reduced streamflow lowers dry-season growth of rainbow trout in a small stream. *Transactions of the American Fisheries Society* **135**: 998–1005.

Hayes, J. W. 1988. Comparative stream residence of juvenile brown and rainbow trout in a small lake inlet tributary, Scott Creek, New Zealand. *New Zealand Journal of Marine and Freshwater Research* **22**: 181–188.

Hayes, J. W. 1995. Spatial and temporal variation in the relative density and size of juvenile brown trout in the Kakanui River, North Otago, New Zealand. *New Zealand Journal of Marine and Freshwater Research* **29**: 393–407.

Hayes, J. W. 2013. Brown trout growth models: User guide Version 2.1. Cawthron Report No. 571A. 46 P. plus appendices.

Hayes, J. W., Stark, J. D. and Shearer, K. A. 2000. Development and test of a whole-lifetime foraging and bioenergetics growth model for drift-feeding brown trout. *Transactions of the American Fisheries Society* **129**: 315–332.

Hayes, J. W., Olsen, D. A. and Hay, J. 2010. The influence of natural variation in discharge on juvenile brown trout population dynamics in a nursery tributary of the Motueka River, New Zealand. *New Zealand Journal of Marine and Freshwater Research* **44**: 247–269.

Hayes, J. W., Goodwin, E. O., Shearer, K. A., Hay, J. and Kelly, L. 2016. Can Weighted Useable Area Predict Flow Requirements of Drift-Feeding Salmonids? Comparison with a Net Rate of Energy Intake Model Incorporating Drift–Flow Processes. Transactions of the American Fisheries Society **145**: 589–609.

Hayes, J. W., Goodwin, E. O., Shearer, K. A. and Hicks, D. M. 2019. Relationship between background invertebrate drift concentration and flow over natural flow recession and prediction with a drift transport model, Canadian Journal of Fisheries and Aquatic Sciences **76**: 871–885.

Hayes, S. A., Hanson, C. V., Pearse, D. E., Bond, M. H., Garza, J. C. and MacFarlane, B. R. 2012. Should I stay or should I go? The influence of genetic origin on emigration behaviour and physiology of resident and anadromous juvenile *Oncorhynchus mykiss*. *North American Journal of Fisheries Management* **32**: 772–780.

Healey, M. C. 1991. Life history of chinook salmon (*Oncorhynchus tshawytscha*). In: Groot, C. and Margolis, L. (Editors), Pacific salmon life histories. University of British Columbia Press, Vancouver, BC, pp. 313–393.

Healey, M. C. 2009. Resilient salmon, resilient fisheries for British Columbia, Canada. *Ecology and Society* **14**: 1–13. Available online at: http://www.ecologyandsociety.org/vol14/iss1/art2/

Hegg, J. C., Kennedy B. P., Chittaro P. M. and Zabel R. W. 2013. Spatial structuring of an evolving life-history strategy under altered environmental conditions. *Oecologia* **172**: 1017–1029.

Hegg, J.C. 2017. Movement, migration, and melody: an interdisciplinary study of juvenile salmon ecology through otolith chemistry. Ph.D. dissertation, University of Idaho.

Heintz, R. A., Nelson, B. D., Larsen, M., Holland, L., Wipfli, M. S. and Hudson, J. P. 2004. Effects of salmon carcasses on the lipid class and fatty acid composition of juvenile coho salmon. *Transactions of the American Fisheries Society* **133**: 559–567.

Hendry, A. P. and Stearns, S. C. 2004. Evolution illuminated: salmon and their relatives. Oxford University Press, Oxford, UK.

Hickford, M. J. H. and Schiel, D. R. 2011. Population sinks resulting from degraded habitats of an obligate life-history pathway. *Oecologia* **166**: 131–140.

Hicks, A. S., Closs, G. P. and Swearer, S. E. 2010. Otolith microchemistry of two amphidromous galaxiids across an experimental salinity gradient: A multi-element approach for tracking diadromous migrations. *Journal of Experimental Marine Biology and Ecology* **394**: 86–97.

Hicks, B. J. and Tana, R. 2013. Life history analysis of chinook salmon (*Oncorhynchus tshawytscha*) from lakes Mapourika and Paringa, West Coast, South Island, NZ, by otolith microchemistry. Environmental Research Institute Report No. 24, 14pp. Prepared for West Coast Fish and Game Council. Environmental Research Institute, Faculty of Science and Engineering, The University of Waikato, Hamilton.

Hilborn, R., Quinn, T. P., Schindler, D. E. and Rogers, D. E. 2003. Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Sciences of the United States of America* **100**: 6564–6568.

Hobson, K. A., Barnett-Johnson, R. and Cerling, T. 2010. Using isoscapes to track animal migration. *In*: J. B. West et al., (Editors) 2010. Isoscapes:

understanding movement, pattern, and process on earth through isotope mapping.

Hodell, D. A., Mueller, P. A., McKenzie, J. A. and Mead, G. 1989. Strontium isotope stratigraphy and geochemistry of the late Neogene ocean. *Earth and Planetary Science Letters* **92**: 165–178.

Hoff, G. R. and Fuiman, L. A. 1995. Environmentally induced variation in elemental composition of red drum (*Sciaenops ocellatus*) otoliths. *Bulletin of Marine Science* **56**: 578−591.

Holmes, R., Hayes, J. W., Jiang, W., Quarterman, A. and Davey, L. N. 2014. Emigration and mortality of juvenile brown trout in a New Zealand headwater tributary. *Ecology of Freshwater Fish* **24**: 631–643.

Horrell, G. 2014. Review of the science supporting the proposed minimum flow regime for the Lindis River. NIWA client report CHC2014-146. Prepared for Otago Regional Council.

Hughes, N. F. 1998. A model of habitat selection by drift-feeding stream salmonids at different scales. *Ecology* **79**: 281–294.

Hutchings, J. A. 2002. Sustaining Atlantic Salmon in the Northwest Atlantic: Considerations from a life history perspective. *In*: Lynch, K. D., Jones, M. L., and Taylor, W. W. (Editors) Sustaining North American salmon: perspectives across regions and disciplines. American Fisheries Society, Bethesda, Maryland, pp. 33–60.

Jaecks, T., Bond, M. H. and Quinn, T. P. 2016. Can dietary reliance on Pacific salmon eggs create otolith Sr/Ca signatures that mimic anadromy in resident salmonids? *Environmental Biology* **99**: 237–247.

Jeffres, C. and Moyle, P. 2012. When good fish make bad decisions: coho salmon in an ecological trap. *North American Journal of Fisheries Management* **32**: 87–92.

Jellyman, D. J. and Bonnett, M. L. 1992. Survey of juvenile trout in the Lindis and Cardrona rivers, and Clutha River in the vicinity of Cromwell, March 1992, including a review of previous surveys, Christchurch [N.Z.]. Report to Otago Fish and Game Council. *New Zealand Freshwater Fisheries Miscellaneous Report* **120**, 48 p.

Jellyman, D. and Graynoth, E. 1993. Summary of results of fisheries survey of Lake Dunstan March 1994. Consultancy report no. ELE027 NIWA, Christchurch.

Jensen, A. J., Forseth, T. and Johnson, B. O. 2000. Latitudinal variation in growth of young brown trout *Salmo trutta*. *Journal of Animal Ecology* **69**: 1010–1020.

Johnson, R. C., Weber, P. K., Wikert, J. D., Workman, M. L., MacFarlane, R. B., Grove, M. J. and Schmitt, A. K. 2012. Managed metapopulations: do salmon hatchery 'sources' lead to in-river 'sinks' in conservation? *PLoS ONE* **7**: e28880. Available online at: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0028880

Johnson, R. C., Garza, J. C., MacFarlane, B., Grimes, C. B., Phillis, C. C., Koch, P. L., Weber, P. K. and Carr, M. H. 2016. Isotopes and genes reveal freshwater origins of Chinook salmon *Oncorhynchus tshawytscha* aggregations in California's coastal ocean. Marine Ecology Progress Series **548**: 181–196.

Jonsson, B. and Jonsson, N. 1993. Partial migration: niche shift versus sexual maturation in fishes. *Reviews in Fish Biology and Fishes* **3**: 348–365.

Jonsson, B. and Jonsson, N. 2011. Ecology of Atlantic salmon and brown trout: habitat as a template for life histories. Fish and Fisheries Series 33. Springer-Verlag, Dordrecht.

Jonsson, B., Jonsson, N. and Ugedal, O. 2011. Production of juvenile salmonids in small Norwegian streams is affected by agricultural land use. *Freshwater Biology* **56**: 2529–2542.

Jönsson, E., Johnsson, J. I. and Björnsson, B. T. 1996. Growth hormone increases predation exposure of rainbow trout. *Proceedings Royal Society Biological Sciences* **263**: 647–651

Kalish, J. 1990. Use of otolith chemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. *Fisheries Bulletin* **88**: 657–666.

Kendall, N. W., McMillan, J. R., Sloat, M. R., Buehrens, T. W. and Quinn, T. P. 2015. Anadromy and residency in steelhead and rainbow trout (*Oncorhynchus mykiss*): a review of the processes and patterns. *Canadian Journal of Fisheries and Aquatic Sciences* **72**: 319–342.

Kennedy, B. P., Folt, C. L., Blum, J. D. and Chamberlain, C. P. 1997. Natural isotope markers in salmon. *Nature* **387**: 766–767.

Kennedy, B. P., Blum, J. D., Folt, C. L. and Nislow, K. H. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. *Canadian Journal of Aquatic Sciences* **57**: 2280–2292.

Kennedy, B. P., Klaue, A., Blum, J. D., Folt, C. L. and Nislow, K. H. 2002. Reconstructing the lives of fish using Sr isotopes in otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **59**: 925–929.

Kim, J. P. and Hunter, K. A. 1997. Aqueous chemistry of major ions and trace metals in the Takaka-Cobb River system, New Zealand. *Marine and Freshwater Research* **48**: 257–266.

Klemetsen, A., Amundsen, P. A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F. and Mortensen, E. 2003. Atlantic salmon *Salmo salar* L. brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecology of Freshwater Fish* **12**: 1–59.

Koski, K V. 2009. The fate of coho salmon nomads: the story of an estuarine rearing strategy promoting resilience. *Ecology and Society* **14**: 4. Available online at: http://www.ecologyandsociety.org/vol14/iss1/art4/

Kristensen, E. A. 2006. Population dynamics, spawning and movements of brown trout in Taieri River tributary streams. PhD Thesis, University of Otago, Dunedin, New Zealand.

Kristensen, E. A. and Closs, G. P. 2008. Environmental variability and population dynamics of juvenile brown trout (*Salmo trutta*) in an upstream and downstream reach of a small New Zealand river. *New Zealand Journal of Marine and Freshwater Research* **42**: 57–71.

Kristensen, E. A., Closs, G. P., Olley, R., Kim, J., Reid, M. and Stirling, C. 2011. Determining the spatial distribution of spawning by anadromous and resident brown trout *Salmo trutta* L using strontium content of eggs collected from redds. *Ecology of Freshwater Fish* **20**: 377–383.

Kritzer, J. P. and Sale, P. F. 2004. Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. *Fish and Fisheries* **5**: 131–140.

Krkošek, M. and Lewis, M. 2010. An R_0 theory for source–sink dynamics with application to *Dreissena* competition. *Theoretical Ecology* **3**: 24–43.

Lacroix, G. L. 2013. Population-specific ranges of oceanic migration for adult Atlantic salmon (*Salmo salar*) documented using pop-up satellite archival tags. *Canadian Journal of Aquatic Sciences* **70**: 1011–1030.

Lagarrigue, T., Baran, P., Lascaux, J. M. and Belaud, A. 2001. Growth variability analysis of a brown trout (*Salmo trutta* L.) population in a Pyrenean mountain stream. *Bulletin Franc¸ais de la Peˆche et de la Pisciculture,* **357/360**: 573–594.

Laikre, L., Antunes, A., Apostolidis, A., Berrebi, P., Duguid, A., Ferguson, A., Garcia-Marin, J. L., Guyomard, R., Hansen, M. M., Hindar, K., Koljonen, M. L., Largiader, C., Martinez, P., Nielsen, E. E., Palm, S., Ruzzante, D. E., Ryman, N. and Trianthaphyllidis, C. 1999. Conservation genetic management of brown trout (*Salmo trutta*) in Europe. Report by the concerted action on identification, management and exploitation of genetic resources in the brown trout (*Salmo trutta*). ‘‘TROUTCONCERT’’; EU FAIR CT97-3882. Danmarks fiskeriundersogelser, Silkeborg, 91 pp. Available online at: https://www.researchgate.net/publication/305657027_Conservation_Genetic_Management_of_Brown_Trout_Salmo_trutta_in_Europe

Lange, K., Townsend, C. R., Gabrielsson, R., Chanut, P. C. and Matthaei, C. D. 2014. Responses of stream fish populations to farming intensity and water abstraction in an agricultural catchment. *Freshwater Biology* **59**: 286–299.

Lennox, R. J., Crook, D. A., Moyle, P. B., Structhers, D. P. and Cooke, S. J. 2019. Toward a better understanding of freshwater fish responses to an increasingly drought-stricken world. *Reviews in Fish Biology and Fisheries* **29**: 71–91.

Limburg, K. E. 1995. Otolith strontium traces environmental history of subyearling American shad *Alosa sapidissima*. *Marine Ecology Progress Series* **119**: 25–35.

Lobón-Cerviá, J. and Sanz, N. (Editors) 2017. Brown trout: biology, ecology and management. ISBN: 978-1-119-26831-4, 808 pages.

Lowe, M. R., DeVries, D. R., Wright, R. A., Ludsin, S. A. and Fryer, B. J. 2009. Coastal largemouth bass (*Micropterus salmoides*) movement in response to changing salinity. *Canadian Journal of Aquatic Sciences* **66**: 2174–2188.

Mann, C. C. and Plummer, M. L. 2000. Can science rescue salmon? *Science* **289**: 716–719.

Marohn, L., Prigge, E., Zumholz, K., Klugel, A., Anders, H. and Hanel, R. 2009. Dietary effects on multi-element composition of European eel (*Anguilla anguilla*) otoliths. *Marine Biology* **156**: 927−933.

Martin, J., Bareille, G., Berail, S., Pecheyran, C., Daverat, F., Bru, N., Tabouret, T. and Donard, O. 2013. Spatial and temporal variations in otolith chemistry and relationships with water chemistry: a useful tool to distinguish Atlantic salmon *Salmo salar* parr from different natal streams. *Journal of Fish Biology* **82**: 1556–1581.

McDowall, R. M. 1990. New Zealand freshwater fishes: a natural history and guide. Heinemann Reed, Auckland.

McDowall, R. M. 1994. Gamekeepers for the nation. Canterbury University Press, Christchurch.

McDowall, R. M. 1997. The evolution of diadromy in fishes (revisited). *Reviews in Fish Biology and Fisheries* **7**: 443–462.

McCullough, D. R. (Editor) 1996. Metapopulations and wildlife conservation. Washington, DC: Island Press.

McKenzie, J., Parsons, B., Seitz, A., Keller Kopf, R., Mesa, M., and Phelps, Q. (Editors) 2012. Advances in fish tagging and marking technology. American Fisheries Society, Symposium 76, Bethesda, Maryland. 560 pp. ISBN: 978-1-934874-27-1

McMeel, O. and Ferguson, A. 1997. The genetic diversity of brown trout in the River Dove. Unpublished Report by Queen's University of Belfast to the Environment Agency, Lichfield, Staffs.

McMichael, G., Rakowski, C., James, B. and Lukas, J. 2005. Estimated fall chinook salmon survival to emergence in dewatered redds in a shallow side channel of the Columbia River. *North American Journal of Fisheries Management* **25**: 876–884.

Metcalfe, N. B., Taylor, A. C. and Thorpe, J. E. 1995. Metabolic rate, social status and life-history strategies in Atlantic salmon. *Animal Behaviour* **49**: 431–436.

Metcalfe, J. D., Arnold, G., McDowall, R. 2002. Migration. Pages 175–199 *In:* P. J. B. Hart and J. D. Reynolds (Editors). Handbook of fish biology and fisheries, volume 1. Blackwell Scientific Publications, Oxford, UK.

Miller, J., Gray, A. and Merz, J. 2010. Quantifying the contribution of juvenile migratory phenotypes in a population of chinook salmon *Oncorhynchus tshawytscha*. *Marine Ecology Progress Series* **408**: 227–240.

Miller, J. A. and Kent, A. J. R. 2009. The determination of maternal run time in juvenile chinook salmon (*Oncorhynchus tshawytscha*) based on Sr/Ca and $^{87}Sr/^{86}Sr$ within otolith cores. *Fisheries Research* **95**: 373–378.

Milton, D. A. and Chenery, S. R. 2001. Sources and uptake of trace metals in otoliths of juvenile barramundi (*Lates calcarifer*). *Journal of Experimental Marine Biology and Ecology* **264**: 47–65.

Mo T. A., Thorstad E. B., Sandlund O. T., Berntsen H. H., Fiske P., and Uglem I. 2018. The pink salmon invasion: a Norwegian perspective. *Journal of Fish Biology* **93**: 5–7.

Molony, B. W., Lenanton, R., Jackson, G. and Norris, J. 2003. Stock enhancement as a fisheries management tool. *Reviews in Fish Biology and Fisheries* **13**: 409–432.

Moore, J. W., Yeakel, J. D., Peard, D., Lough, J. and Beere, M. 2014. Life-history diversity and its importance to population stability and persistence of a migratory fish: steelhead in two large North American catchments. *Journal of Animal Ecology* **83**: 1035–1046.

Morinville, G. R. and Rasmussen, J.B. 2003. Early juvenile bioenergetic differences between 554 anadromous and resident brook trout (*Salvelinus fontinalis*). *Canadian Journal of Aquatic Sciences* **60**: 401–410.

Morita, K., Saito, T., Miyakoshi, Y., Fukuwaka, M., Nagasawa, T. and Kaeriyama, M. 2006. A review of Pacific salmon hatchery programmes on Hokkaido Island, Japan. *ICES Journal of Marine Science* **63**: 1353–1363.

Mosley, M. P. 1982. New Zealand river temperature regimes. New Zealand Ministry of Works and Development, Water and Soil Miscellaneous Publication No. 36.

Muhlfeld, C. C., Thorrold, S. R., McMahon, T. E. and Marotz, B. 2012. Estimating westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) movements in a river network using strontium isoscapes. *Canadian Journal of Fisheries and Aquatic Sciences* **69**: 906–915.

Myers, K. W., Kope, R. G., Bryant, G. J., Teel, D., Lierheimer, L. J., Wainwright, T. C., Grant, W. S., Waknitz, F. A, Neely, K., Lindley, S. T. and Waples, R. S. 1998. Status review of chinook salmon from Washington, Idaho, Oregon and California. National Marine Fisheries Service. NOAA Technical Memorandum NMFS-NWFSC-35. Seattle, WA.

Nakano S., Miyasaka, H. and Kuhara, N. 1999. Terrestrial-aquatic linkages: riparian arthropod inputs alter tropic cascades in a stream food web. *Ecology* **80**: 2435–2441.

Nicola, G. and Almodovar, A. 2004. Growth patterns of stream-dwelling brown trout under contrasting thermal conditions. *Transactions of the American Fisheries Society* **133**: 66–78.

Nicola, G., Almodovar, A. and Elvira, B. 2009. Influence of hydrologic attributes on brown trout recruitment in low-latitude range margins. *Oceologia* **160**: 515–524.

Nislow, K. H. and Armstrong, J. D. 2012. Towards a life-history-based management framework for the effects of flow on juvenile salmonids in streams and rivers. *Fisheries Management and Ecology* **19**: 451–463.

Northcote, T. G. 1997. Potamodry in Salmonidae – living and moving in the fast lane. *North American Journal of Fisheries Management* **17**: 1029–1045.

Olley, R., Young, R. G., Closs, G. P., Kristensen, E. A., Bickle, T. O., Deans, N. A., Davey, L. N. and Eggins, S. M. 2011. Recruitment sources of brown trout identified by otolith trace element signatures. *New Zealand Journal of Marine and Freshwater Research* **45**: 395–411.

Olsson, I. C., Greenberg, L. A., Bergman, E. and Wysujack, K. 2006. Environmentally induced migration: the importance of food. *Ecology Letters* **9:** 645–651.

O'Neal, S. L. and Stanford, J. A. 2011. Partial migration in a robust brown trout population of a Patagonian River. *Transactions of the American Fisheries Society* **140**: 623–635.

Otago Regional Council. 2008. Management flows for aquatic ecosystems in the Lindis River. Technical report prepared by staff at Otago Regional Council, Dunedin, New Zealand.

Otago Regional Council. 2014. Lindis catchment water resource study River flows, water use and flow statistics for the Lindis catchment October 2012 to April 2014. Technical report prepared by staff at Otago Regional Council, Dunedin, New Zealand.

Otago Regional Council. 2017. Measured and naturalised low-flow statistics calculated by Otago Regional Council for the Lindis River hydrological sites (Jul-Jun, inclusive) based on flow records from the 25 September 1976 to 21 March 2017 File Note Document Id: A995461.

Outridge, P. M., Chenery, S. R., Babaluk, J. A. and Reist, J. D. 2002. Analysis of geological Sr isotope markers in fish otoliths with subannual resolution using laser ablation-multicollector-ICP-mass spectrometry. *Environmental Geology* **42**: 891–899.

Pain, S. and McConchie, J. 2015. Hydrological analysis to support an economic assessment of the potential impact of a minimum flow regime for the Lindis River. Technical report prepared for Otago Regional Council by Opus International Consultants Limited.

Pascual, M. A. and Ciancio, J. E. 2007. Introduced anadromous salmonids in Patagonia: risks, uses, and a conservation paradox. *In:* Bert TM (Editor) Ecological and genetic implications of aquaculture activities. Springer, Netherlands.

Phelps, Q. E., Whiteledge, G. W., Tripp, S. J., Smith, K. T. and Garvey, J. E. 2012 Identifying river of origin for age-0 *Scaphirhynchus* sturgeons in the Missouri and Mississippi rivers using fin ray microchemistry. *Canadian Journal of Fisheries and Aquatic Sciences* **69**: 930–941.

Phillis, C. C., Sturrock, A. M., Johnson, R. C. and Weber, P. K. 2018. Endangered winter-run chinook salmon rely on diverse rearing habitats in a highly altered landscape. *Biological Conservation* **217**: 358–362.

Pin, C. and Bassin, C. 1992. Evaluation of a strontium-specific extraction chromatographic method for isotopic analysis in geological materials. *Analytica Chimica Acta* **269**: 249–255.

Polis, G. A., Anderson, W. B. and Holt, R. D. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Review of Ecology and Systematics* **28**: 289–316.

Polis, G. A. and Hurd, S. D. 1996. Linking marine and terrestrial food webs: allochthonous input from the ocean supports high secondary productivity on small islands and coastal land communities. *American Naturalist* **147**: 396–423.

Potts, W. and Rudy, P. 1969. Water balance in the eggs of the Atlantic salmon (*Salmo salar*). *Journal of Experimental Biology* **50**: 223–237.

Press, G. R., Hilborn, R., Kloehn, K. and Quinn, T. P. 2012. The influence of population dynamics and environmental conditions on pink salmon (*Oncorhynchus gorbuscha*) recolonization after barrier removal in the Fraser River, British Columbia, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **69**: 970–982.

Quinn, T. P. 2005. The behaviour and ecology of Pacific salmon and trout. University of Washington Press, Seattle, Washington, USA.

Quinn, T., Kinnison, M. and Unwin, M. 2001. Evolution of chinook salmon (*Oncorhynchus tshawytscha*) populations in New Zealand: Patterns, rate, and process. *Genetica* **112/113**: 493–513.

Quinn, T. and Unwin, M. 1993. Variation in life history patterns among New Zealand chinook salmon (*Oncorhynchus tshawytscha*) populations. *Canadian Journal of Fisheries and Aquatic Sciences* **50**: 1414–1421.

Quiñones, R. M., Grantham, T. E., Harvey, B. N., Keirnan, J. D., Klasson, M., Wintzer, A. P. and Moyle, P. B. 2015. Dam removal and anadromous salmonid (*Oncorhynchus* spp.) conservation in California. *Reviews in Fish Biology and Fisheries* **25**: 195–215.

Quiñones, R. M., Johnsson, M. L. and Moyle, P. B. 2013. Hatchery practices may result in replacement of wild salmonids: adult trends in the Klamath basin, California. *Environmental Biology of Fishes* **97**: 233–246.

R Development Core Team 2014. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. ISBN 3-900051-07-0, ULR www.R-project.org

Radinger, J. and Wolter, C. 2014. Patterns and predictors of fish dispersal in rivers. *Fish and Fisheries* **15**: 456–473.

Ricker, W. E. 1972. Hereditary and environmental factors affecting certain salmonid populations. *In:* R.C. Simon and P.A. Larkin (eds.) 1972. The stock concept in Pacific salmon. H. R. MacMillan Lectures in fisheries, University of British Columbia Press, Vancouver, BC, pp. 19–160.

Rieman, B. E., Myers, D. L. and Nielsen, R. L. 1994. Use of otolith microchemistry to discriminate *Oncorhynchus nerka* of resident and anadromous origin. *Canadian Journal of Fisheries and Aquatic Sciences* **51**: 68–77.

Rieman, B. E. and Dunham, J. B. 2000. Metapopulations and salmonids: a synthesis of life history patterns and empirical observations. *Ecology of Freshwater Fish* **9**: 51–64.

Riva-Rossi, C. M., Pascual, M. A., Aedo Marchant, E., Basso, N., Ciancio, J. E., Mezga, B., Fernandez, D. A. and Ernst-Elizalde, B. 2012. The invasion of Patagonia by chinook salmon (*Oncorhynchus tshawytscha*): inferences from mitochondrial DNA patterns. *Genetica* **140**: 439–453.

Rustadbakken, A., L'Abee-Lund, J. H., Arnekleiv, J. V. and Kraabøl, M. 2004. Reproductive migration of brown trout in a small Norwegian river studied by telemetry. *Journal of Fish Biology* **64**: 2–15.

Ruttenberg, B. I., Hamilton, S. L., Hickford, M. J. H., Paradis, G. L., Sheehy, M. S., Standish, J. D., Ben-Tzvi, O. and Warner, R. R. 2005. Elevated levels of trace elements in cores of otoliths and their potential for use as natural tags. *Marine Ecology Progress Series* **297**: 273–281.

Sawada, H., Shigeta, K., Kawakami, M., Yuma, M. and Maruyama, A. 2019. Isotope analysis reveals proportional change and site-selection variation of river-

and lake-produced eggs of a landlocked migratory fish. Journal of Fish Biology **96**: 168-174.

Schmutz, S., Bakken, T. H., Friedrich, T., Greimel, F., Harby, A., Jungwirth, M., Melcher, A., Unfer, G. and Zeiringer, B. 2015. Response of fish communities to hydrological and morphological alterations in hydropeaking rivers of Austria. *River Research and Applications* **31**: 919–930.

Schtickzelle, N. and Quinn, T. P. 2007. A metapopulation perspective for salmon and other anadromous fish. *Fish and Fisheries* **8**: 297–314.

Secor, D. H. 2010. Is otolith science transformative? New views on fish migration. *Environmental Biology of Fishes* **89**: 209–220.

Secor, D. H. 2015. Migration ecology of marine fishes. Johns Hopkins University Press, Baltimore.

Seddon, P. J., Armstrong D. P. and Maloney R. F. 2007. Developing the science of reintroduction biology. *Conservation Biology* **21**: 303–312.

Shaw, B., Buckley, H., Summerhayes, G., Anson, D., Garling, S., Valentin, F., Mandui, H., Stirling, C. and Reid, M. 2010. Migration and mobility at the Late Lapita site of Reber–Rakival (SAC), Watom Island using isotope and trace element analysis: a new insight into Lapita interaction in the Bismarck Archipelago. *Journal of Archaeological Science* **37**: 605–613.

Smedbol, R. K., McPherson, A., Hansen, M. M. and Kenchington, E. 2002. Myths and moderation in marine 'metapopulations'? *Fish and Fisheries* **3**: 20–35.

Soto, D., Jara, F. and Moreno, C. A. 2001. Escaped salmon in the Chiloe and Aysén inner seas, southern Chile: facing ecological and social conflicts. *Ecological Applications* **11**: 1750–1762.

Soto, D., Arismendi, I., Gonzalez, J., Guzman, E., Sanzana, J., Jara, F., Jara C. and Lara, A. 2006. Southern Chile, trout and salmon country: conditions for invasion success and challenges for biodiversity conservation. *Revista Chilena de Historia Natural* **79**: 97–117.

Soto, D, Arismendi, I., Di Prinzio, C. and Jara, F. 2007. Establishment of chinook salmon (*Oncorhynchus tshawytscha*) in Pacific basins of southern South America and its potential ecosystem implications. *Revista Chilena de Historia Natural* **80**: 81–98.

Stanley, E. H., Fisher, S. G. and Grimm, N. B. 1997. Ecosystem expansion and contraction in streams. *Bio Science* **47**: 427–435.

Stead, S. M. and Laird, L. 2002. Handbook of salmon farming. Chichester: Praxis Publishing Ltd.

Stevenson, H. 2010. Salmon scale analysis from the Glenavy Hotel competition. Technical report prepared for Central South Island Fish and Game Council, New Zealand. 6p.

Stockhoff B. A. 1991. Starvation resistance of gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: *Lymantriidea*): trade-offs among growth, body size, and survival. *Oecologia* **88**: 422–429.

Trotter, M. J. 2016. Juvenile trout survival and movement during the summer low flow abstraction period in the Lindis River, Central Otago. A thesis submitted for the degree of Master of Science, University of Otago, NZ

Thorrold, S. R., Jones, C. M., Campana, S. E., McLaren, J. W. and Lam, J. W. H. 1998. Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). *Limnology and Oceanography* **43**: 1826–1835.

Thresher, R. E. 1999. Elemental composition of otoliths as a stock delineator in fishes. *Fisheries Research* **43**: 165–204.

Unwin, M. 2009. Angler usage of lake and river fisheries managed by Fish & Game New Zealand: results from the 2007/08 National Angling Survey. NIWA Client Report: CHC2009-046. Prepared for Fish & Game New Zealand. 110 p.

Unwin, M. 2016. Angler usage of New Zealand lake and river fisheries. Results from the 2014/15 National Angling Survey. NIWA Client Report 2016021CH. Prepared for Fish & Game New Zealand. 143 p.

Unwin, M. and Gabrielsson, R. 2018. Review of historical hatchery releases of chinook salmon in New Zealand. Prepared for Contact Energy Limited. Cawthron Report No. 3111. 39 p.

Unwin, M. and Glova, G. 1997. Changes in life history parameters in a natural spawning population of chinook salmon (*Oncorhynchus tshawytscha*) associated with releases of hatchery-reared fish. *Canadian Journal of Fisheries and Aquatic Sciences* **54**: 1235–1245.

Unwin, M. J., Quinn, T. P., Kinnison, M. T. and Boustead, N. C. 2000. Divergence in juvenile growth and life history in two recently colonized and partially isolated chinook salmon populations. *Journal of Fish Biology* **57**: 943–960.

Unwin, M. J., Webb, M., Barker, R. J. and Link, W. A. 2005. Quantifying production of salmon fry in an unscreened irrigation system: A case study on the Rangitata River, New Zealand. *North American Journal of Fisheries Management* **25**: 619–634.

Veinott, G., Westley, P. A. H., Warner, L. and Purchase, C. F. 2012. Assigning origins in a potentially mixed-stock recreational sea trout (*Salmo trutta*) fishery. *Ecology of Freshwater Fish* **21**: 541–551.

Veinott, G., Westley, P. A. H., Purchase, C. F. and Warner, L. 2014. Experimental evidence simultaneously confirms and contests assumptions implicit to otolith microchemistry research. *Canadian Journal of Fisheries and Aquatic Sciences* **71**: 356–365.

Venables, W. N. and Ripley, B. D. 2002. Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0.

Verburg, P., Hamill, K., Unwin, M. and Abell, J. 2010. Lake water quality in New Zealand 2010: Status and trends. Report prepared for Ministry for the Environment by National Institute of Water & Atmospheric Research Ltd, Christchurch.

Volk, E. C., Blakley, A., Schroder, S. L. and Kuehner, S. M. 2000. Otolith microchemistry reflects migratory characteristics of Pacific salmonids: using otolith core chemistry to distinguish maternal associations with sea and freshwaters. *Fisheries Research* **46**: 251–266.

Waite, E. M., Closs, G. P., Kim, J. P., Barry, B., Markwitz, A. and Fitzpatrick, R. 2008. The strontium content of roe collected from spawning brown trout *Salmo trutta* L. reflects recent otolith microchemistry. *Journal of Fish Biology* **72**: 1847–1854.

Walther, B. D. and Thorrold, S. R. 2008. Continental-scale variation in otolith geochemistry of juvenile American shad. *Canadian Journal of Fisheries and Aquatic Science*s **65**: 2623–2635.

Walther, B. D., Thorrold, S. R. and Olney, J. E. 2008. Geochemical signatures in otoliths record natal origins of American shad. *Transactions of the American Fisheries Society* **137**: 57–59.

Walther, B. D. and Limburg, K. E. 2012. The use of otolith chemistry to characterize diadromous migrations. *Journal of Fish Biology* **81**: 796–825.

Waples, R. S., Beechie, T. and Pess, G. R. 2009. Evolutionary history, habitat disturbance regimes, and anthropogenic changes: What do these mean for resilience of Pacific salmon populations? *Ecology and Society* **14**: 1–18. Available online at: http://www.ecologyandsociety.org/vol14/iss1/art3/

Webster, M. S., Marra, P. P., Haig, S. M., Bensch, S. and Holmes, R. T. 2002. Links between worlds: unravelling migratory connectivity. *Trends in Ecology and Evolution* **17**: 76–83.

Willmes, M., Hobbs, J. A., Sturrock, A. M., Bess, Z., Lewis, L. S., Glessner, J. J. G., Johnson, R. C., Kurth, R. and Kindopp, J. 2018. Fishery collapse, recovery, and the cryptic decline of wild salmon on a major California river. *Canadian Journal of Fisheries and Aquatic Sciences* **75**: 1836–1848.

Wipfli, M. S. 1997. Terrestrial invertebrates as salmonid prey and nitrogen sources in streams: contrasting old-growth and young-growth riparian forests in southeastern Alaska, USA. *Canadian Journal of Fisheries and Aquatic Sciences* **54**: 1259–1269.

Wipfli, M. S. 2009. Food supplies of stream-dwelling salmonids. *American Fisheries Society Symposium* **70**: 39–52.

Wipfli, M. S., Hudson, J. P., Caouette, J. P. and Chaloner, D. T. 2003. Marine Subsidies in Freshwater Ecosystems: Salmon Carcasses Increase the Growth Rates of Stream-Resident Salmonids. *Transactions of the American Fisheries Society* **132**: 371–381.

Woodhead, J., Swearer, S., Hergta, J. and Maasa, R. 2005. In situ Sr-isotope analysis of carbonates by LA-MC-ICP-MS: interference corrections, high spatial resolution and an example from otolith studies. *Journal of Analytical Atomic Spectrometry* **20**: 22–27.

Young, P. S., Cech, J. J. and Thompson, L. C. 2011. Hydropower-related pulsed-flow impacts on stream fishes: a brief review, conceptual model, knowledge gaps, and research needs. *Reviews in Fish Biology and Fisheries* **21**: 713–731.

Young, R., Smart, G. and Harding, J. 2004. Impacts of hydro-dams, irrigation schemes and river control works. *In*: Harding, J. S.; Mosely, M. P.; Pearson, C. P.; and Sorrell, B. K. (Editors) 2004. Freshwaters of New Zealand. New Zealand Hydrological Society Inc. and New Zealand Limnological Society Inc., Christchurch, New Zealand. 764 pp. ISBN 0-476-0078-9.

Zimmerman, C. E. 2005. Relationship of otolith strontium-to-calcium ratios and salinity: experimental validation for juvenile salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* **62**: 88–97.

Zimmerman, C. E. and Reeves, G. H. 2000. Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. *Canadian Journal of Fisheries and Aquatic Sciences* **57**: 2152–2162.

Zimmerman, C. E. and Reeves, G. H. 2002. Identification of steelhead and resident rainbow trout progeny in the Deschutes River, Oregon, revealed with otolith microchemistry. *Transactions of the American Fisheries Society* **131**: 986–993.