## ELEMENTAL SIGNATURES IN BONE TO DETERMINE LIFE HISTORY

CHARACTERISTICS IN FISH

by

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## Abstract

We<sup>1</sup> used Laser-Ablation-Inductively-Coupled-Plasma-Mass-Spectrometry (LA-ICP-MS) to determine if trace metals deposited in calcified structures could be used to infer the life histories of three different species of fish. We were successful in resolving the movements and age at maturity for an anadromous species, the eulachon (*Thaleicthys pacificus*) using both Strontium (Sr):Calcium (Ca) and Barium (Ba):Calcium ratios deposited in otoliths. Stream of residence was identified for a non-migratory freshwater species, the slimy sculpin (*Cottus cognatus*) in the Williston Reservoir by matching the chemical fingerprint measured in the otolith to the water chemistries where they were captured. We could not reveal, however, movement of bull trout (*Salvelinus confluentus*) in the Morice River watershed. Water chemistry was similar throughout the length of the Morice River and movements within the mainstem of this river, therefore could not be distinguished. We conclude that chemical ratios measured in calcified structures are useful for quantifying life-history in fish providing that sufficient differences exist in the fishes ambient chemical environment.

<sup>&</sup>lt;sup>1</sup> Clarke, A.D., Shrimpton, J.M., and Telmer, K. are all contributing authors for: Chapter 2 Life History and Patterns of Movement Between Fresh- and Seawater in Eulachon (*Thaleicthys pacificus*); Chapter 3 Discrimination of Habitat use by Slimy Sculpins (*Cottus cognatus*) in Tributaries of the Williston Reservoir using Natural Elemental Signatures; Chapter 4 Movement Patterns of Bull Trout (*Salvelinus confluentus*) in the Morice Watershed using Chemical Signatures Deposited Spatially in Fin Rays.

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## **Chapter 1: Prologue**

Tracking fish through multiple life-history stages with conventional tagging techniques has contributed useful information to identify the timing and duration of habitat utilization. Additionally, stocks are usually defined by geographic separation and tagging information has been useful to discriminate between stocks. It has been determined, however, that there is an inherent bias in many tagging techniques as tagging programs often end up concentrating on the re-captured, non-mobile portion of the population (migrating fish often leave the study area), or on the members that are physically large enough to receive tags (Gowan et al. 1994). Moreover, some species that are susceptible to injury from handling may experience higher mortality rates resulting directly from the application of physical tags. Partly, for these reasons, the amount of dispersal and timing of migration for various species and different age classes is poorly understood. Other techniques have to be developed to determine habitat use and movement of small fish species and of individual fish throughout their entire life cycle.

One method that has shown promise in providing habitat-utilization data for fish is elemental analysis of bony structures. Bony structures have been used to assess movement of anadromous fish between freshwater and seawater due to the large differences in the chemical composition between these two media (Coutant and Chen 1993; Friedland et al. 1998; Limburg 1998; Veinott et al. 1999). Recently, distinct chemical signatures have been found to vary in bony structures of saltwater fish as a

result of variable ion content (inshore/offshore), temperature, and sources of food (Kalish 1991; Elsdon and Gillanders 2002); therefore, seasonal cycles in elemental concentration are often apparent when measured in the structure.

Distinctive chemical signatures have also been found among freshwater watersheds. Specific elemental and isotopic stream signatures are dependent on the underlying bedrock geology. Kennedy et al. (2000) were able to discriminate location of origin for Atlantic salmon (Salmo salar) among several streams in Vermont using stable isotopes of Strontium (Sr). Stable isotopes vary with composition and age of bedrock and Kennedy et al. (2000) argue that isotopic ratios are better suited for determining fish movement with geochemistry than elemental concentrations. These authors indicate that the elemental composition of stream waters may vary too much over time and tend to have low spatial variability, limiting the use of elemental fingerprints in resolving fish movement. This finding is contradicted by Wells et al. (2003), who determined that stream chemistries were stable seasonally and over a two-year duration. Wells et al. (2003) were able to use elemental signatures to determine origin for Westslope cutthroat trout (Oncorhynchus clarki lewisi) from three populations within the Couer d'Alene river system. Cutthroat trout could be differentiated using the combination of barium (Ba):calcium (Ca), Sr:Ca, and magnesium (Mg):Ca ratios. Elemental signatures, therefore, may provide an alternative to the examination of isotopic stream signatures as a whole range of elements can be examined (Wells et al. 2003). Furthermore, the cost of analyzing multiple elements, as opposed to different isotopes of specific elements, is

much lower. The lower cost associated with analysis should allow researchers the opportunity to increase the number of samples examined in a particular study.

Three structures that show promise in tracking the environmental life-histories of fish are otoliths, pectoral fin-rays, and scales. Otoliths are located within the semicircular canals of the inner ear in fish. There are three pairs of otoliths, the sagittae, lapilli, and asterisci; the sagittae is the largest. The main functions of the otolith complex in fish are equilibrium and sound detection (Moyle and Cech 2004). Growth of fish otoliths is a continual process (Campana and Thorrold 2001), resulting from successive deposits of CaCO<sub>3</sub> with residual proteins (Campana and Nielson 1985). Campana and Nielson (1985) suggest that otoliths are metabolically inert even under extreme stress. Further, otoliths are unique when compared to other bony structures found in fish as daily increments are often visible. For this reason spatial resolution using a probe-based technique for chemical analysis is very good due to the chronological properties of the otolith (Pannella 1971; Campana and Nielson 1985; Campana and Thorrold 2001). A major disadvantage of using otoliths for chemical analysis is the fact that the animal must be sacrificed.

Fin-rays offer a non-lethal alternative for chemical investigations examining lifehistory in fish. These structures support fish fins, but do not necessarily grow continuously like otoliths. Fin-rays grow incrementally and show alternating opaque and translucent zones that correspond to winter and summer growth respectively. There is the potential that material may reabsorb and be mobilized in bone after deposition; a

process that does not occur in otoliths, and may subsequently limit the utility of investigations using fin-rays to trace environmental life histories (Campana and Nielson 1985; Veinott and Evans 1999). Nevertheless, fin-rays are potentially still useful in rebuilding environmental life histories, as Sr, Mg, lead (Pb), bromine (Br), zinc (Zn), Ba, tin (Sn), manganese (Mn), and sodium (Na) have been shown to remain stable in fin-rays of white sturgeon (*Acipenser transmontanus*) over time (Veinott and Evans 1999).

Elemental analysis on scales also shows promise for rebuilding the environmental life histories of fish using natural chemical markers. For instance, measured Sr values in scales reflect proportional incorporation from the aquatic environment (Eek and Bohlin 1997; Wells et al. 2000b; Wells et al. 2003). Scales offer a similar advantage to fin-rays, as fish do not need to be sacrificed in order to examine life-history. These structures have also been used routinely to age fish in the past and are adequate to resolve a chemical time series. Farrell et al. (2000) successfully resolved the spatial heterogeneity of trace metals in Arctic grayling (Thymallus arcticus) scales. One problem with scales is that controversy still surrounds the potential for resorption of apatite (Bilton and Robbins 1971); however, other studies have determined that trace elements remain metabolically inert in scales and can be used to rebuild environmental life histories (Yamada and Mulligan 1982; Well et al. 2000b). Thus, otoliths are likely the best choice for an examination of life-history using natural chemical tags; however, when life-history information is required on fish species that cannot be lethally sampled, both fin-rays and scales may offer non-lethal alternatives.

Physiological regulation and transport of ions differs among otoliths, fin-rays, and scales; therefore, the mechanisms of incorporation are outlined below. The processes involved are difficult to track in some instances, as fish incorporate elements from both the water in which they live and the nutrients they acquire. Changes in the elemental composition across bony structures generally reflect ambient water chemistry; however, some trace elemental and isotopic variations are due to the physiology of the animal during a specific time period (Kalish 1990; Kennedy et al. 2000). Most ion exchange occurs from direct contact with water across the gills; however, some occurs across the intestine, particularly in saltwater fish where there is continual intake of water to compensate for water losses (Fenwick 1989). The incorporation of ions into otoliths differs from fin-rays and scales and follows from two distinctive pathways. Otoliths are immersed in the endolymph fluid where ion concentrations show less variation than in the blood (Campana et al. 2000). Ions crystallize directly onto the otolith from the endolymph fluid (Campana and Nielson 1985), while fin-rays and scales incorporate ions directly into the crystal lattice from the blood (Veinott et al. 1999).

One major assumption of using elemental signatures to infer life-history is that trace element deposition onto the bony structure is mainly a result of branchial uptake directly from the water (Fenwick 1989) and not a result of elemental deposition due to diet. Further to this, the researcher must be certain that the chemical composition of the aquatic environment is influencing the deposition onto the structure, rather than the physiology of the animal. To date, the following studies have determined that non-

essential trace elements are related proportionately to water chemistry and not diet: Sr:Ca ratios in juvenile black bream (*Acanthopagrus butcheri*) (Elsdon and Gillanders 2002;Elsdon and Gillanders 2003); Sr:Ca and Ba:Ca in Westslope cutthroat trout (*Oncorhynchus clarki lewisii*) (Wells et al. 2003); and Sr:Ca, Ba:Ca, and Cd:Ca in juvenile spot (*Leiostomus xanthurus*) (Wells et al. 2000a; Bath et al. 2000). Thus, it appears that trace metal deposition in the otolith must be proportional to the dissolved elements in the ambient aquatic environment in order to link habitat use, and therefore, rebuild fish environmental life histories. The use of elemental signatures shows promise for determining temporal locations of fish, both in seawater and freshwater, from the elemental profile in bony structures.

The ability to analyze the elemental composition of a sample spatially is key to this technique, as it provides an indication of which habitats were utilized over the life of the animal. A method that gives good resolution for determining elemental ratios over different life-history stages is Laser-Ablation-Inductively-Coupled-Plasma-Mass-Spectrometry (LA-ICP-MS). LA-ICP-MS is a micro-analytical tool that enables a spatial analysis of element distribution in a sample (Wang et al. 1994). This technique combines the beam capabilities of a high-energy laser with the analytical capabilities of ICP-MS (Denoyer et al. 1991). The laser is directed at a small area on the sample where photon energy is converted to thermal energy and a small portion of the structure is vaporized to a depth of a few micrometers (Fowler et al. 1995). The vaporized material is then carried by a flow of argon gas into the plasma of an ICP and the elemental and isotopic

composition is determined by the mass spectrometer (Campana et al. 1997). LA-ICP-MS can discriminate between spatial regions on bony structures that are as small as 30  $\mu$ m. Resolution of trace elements can be identified as low in concentrations as 0.1 to  $0.01 \mu g/g$ in multi-element analyses (Wang et al. 1994). There have been some problems associated with this technique in the past. These problems, however, appear to be linked to specific laboratories performing the analytical procedures. A comparison of techniques was made between numerous laboratories using a standardized sample measured with the electron microprobe, X-Ray diffraction, and LA-ICP-MS. Both the electron microprobe and LA-ICP-MS were determined to be valid techniques in reconstructing environmental life histories (Campana et al. 1997). Furthermore, LA-ICP-MS has been reliable for determining the environmental life histories of white sturgeon (Veinott et al. 1999), Atlantic cod (Gadus morhua) (Campana et al. 1994), striped bass (Morone saxatilis) (Coutant and Chen 1993), and several species of salmonids (Kalish 1990; Thorrold and Shuttleworth 2000). Recently, Sanborn and Telmer (2003) have greatly increased the spatial resolution of this technique, while substantially reducing the cost by using continuous line scans with LA-ICP-MS. The authors determined that line scans are at a minimum equivalent to discrete spot analysis and likely provide better information about the distribution of elements in heterogeneous solids.

The above methods use a quantitative examination of metal deposition in bone. An alternate approach for examination of elemental deposition in bony structures is Cathodoluminescence (CL) microscopy. CL microscopy can potentially be used to

complement otolith chemistry investigations in a qualitative manner. CL is very sensitive to the presence of low levels of trace elements that provide a unique visualization of colour changes in the otolith which is attributed to variations in chemical zonation. CL emissions, therefore, are a useful method for understanding the chemical variation experienced by fish (Halden 2001). The variation in luminescence likely results from changes in the chemical environment or physiology experienced by the fish. Typically, Mn causes most of the luminescence associated with carbonates (Marshall 1988); but Sr may have contributed to the luminescence found in whitefish otoliths (Halden 2001). To date, there has been very little research done using this technique on otoliths to determine if it would be useful for rebuilding the environmental life histories of various fish species.

This project utilized discrete chemical signatures deposited spatially in bony structures of three different species of fish over the duration of their life-history. Elemental analysis was used to match specific signatures to the ambient water chemistry in order to examine life histories of an anadromous species, a freshwater non-migratory species, and a freshwater migratory species.

In the first part of this study (Chapter 2), large chemical differences in saltwater and freshwater were used to examine the life-history of the eulachon (*Thaleicthys pacificus*) a small osmerid species. Sr:Ca ratios have been shown to be effective in observing movements in anadromous fish between these two media. As well, age

information for eulachon is uncertain to date. Seasonal fluctuations in elemental concentration were examined in eulachon otoliths to attempt to age these animals correctly.

The second portion of this project (Chapter 3) examined the chemical heterogeneity in a freshwater watershed and determined that the different chemicalstream signatures corresponded to the otoliths of fish from each specific area. Slimy sculpins (*Cottus cognatus*) in the Williston watershed are a non-migratory fish species and were used to determine if chemical signatures are geographically distinct by linking specific water chemistries to the elemental signatures found in otoliths.

The final portion of this examination (Chapter 4), determined if distinct chemical signatures deposited spatially in fin-rays could be used to assess the movements of a migratory fish. Bull trout (*Salvelinus confluentus*) a provincially blue-listed species from the Morice watershed has been classified as 'movers' and 'non-movers' from a radio-telemetry study (Bahr 2002). Ten fish from the Morice River telemetry study were examined to see if chemical signatures could be used to assess movements within the watershed. Water chemistries were determined from tributaries of the Morice River and within the Morice River itself to determine if migrations highlighted by variations in measured elemental ratios across the fin-ray could be linked to specific regions within the watershed. This project should aid in our ability to reconstruct the movement of an

individual's entire life-history and provide important information and a potential tool which can be used towards the conservation of threatened fish species.

# Chapter 2: Life-history and Patterns of Movement Between Fresh- and Sea-water in Eulachon (*Thaleicthys pacificus*)

#### ABSTRACT

Populations of eulachon (Thaleicthys pacificus) have declined significantly in recent years and it is crucial to further our understanding of their life-history. The main objectives of this study were to determine the age at maturity and repeat spawning potential for eulachon, two aspects of eulachon life-history that are not known, but are important for successful management of this species. Trace-element analysis of bone can be used to reconstruct many life-history characteristics because elements are incorporated from the water as fish grow. We used Laser-Ablation-Inductively-Coupled-Plasma-Mass-Spectrometry (LA-ICP-MS) to reconstruct the Ba:Ca and Sr:Ca molar ratios deposited spatially into the otolith, a bone located in the inner ear. Spawning eulachon examined in this study were at least 160 mm in length and 30 g in weight (suggesting that eulachon spawn after reaching a minimum size). Age at maturation, however, differed among populations examined. Three full cycles of fluctuations in Ba:Ca molar ratios were observed in the majority of otoliths from spawning fish, indicating that eulachon mature at three years of age. Based on the seasonal fluctuations in Ba:Ca molar ratios, we determined that most Columbia River eulachon spawn after two years, while Fraser, Kemano, and Skeena River eulachon generally mature after three years. Two Skeena River eulachon matured after four years. In contrast to the Ba:Ca molar ratios in the otolith, Sr:Ca molar ratios maintained a relatively flat profile over the life of the eulachon. The lack of a change in Sr:Ca ratios within the otolith, the single size class of spawners

across all river systems, and the single age class within most rivers strongly suggest that eulachon from the populations in our study are semelparous. Thus, examination of otolith microstructure was successful in identifying two important life-history characteristics, age at maturity and repeat spawning potential, furthering our understanding of the eulachon.

## INTRODUCTION

The eulachon (*Thaleicthys pacificus*) is a small anadromous smelt that spawns from northern California to the southern Bering Sea. Despite the wide geographic range, there is much unknown regarding the general biology and life-history of this species. There is a pressing need to acquire more information regarding this species as populations of eulachon have shown a dramatic decline in numbers of spawners over much of their geographic range in the last 20 years. This trend has become particularly apparent since the mid-1990s and reasons for the decline are unknown. It is likely, however, that many factors are responsible for this trend. Information on the life-history of eulachon is needed to determine what mechanisms are responsible for the observed declines.

The eulachon exhibits an anadromous life-history (McAllister 1963) and has been observed to spawn in many of British Columbia's rivers during March and April (Barraclough 1964). Other observations have shown that eulachon enter the Skeena and Nass Rivers as early as February and some Alaskan rivers as late as May (Hay and McCarter 2000). The spawning habitats of the eulachon range from Northern California in streams of the Klamath River, to the Nushagak River in Alaska (McAllister 1963). Eulachon are found in 33 rivers in British Columbia; of these, only 14 have regular runs (Hay and McCarter 2000). Barraclough (1964) determined that eulachon begin maturing at age two and spawn at three years of age.

Eulachon spawn in rivers that have spring freshets characterized by headwaters with large snow packs or glaciers (Hay and McCarter 2000). Spawning occurs a short distance upriver in areas that are covered by coarse sand (Hart and McHugh 1944). In the Nass River, eulachon migrate a maximum of 24-32 km upstream and spawn after the ice breaks up, when water temperatures are between 4.4-7.8 °C (Scott and Crossman 1973). The Bella Coola River has eulachon spawning in the first 6 km (Pootlace and Siwallace 2000) and in the Kowesis River, eulachon have been found 5 km upstream (Kelson 2000). Eulachon generally spawn at night (Kelson 2000) and remain in some river systems for approximately three weeks (Pootlace and Siwallace 2000). Females produce between 17,000 and 40,000 eggs, each egg having an adhesive outer membrane that sticks to the coarse sand particles (Hart and McHugh 1944). The fertilized eggs take approximately two to three weeks to hatch depending on water temperature (Shepherd and Vroom 1977). It took 41 days for eulachon to hatch at water temperatures between 4-7 °C in the Kitimat River (Farara 2000). After the eggs hatch, the newly emerged larvae are carried down to the ocean by the current (Hart and McHugh 1944). As a consequence, eulachon spend very little time in the freshwater environment. The freshwater residence of larval eulachon, however, may be a critical stage for development before the fish enter the ocean and also after they return to spawn and complete their life-cycle.

The marine life-history of eulachon begins when the larvae that have been swept downstream by the current enter the ocean. Larval stages may remain in estuarine and

marine waters close to natal rivers for several months or longer. This leaves very little time for eulachon to imprint to the freshwater environment, and it is possible that imprinting does not occur at all (McLean et al. 1999). Hay and McCarter (2000) indicate that any imprinting is likely to occur in the estuarine environment adjacent to spawning rivers. McLean et al. (1999) found that population subdivision was more likely to occur as a result of the marine environment, rather than factors that affect other anadromous species (e.g. a distinct chemical cue present in natal stream), because there was an apparent high level of gene flow. Other anadromous species, such as salmon, imprint to chemical signatures from a specific stream or river and generally show high fidelity when returning to natal rivers to spawn.

Population structure is weakly developed in eulachon with < 2% total genetic difference found among populations from the Columbia River, Cowlitz River, Fraser River, Franklin and Klinaklina Rivers in Knight Inlet, Kowesas, Kitimat, and Kemano Rivers from Gardner Canal, Nass River, Cook Inlet, and the Bering Sea (McLean et al. 1999). Additional evidence that extensive mixing of stocks may occur arises from observations of abundance. There have been reports that eulachon will repeat-spawn in a river system over subsequent years, disappearing from the system and returning years later (Stacey 1998 cited in Hay and McCarter 2000).

One aspect of eulachon life-history that is poorly understood is age at maturity. Commonly, ages of fish are determined from the spatial deposition of rings on bony

structures such as otoliths. Estimates of age at maturity for eulachon are between four and six years (Delacey and Batts 1963), but there is considerable controversy. Hay and McCarter (2000) suggest that age estimates from otoliths may be unreliable from eulachon. This conclusion is based on the high variation in age analysis using otoliths in previous studies. Ricker et al. (1954) compared scales and otoliths from eulachon and showed that age estimates from otoliths were one to two years higher than from scales. Hay and McCarter (2000) further note that DeLacy and Batts (1963) obtained much higher ages for eulachon using otoliths than other studies. An interesting observation put forward by Hay and McCarter (2000) is that length and weight do not vary between eulachon aged between two and six years; a finding that indicates the determined ages may not be accurate. This observation may not be warranted for all eulachon populations, as repeat spawning potential is still undetermined. Fish often return to normal feeding after spawning, but growth is restricted (McDowall 1987); furthermore, older members of a population typically grow slower than younger members. Confirmation of age for eulachon may provide insight to the variability in age at spawning, with younger members of the population potentially returning to repeat spawn at a later date. Hay and McCarter (2000) felt that using otoliths for ageing will not be reliable unless further studies verify otolith age determination with other means of analyses.

There are many methods that have been used to verify age estimates, or at least to correlate annuli deposition. Campana (2001) outlines the use and misuse of these

many techniques. The techniques for validating age estimations outlined include: release of known age and marked fish, mark-recapture, radio-chemical dating, marginal increment analysis, and captive rearing. For the most part, these techniques are not suitable for use in validating eulachon ages, as eulachon cannot be effectively marked and recaptured, they are potentially short lived, and captive rearing would require development of rearing techniques and elaborate holding facilities. One method that may be appropriate for age validation in eulachon is observation of elemental signals. This procedure may be viewed as only corroborating the frequency of annuli formation, as elemental signatures may not fluctuate regularly or annually (Campana 2001). One way around this is to utilize chemical signatures that are shown to have regular seasonal variation that is represented by fish migration or intra-seasonal variation due to temperature effects.

Chemical signatures of otoliths from salmon have shown periodicity as fish migrate between different regions of the ocean. Distinct chemical signatures have been found to vary in bony structures of saltwater fish as a result of variable ion content (inshore/offshore), temperature, and food sources (Kalish 1991; Elsdon and Gillanders 2002; Bath-Martin et al. 2004); therefore, seasonal cycles are often apparent. Seasonal variation among elements in the marine environment has been reported for Na, Sr, K, S (Kalish 1991), Li, Mg, Sr, and Ba (Elsdon and Gillanders 2002).

Another aspect of eulachon life-history that requires further examination is the potential for eulachon to repeat the spawning cycle. Some of the available data suggest that eulachon spawn once and die (semelparous). Hay and McCarter (2000), indicate that most eulachon are semelparous because: (1) they re-absorb their teeth during spawning; (2) spawning eulachon are larger than those seen in marine waters, (3) to date, no toothless eulachon, or eulachon with regenerating teeth have been found in the sea, and (4) substantial post-spawning mortality has been documented in most BC rivers. This information is evidence that most eulachon, and particularly the largest of spawning eulachon, do not survive to return to the sea. Bailey (2000) concurs with the hypothesis that eulachon are semelparous, as beaches of the Fraser River are white with dead eulachon following spawning. It has been suggested, however, that eulachon are iteroparous (spawn more than once) in some river systems. Pootlace and Siwallace (2000) indicated that carcasses were not observed on the beaches of rivers in Dean and Burke Channel in northwestern British Columbia after spawning and suggested that eulachon return to the ocean following spawning. Additionally, Barraclough (1964) reported that eulachon were caught off the Fraser River after they had spawned once and were in good condition and he hypothesized that they may spawn a second time. There is morphological data that suggests iteroparity may increase in the more northern latitudes. Eulachon, over much of their distribution, re-absorb their teeth during spawning, whereas fish caught in the marine environment typically have large pronounced teeth (Hay and McCarter 2000). Fish caught in rivers from Alaska retain

their teeth while in freshwater and during spawning. Hay and McCarter (2000) hypothesized that these more northerly populations could be iteroparous, however much firmer conclusions are necessary.

Determination of movement patterns and multiple spawning, however, is difficult for eulachon. Length-frequency relationships have been used in some fish species to examine whether fish spawn more than once, but multiple age classes may overlap in size (McDowall 1987) and considerable controversy exists for age at maturity in eulachon (Hay and McCarter 2000). An alternate approach to determine multiple spawning is to assess movement patterns between fresh- and sea-water from incorporation of elemental signatures in bony structures. Elemental analysis of bony structures can be used to determine a variety of life-history traits in fish. Different chemical signatures exist between marine, estuarine, and freshwater environments and these different signatures are incorporated into bony structures as fish grow and move between these environments. Bony structures, therefore, provide a spatial elemental record corresponding to habitat utilization at each life stage of a specific population or individual. For this reason, elemental analysis of bony structures can be used to detect migration and movement patterns between freshwater and seawater. A number of elements have proven useful in elemental analysis, most commonly Ca, Ba, and Sr. The ratios of Ba:Ca, and Sr:Ca, vary between fresh- and sea-water and these differences are incorporated into bony structures.

In this study we investigated the potential for using chemical signatures naturally deposited in otoliths to further our understanding of eulachon life-history. The objectives of our study were to acquire eulachon otoliths from a wide geographic range including the Skeena, Kemano, Fraser, and Columbia Rivers, to investigate otoliths for seasonal fluctuations in elemental concentration and link seasonal changes in elemental concentration with annual increment formation on the otoliths to assess age, and to ascertain if eulachon are semelparous or iteroparous by examining elemental ratios deposited in eulachon otoliths.

### MATERIALS AND METHODS

Eulachon were collected from the Skeena, Kemano, Fraser, and Columbia Rivers between late January and May 2003. All samples came from First Nation's harvest fisheries donated after capture. In the Skeena and Fraser River eulachon were captured in gill-nets, purse-seined in the Kemano River, and dip-netted in the Columbia River. Twenty individuals were randomly selected from each sample to be used in the study. Only male eulachon were available from both the Skeena and Columbia Rivers. Male and female eulachon were examined for the Kemano and Fraser Rivers; however, there were more male fish available for the analysis. In addition, 10 ocean eulachon captured in July 2001 from Area 23-6 (Barkley Sound) were included in the analysis. Eulachon from Area 23-6 were captured by a commercial shrimp trawler. The ocean fish were immature and sex could not be determined. Length (L) and weight (W) were measured on all fish and condition factor (index of plumpness (K)) was calculated using the

equation  $K = W / L^3 * 100\ 000$  (Moyle and Cech 2004). Both L and W were analyzed with a one-way Anova using both Tukey's HSD post hoc test (SPSS version 11.5, Sep 2002 Chicago, IL.). All data are presented with the standard error of the mean.

Both the right and left sagittal otoliths were removed from the fish and sonicated for five minutes in ultra-pure water. Otoliths were then embedded in epoxy resin (Allied High Tech embedding medium, Rancho Domiguez, CA) and ground in the transverse plane with 1200  $\mu$ m silicon carbide paper until the core was exposed. Wet grinding using ultra-pure water prevented external contamination of the samples. Otoliths were then sequentially polished with 6  $\mu$ m, 1  $\mu$ m, and finally 0.05  $\mu$ m diamond suspension to ensure an adequate surface for ablation with the laser. During preparation and transfer of polished otoliths, some otoliths were damaged and could not be used, reducing the sample size.

LA-ICP-MS analysis was conducted following the protocol outlined in Sanborn and Telmer (2003). Material was extracted from the otolith with a PQ II S+ high sensitivity ICP-MS (VG Elemental) coupled to a UV laser ablation system (Merchantek). The laser system operated with an output of 266 nm that has a maximum energy output of 4 mJ. Optimization was conducted using Standard Reference Material (SRM) 613 NIST glass, containing  $\sim 50 \,\mu$ g/g total trace elements. All analyses were conducted at a frequency of 20 Hz with 75% power and the aperture of the laser set at one. Average energy while operating at these conditions was 0.70 mJ. We measured the width of the

laser scan after analysis with a microscope mounted micrometer and determined it to be 25-32  $\mu$ m. All otoliths scans were completed by us by tracking the laser across the otoliths at 5.3  $\mu$ m/sec. The isotopes measured in the otoliths included <sup>43</sup>Ca, <sup>86</sup>Sr, <sup>137</sup>Ba, <sup>25</sup>Mg, and <sup>66</sup>Zn. Ca was used as the internal standard, due to the otoliths aragonite (CaCO<sub>3</sub>) composition. Ca is 40% of the molecular weight of aragonite. An internal standard was required to account for variations in aerosol production caused by the variation in the amount of material being extracted from the otolith by the laser. Background intensities were collected for 30 seconds prior to running the laser.

Data collection and reduction were completed using VG Thermo Electron PlasmaLab Software 2003 (Version 1.06.007, Burlington, ON). The Fully Quantitative Analysis option was chosen and an SRM 613 NIST glass was selected as the known standard. Two SRM 613 NIST glasses were analyzed, both at the beginning and end of each run. These certified standards were used to complete an external drift correction to compensate for any changes in machine sensitivity. Five otoliths were analyzed between each set of standards. An SRM 611 NIST glass was also analyzed as an unknown sample during each run of five otoliths to help ensure measurement accuracy and precision.

#### RESULTS

Length-weight relationships (Figure 2.1) and condition factor (Figure 2.2) were plotted for the five eulachon populations examined in this study. There was a significant difference in fork length for the populations examined F (4,182) = 44.123, p < 0.05 and



Figure 2.1. Relationship between length and weight for the populations of eulachon examined. Solid symbols are males, open symbols are females. Area 23-6 fish were immature. Tukey's HSD post hoc test (subset for alpha 0.05) revealed significant differences among fish caught from different locations. Mean length of eulachon was  $151 \pm 5$  mm for Area 23-6,  $175 \pm 3$  mm for Columbia,  $183 \pm 3$  mm for Fraser,  $189 \pm 2$  mm for Skeena, and  $196 \pm 3$  for Kemano.



Figure 2.2. Condition factor (index of plumpness) as a function of length for the Columbia, Fraser, Kemano, Skeena, and Area 23-6 fish examined. Solid symbols are males, open symbols are females. Area 23-6 fish were immature.

weight F (4,182) = 48.788, p< 0.05 among the mature samples collected from the four rivers and one ocean group. Tukey's HSD post hoc test (subset for alpha 0.05) revealed that Area 23-6 eulachon were significantly smaller than the other populations by length; mean length was 152  $\pm$  3 mm. Spawning eulachon were larger than the fish caught in the ocean, although, there were significant differences among the populations. Columbia River fish were 175  $\pm$  3 mm and did not differ significantly from the Fraser River fish, 183  $\pm$  3 mm. The Skeena and Kemano River fish were significantly larger than the Columbia River fish. Skeena and Kemano eulachon were 189  $\pm$  2 mm and 196  $\pm$  3 mm, respectively. Condition factors are shown for each of the river systems (Figure 2.2). The group of four fish at the bottom left of the figure captured in Area 23-6, off the west coast of Vancouver Island, had the lowest values for condition factor.

There was an oscillation in Ba:Ca deposition in the otolith that appeared to correspond with seasonal ocean temperature. During the summer (July-September) when ocean temperature was highest, Ba:Ca deposition in the otolith was also highest (Figure 2.3 A). This was observed in Area 23-6 eulachon (collected in July), which had the highest Ba:Ca values (referred to as peaks) at the outside edge of the otolith. All of the eulachon captured on their spawning migration during the winter and spring were characterized by low values of Ba:Ca at the outside edge of the otolith. This otolith region represented the chemical environment the fish were exposed to near the time of sampling, as the 30  $\mu$ m resolution attained in this study corresponded to approximately two weeks of growth. Sea surface temperatures (Figure 2.3 B) represented oscillations in 25
ocean temperature, however these values did not necessarily correlate with measured values in the otolith. Figure 2.4 illustrates the number of Ba:Ca peaks measured in southern eulachon populations. Eulachon captured in Area 23-6 (ocean) had 1.5 and 2.5 peaks, Fraser River eulachon were all characterized by three peaks in Ba:Ca, and Columbia River eulachon exhibited two or three peaks in Ba:Ca. Figure 2.5 illustrates the number of Ba:Ca peaks measured in otoliths from northern populations of eulachon. All of the fish in the Kemano and Skeena rivers examined were characterized by three peaks in Ba:Ca peaks. The number of peaks in Ba:Ca observed in eulachon otoliths tends to increase with increasing latitude.

Cross-sectional and whole-mount views of otoliths are shown in Figures 2.6.1, 2.6.2, and 2.6.3. Adjacent to the pictures of each otolith are the elemental signatures determined for Ba:Ca, Sr:Ca, Mg:Ca, and Zn:Ca. Ba:Ca ratios correlate well with opaque zones visible after polishing many of the transversely sectioned otoliths; however, the profile for Sr:Ca remains relatively flat over the life of the animal. Both Mg:Ca and Zn:Ca ratios are higher in the core and then decline with age and growth of the fish. Ratios of Mg and Zn do not return to values observed near the core of the otolith.

Figure 2.7 illustrates some of the difficulties regarding age estimation of eulachon by counting the number of annuli that are either on cross sections or whole otoliths. We would estimate the ages of the fish to be from one to four years old based on the number



Figure 2.3 (A) Representative line scans showing Ba:Ca chemical ratios over the lifetime of individual eulachon from the Columbia River, Kemano River, Skeena River, Fraser River, and Area 23-6. Winter (W) periods are marked on the graphs and correspond to the lowest measured Ba:Ca chemical ratios. Figure 2.3 (B) Sea surface temperature data was adapted from Hay et al. 2003. Ba:Ca measured in the otoliths appears to correlate well with seasonal variations in ocean temperature. Eulachon spawning in the rivers were collected from January to late April while Area 23-6 eulachon were captured in July.



Figure 2.4 Fluctuations in Ba:Ca ratios measured in this study for southern populations of eulachon. Area 23-6 eulachon are characterized by two and three peaks of Ba:Ca. Fraser River eulachon had three peaks of Ba:Ca while the majority of Columbia River eulachon had two peaks (three fish had three peaks of Ba:Ca).



Figure 2.5. Ba:Ca profiles demonstrating seasonal variation in northern eulachon populations. All Kemano River and most of the Skeena River eulachon had three peaks of Ba:Ca, however two Skeena eulachon exhibited four peaks.

**Figure 2.6-1.** Otolith images and elemental profiles for an eulachon sampled from the Skeena River (S35). A and B are cross-section and whole mount views of otoliths showing annuli. For A the black line indicates the location and length (689  $\mu$ m) of the laser line scan. C, D, E, and F are scan lines of elemental signatures for Ba:Ca, Sr:Ca, Mg:Ca and Zn:Ca.







**Figure 2.6-2**: Otolith images and elemental profiles for an eulachon sampled from the Skeena River (S45). A and B are cross-section and whole mount views of otoliths showing annuli. For A the black line indicates the location and length (731  $\mu$ m) of the laser line scan. C, D, E, and F are scan lines of elemental signatures for Ba:Ca, Sr:Ca, Mg:Ca and Zn:Ca.







**Figure 2.6-3.** Otolith images and elemental profiles for an eulachon sampled from the Kemano River (K23). A and B are cross-section and whole mount views of otoliths showing annuli. For A the black line indicates the location and length (684  $\mu$ m) of the laser line scan C, D, E, and F are scan lines of elemental signatures for Ba:Ca, Sr:Ca, Mg:Ca and Zn:Ca.







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	Fish	Length (mm)	Weight (g)	# of summers at sea
A	K31	209	83.5	3
В	O13	179	41.4	2.5
С	O4	146	21.9	2.5
D	O8	137	17.2	1.5

Figure 2.7 Cross sections of eulachon otoliths showing pseudo-annuli (dots) that appear to represent yearly growth. Tabulated information below figure indicates origin and fish number, size and age estimate based on number of peaks in Ba:Ca ratio. White line indicates 1mm. (K) refers to the Kemano River and (O) refers to Area 23-6 eulachon.

of annuli. Our estimates of age for these fish from oscillations in Ba:Ca differs, as shown in the tabulated values below (Figure 2.7). Polishing time and the thickness of the polished section appeared to affect the number of annuli visible for many of the samples examined.

The data obtained in our study suggest that there may be a minimum size that eulachon must reach prior to the onset of maturity and spawning (Figure 2.8). Area 23-6 fish characterized by 1.5 peaks of Ba:Ca have likely not yet reached the minimum size necessary to migrate to the spawning grounds, therefore, we estimate these fish are more than one-year old. Area 23-6 fish that have 2.5 peaks of Ba:Ca are more than two years old and will likely reach the minimum size needed to mature by the following spring at three years of age. Spawning eulachon, therefore, appear to belong to a single size class.

A single age class of fish was also observed to spawn in two of the systems examined in this study; only three-year old eulachon were observed from the spawning populations in the Fraser and Kemano Rivers (Figures 2.8). The majority of fish for the Columbia and Skeena Rivers was also composed of a single age class; two and three year olds from the Columbia and Skeena Rivers, respectively. The dominance of a single age class and single size class of fish observed spawning strongly suggest that eulachon spawn once.



Figure 2.8 The relationship between number of peaks in Ba:Ca elemental ratio and fork length of eulachon from the four river systems examined and for ocean fish caught off the coast of Vancouver Island. Solid symbols are males, open symbols are females. Area 23-6 fish were immature.

#### DISCUSSION

Spawning eulachon (Area 23-6 fish are not included as they were captured in the ocean) in our study were at least 160 mm in length and greater than 30 g in weight; it appears that eulachon spawn after reaching a minimum size. Our analysis indicates that the age when fish reach this 160 mm threshold and mature varies with latitude (as shown by Columbia River fish (the most southerly in latitude) spawning at the earliest ages and the Skeena River fish (most northerly river in latitude) spawning at the oldest ages. Eulachon spawn after two or three years in the Columbia River, three years in the Fraser and Kemano populations, and three or four years in the Skeena population. The size threshold and lack of an appreciable change in elemental signatures associated with freshwater movement strongly suggest that eulachon are also semelparous. We will discuss our findings in relation to what is known regarding other species of smelts, validate our method to assess age at maturity, and assess semelparity within this species.

### Comparison of Life-history to other smelts

The results of our study indicate that eulachon have similar life-history characteristics to other smelts. Most smelts are short lived, demonstrate a mainly semelparous life-history, and utilize distinct habitats for spawning. Examination of some of these life-history patterns may allow for an increased understanding of eulachon lifehistory. Within the Family Osmeridae (or the "true" smelts), there are only 13 species (Moyle and Cech 2004). The true smelts are abundant in the coastal areas of the

northern hemisphere. Some species are entirely marine, some live entirely in freshwater or brackish-water, and some are anadromous. In addition to the eulachon, there are three species of true smelt that have been extensively studied, although knowledge gaps still exist for these species. An examination of life-history traits within these species will aid our understanding of potential life-history patterns that may exist for the eulachon. These three species are the rainbow smelt (*Osmerus mordax*), the ayu (*Plecoglossus altivelis*), and the delta smelt (*Hypomesus transpacificus*).

The rainbow smelt shows considerable variation in life-history traits. Some populations of rainbow smelt are anadromous, although other populations are lacustrine (live in lakes) (Taylor and Bentzen 1993). Anadromous rainbow smelt are generally 150 – 300 mm in length at maturity (Copeman 1977). Anadromous forms of this species enter coastal streams and deposit adhesive eggs onto shallow riffle areas. Eggs hatch in eight days at a mean water temperature of 15 °C (Cooper 1978). After the eggs hatch, the larvae are carried downstream to the estuary or the open ocean (Akielazek et al. 1985). The distribution of rainbow smelt expands from Vancouver Island to the Canadian Arctic (McPhail and Lindsey 1970). Prey of rainbow smelt consists of mysids and amphipods, however, they also demonstrate piscivory (Haldorson and Craig 1984). Ages determined for mature fish are extremely variable in rainbow smelt. Rainbow smelt have been reported to mature at two to four years of age in Lake Huron (Frie and Spangler 1985) and six to seven years (with a maximum age of 15 years) in the Beaufort Sea (Haldorson and Craig 1984). The variation in age at maturity reported may reflect

difficulties in ageing smelts, similar to what has been argued by Hay and McCarter (2000) and what we have shown in eulachon. In Lake Huron male rainbow smelt demonstrated higher natural mortality (M = 1.3) than females (M = 0.9) during spawning (Frie and Spangler 1985). This shows that females have a higher capability to spawn again, which is important as fish generally demonstrate higher fecundity as they get larger. The similarity that exists between eulachon and anadromous rainbow smelt life-history traits is considerable. Both species spawn in coastal streams, deposit adhesive eggs, and larvae are carried to the ocean immediately after hatching.

The ayu, is another osmerid smelt; however, it displays an amphidromous lifehistory. These fish move between fresh and salt water for purposes other than spawning. The ayu is common in eastern Asia and demonstrates a one-year life cycle. These smelts are similar to eulachon in that they also spawn in the lower reaches of rivers and the larvae are then carried to the ocean after hatching. Ayu generally spawn in the autumn after migrating downstream to the lower river reaches (Nishida, 1978, cited in Katano and Iguchi 1996). During their ocean residence, ayu mainly feed on zooplankton (Katano and Iguchi 1996). Throughout the spring, the young ayu migrate back into the river and forage on algae. Ayu are semelparous spawners that mature after one year regardless of body size (McDowall 1992). This species is different from the rainbow smelt that show repeat spawning and more plasticity in timing of maturation. Our data suggest that eulachon are most likely semelparous (like ayu), but show some plasticity in timing of maturation similar to rainbow smelt.

Delta smelt only occur in Suisan Bay located in the Sacremento-San Joaquin estuary (Moyle et al. 1992). Spawning takes place in fresh water from late February to May, when the water temperature is between 7-15 °C. Eggs are adhesive (similar to rainbow smelt and eulachon) (Moyle 1976) and larvae are carried to the estuary after hatching. Delta smelt mainly feed on zooplankton in the estuary, staying in the estuarine environment until the following winter when they migrate from 10-100 km upstream as maturing adults (Swanson et al. 1998). Delta smelt mature at 55-80 mm of length and most adults die after spawning, having completed a one-year life cycle (Moyle et al. 1992). Delta smelt are listed as a threatened species; habitat disturbance is likely the main factor for the decline of the delta smelt. Sacremento-San Joaquin River system is extremely disturbed and most of the anadromous and resident fish in this system have declined severely in recent years. Entrainment of water resulting from diversion appears to be the most significant cause of this decline (Moyle et al. 1992). Delta smelt lifehistory is remarkably similar to that of the eulachon. All of the above examples suggest that the life-history we have proposed for eulachon is consistent with the previously documented characteristics of related smelts.

## Ageing

The seasonal fluctuations in Ba:Ca observed in this study suggest that, to date, eulachon may have been aged incorrectly. An examination of the zonation in whole and transversely polished eulachon otoliths provides an example of the problems

encountered when interpreting age (Figure 2.6). Whole otoliths possess numerous dark bands that have been interpreted as winter growth zones in past ageing attempts. Conversely, some sectioned otoliths viewed under transmitted light reveal fewer zones. Most sectioned otoliths were difficult to interpret, suggesting that ageing by this method is also problematic. Polishing time and thickness greatly affected the readability of the structures and seemed to vary among samples. The use of seasonal variation in elemental signature, therefore, represents an attractive alternative when ageing eulachon otoliths.

Campana (2001) discussed the application of using elemental and isotopic signatures for confirming the ages of growth structures as simply corroborating the periodicity of growth increments. This speculation developed from the assumption that any changes in the deposition resulting in noticeable growth increments would, in theory, also result in changes in chemical signatures. The hypothesis appears valid; however, the problem with ageing eulachon in the past has come from identifying the specific increments in otoliths that correspond to annual zones. Eulachon otoliths possess frequent pseudo-annuli (visible increments formed by an unknown process), making ageing extremely difficult. The goal of this study, therefore, was to determine if chemical signatures could help elucidate the presence of annual increments.

Ba:Ca profiles examined in this study likely represent oscillations that correspond to seasonal variations. Capture dates were reported for the eulachon examined in the

present study. Fish captured in late July in the ocean had peaks of Ba:Ca at the outer edge of the otolith, characteristic of the maximum values measured in the otoliths. Conversely, fish captured between February and March demonstrated Ba:Ca levels in the outer edge of the otolith that represented the minimum values measured. The relationship suggests that eulachon are incorporating higher concentrations of Ba:Ca during the summer and lower concentrations of Ba:Ca during the winter. There are two possible explanations for the seasonal fluctuations observed: that the actual concentration of Ba:Ca fluctuates on a seasonal basis or that Ba:Ca incorporation is regulated by temperature dependent processes.

Bath et al. (2000) demonstrated that Ba:Ca uptake into the aragonitic matrix of the otolith is proportional to the concentration of Ba:Ca in the ambient environment. Fluctuations in this ratio observed in the otoliths, therefore, should correspond to fluctuating Ba levels, presumably on a seasonal basis. Barium in offshore seawater is very low with concentrations between 10 and 45 nM, while riverine Ba inputs can be two to 10 times higher (Chan et al. 1977; Guay and Falkner 1998). Additionally, estuaries can be augmented by Ba discharge (freshwater inputs) and experience high concentrations (Li and Chan 1979). The Ba:Ca profile in this study started high potentially corresponding to a freshwater signal, progressively declined to a magnitude representative of the marine environment, and then went through variable cycles that may represent inshore versus offshore movements. It is also possible that the peaks in Ba:Ca found in the otoliths correspond to times when riverine output is high, such as spring freshet. This could

explain the variation in Ba:Ca observed, as the highest levels of Ba:Ca measured in the otoliths do correspond to times when river output is the highest (May-July).

An alternate explanation for the variation observed in Ba:Ca incorporation in eulachon otoliths could be that branchial (gill) uptake is mediated by temperature dependent processes. Elsdon and Gillanders (2002) determined that the concentration ratio of Ba:Ca increased significantly in juvenile black bream (Acanthopagrus butcheri) otoliths with increasing ambient water temperature. Annual sea surface temperatures recorded on the west coast of British Columbia show a seasonal oscillation that corresponds to lower water temperatures in the winter and higher water temperatures in the summer. Data from the Pacific Region State of the Ocean Report (2003) by Fisheries and Oceans Canada shows that this trend has been stable for many years in both southern and northern sampling locations. The oscillating Ba:Ca ratios in eulachon otoliths may be a reconstruction of life-history information based on the temperature to which the animals were exposed. The seasonal change in Ba:Ca levels measured in eulachon otoliths could be an additive effect of both increased freshwater inputs in the spring and seasonal ocean temperature; however we feel that the seasonal oscillation in temperature is the most likely cause of the Ba:Ca changes measured in the eulachon otoliths.

Strontium:calcium ratios did not appear to vary with temperature in eulachon otoliths as the profile was flat profile throughout their life-history. The finding that Sr:Ca

did not vary with temperature in a similar manner to Ba:Ca is surprising. A possible explanation is that Ba uptake through Ca channels in the gill epithelium may be more sensitive to changes in temperature. Bath-Martin et al. (2004) determined that the Sr:Ca partition coefficient increased linearly with temperatures from 17-26 °C. The water temperatures that eulachon are exposed to are much lower and range from approximately 8-13.5 °C. It is not clear whether water temperature affects Sr uptake in eulachon at these temperatures. Elsdon and Gillanders (2002) found that the ratio of Sr:Ca was greater at both low and high temperatures, but lower in moderate temperatures. Additionally, Sr incorporation into the otoliths is likely mediated by more than just temperature. Strontium concentration is much higher in seawater ( $\sim$ 8 ppm) than Ba concentration ( $\sim 0.015$  ppm). Abundance of Sr in the marine environment may be at a level where branchial uptake is maximal, regardless of small changes in physiological uptake mechanisms that may result from temperature fluctuations. In addition, there were constraints on the resolution of specific elements due to the way that elements from otoliths were measured in our study. Subtle fluctuations in Sr due to temperature change may not have been detectable using our analytical methods. Our data relied on reference material that does not match the matrix of a fish otolith, as no such material is currently available for Laser Ablation investigations. Bath-Martin et al. (2004) utilized a sector-field ICPMS with dissolved otoliths and could rely on more appropriate reference material. Dissolving samples was not an option in our analysis, as we wanted the spatial resolution that is available when using a probe-based instrument.

The results of our study suggest that there is a specific size that eulachon must reach prior to the onset of maturity. If the maturity size threshold is correct, it could explain the variable age classes observed during spawning. The Ba:Ca profile for the Skeena, Kemano, and Fraser Rivers suggest that most eulachon spawn at age three, with some members spawning at age four. Ricker (1954) and McHugh (1939) aged Fraser River eulachon and determined that the most common age at spawning was two years with some fish spawning at age three. Ricker felt that ageing otoliths and scales from eulachon was unreliable due to the difficulties in interpretation of annuli. Ricker's conclusion on a two-year life cycle for Fraser River eulachon was determined through an examination of reproduction success for odd and even years. Eulachon runs spawning in odd years were, on average, higher in density than in even years. The observation correlates with the odd-even alternation in Fraser River pink salmon (Oncorhynchus gorbuscha). Ricker (1954) speculated that eulachon emerging in the spring of even years had to compete with the strong odd-year-returning pink salmon fry, which also emerge in the spring of even years. Ricker (1954) also examined a large flood event in the spring of 1948, where he hypothesized that excessive shifting of the river bottom likely damaged eulachon eggs and lowered survival rates. Interestingly, the lowest return for the period between 1939 and 1953 was the spring of 1950, further suggesting a two-year life-cycle. Potentially, the discrepancy between Ricker's (1954) results and our study, where we found most eulachon to be three years at maturity in the Fraser, is related to ocean productivity. Some of the fish in Ricker's study matured at three years of age

suggesting that most of the fish in the Fraser during 1939-1954 reached the minimum size needed to mature sooner than the fish examined by us.

Columbia River eulachon are an exception in age at spawning as it appears that most fish spawn at age two, with only some members spawning at age three. A possible explanation for this is that most eulachon generally spawn in the Columbia River in late January (Hay and McCarter 2000). Progeny of these fish would, therefore, spend more time in the marine environment when productivity is the highest (Ware and Thompson 1991). Utilization of a winter spawning period may allow Columbia River eulachon the ability to reach the minimum size required to trigger the onset of gonad maturation earlier than eulachon populations that spawn during the spring.

Hay and McCarter (2000) suggest most eulachon spawn at age three. Their finding is a result of an examination of offshore eulachon size classes captured during May 1997 and May 1998, where there is a notable bi-modal age distribution. Hay and McCarter (2000) feel the modes represent age one fish and age two fish; consistent with our findings that most eulachon spawn at age three. One potential problem with their analysis is that the modal ages for 1997 and 1998 are not consistent and show both interannual variation and overlap. The authors attribute this to different rearing conditions, population differences, and geographic differences. Hay and McCarter (2000) also indicate that there are significant differences in eulachon growth rates between populations, further complicating length frequency data. Additionally, it is also suggested

that eulachon larvae from multiple populations mix in different locations. Size classes, therefore, would overlap throughout the marine residency time for eulachon. Interestingly, we have estimated that the ocean fish from Area 23-6 provided to us by Dr. Hay to be 1.5 and 2.5 years old. There is a noticeable difference in size for the two age groups of ocean fish, suggesting that two modal sizes of eulachon do exist simultaneously offshore.

#### Repeat Spawning Potential

Models developed to explain life-history patterns in fish indicate that there are a number of substantive differences between semelparous and iteroparous species. Optimal life-history strategy is a function of both fecundity and the relative survival rate of both adults and juveniles (Charnov and Schaffer 1973). It is generally accepted that there is a trade-off between future growth and reproductive effort. Each individual has a finite energy budget, limiting the amount of resources that can be directed to reproduction if the animal has a chance of survival in the future. In general, iteroparous life-histories are characterized by a long adult life, small clutch size, delayed maturity, large eggs or young, and some form of parental care. Animals with semelparous life histories have a short adult life, large clutch size, small eggs, and no parental care. As well, fish populations with lower densities tend to be iteroparous, although dense populations are semelparous. Our observations suggest that eulachon fit the criteria

describing a semelparous life-history because eulachon are short lived, have high numbers of small eggs, no parental care, and spawn in very high densities.

The Sr:Ca profiles determined in our study also indicate that eulachon are most likely semelparous, as there are no corresponding declines in magnitude that would be due to freshwater movements. Strontium: Calcium ratios are significantly higher in the marine environment than the freshwater environment (Kalish 1990). There have been many studies indicating that migrations from freshwater to estuaries or the marine environment can be detected by high Sr:Ca ratios in bone, otoliths, scales, and pectoral fin-rays (Kalish 1990; Coutant and Chen 1993; Veinott et al. 1999). Factors other than salinity also influence Sr:Ca ratios in some calcified structures. Seasonal changes in reproductive physiology have resulted in variations in Sr:Ca ratios (Kalish 1991). In addition, temperature changes in the ambient environment have resulted in changes to the Sr:Ca ratios (Townsend et al. 1989; Townsend et al. 1995). However, the magnitude of the changes in Sr:Ca ratios when fish migrate from freshwater to seawater are so much greater than variations due to physiology change or temperature. Veinott et al. (1999) examined Sr concentrations in Fraser River white sturgeon (Acipenser transmontanus) pectoral fin-ray annuli using LA-ICPMS and determined that some individuals do make marine migrations into the estuary. Additionally, Howland and Tonn (2001) investigated the Sr profile in inconnu (Stenodus leucichthys) from the Mackenzie River drainage and were able to distinguish between fish that were represented by entirely freshwater, partially anadromous, and completely anadromous life histories. For these reasons, we

expected to see a change in Sr:Ca ratios when eulachon migrated from seawater and into freshwater environments. There was little change, however, in the Sr:Ca ratios incorporated into the otoliths of eulachon over time (Figure 2.6). Despite the short residence time in freshwater, migration to spawning areas should reflect a decline in Sr:Ca measured in the otolith, particularly in areas such as the Fraser and Columbia Rivers where there are heavy influences of freshwater discharge far into the estuary. Furthermore, northern populations of eulachon make extensive migrations through coastal inlets where a decrease in the magnitude of Sr exists from high freshwater inputs. It appears that eulachon spend too short a time in the river during spawning for a distinct freshwater signature to be incorporated into bony structures. In addition, there is anecdotal evidence that much of the time spent in the river before the fish spawn is within the saltwater wedge. The short duration in freshwater and preference for the deeper saltwater wedge appears to preclude a measurable freshwater Sr signal using our probe based technique; as the signal incorporated during the short spawning period would be at the outer edge of the otolith and therefore difficult to detect with our spatial constraints.

Previous observations have shown that some eulachon return to the ocean soon after spawning in the Dean and Burke channels (Pootlace and Siwallace 2000) and in the Gardner Channel from the Kemano River (Adam Lewis, *pers. comm*). These observations, however, do not rule out a semelparous life-history. There may be evolutionary advantages to moving back into coastal inlets prior to senescence, as most of 48 the eulachon larval growth occurs in this environment. Eulachon do not rear in the freshwater environment but are immediately swept downstream into the ocean after hatching (Hay and McCarter 2000). The important nutrients contributed by the large biomass of dead eulachon in the inlets may be important in sustaining early growth at this important developmental stage.

### CONCLUSION

Otolith microchemistry has provided valuable information about the life histories of many fish species in recent years. The spatial resolution of LA-ICP-MS allows for a fine-scale analysis of the chemical environment experienced by fish. Analysis using otolith microchemistry allowed us to address some aspects of eulachon life-history that have been difficult for other researchers to answer to date. The results of our study suggest that most eulachon spawn at age three and are semelparous. Future investigations into eulachon life-history should involve more samples with females to confirm our findings. Regardless, it seems highly unlikely that one sex would be iteroparous, as there would be a highly skewed sex ratio which has not been observed in eulachon populations.

The finding that eulachon are approximately three years of age at spawning contradicts some previous ageing investigations. Difficulties in estimating age for eulachon have been identified. We propose to conduct further analyses on eulachon captured from the Copper River and one of its tributaries in Alaska. These two

populations spawn in January and May, respectively. If Copper River eulachon that spawn in January are one year younger than the cohort that spawns in May, our hypothesis that eulachon need to attain a minimum size prior to maturation will be supported. Independent age analyses of these Copper River eulachon suggests that January spawners are four years old and May spawners are five years old. Perhaps these ages reflect the same errors observed for British Columbia eulachon and the fish are two and three years old respectively.

# Chapter 3: Discrimination of Habitat use by Slimy Sculpins (Cottus cognatus) in Tributaries of the Williston Reservoir using Natural Elemental Signatures

ABSTRACT

Trace-element analysis of bony structures is a technique that has been used to identify location of origin in freshwater fish. This approach may provide fisheries managers with an additional tool when trying to understand the complex movement patterns and life histories of many species. The aim of our study was to correlate chemical signatures deposited in slimy sculpin (Cottus cognatus) otoliths with those measured in the streams where fish were captured. Initially we assessed the chemical stability of water within a river and the differences among rivers for 27 streams within the Williston Watershed, located in northern British Columbia. Stream chemistries remained stable over the duration of the project according to measured values for six sampling events. In addition, Laser-Ablation-Inductively-Coupled-Plasma-Mass-Spectrometry (LA-ICP-MS) and cathodoluminescence (CL) microscopy determined that stream chemistries were consistent from year to year, as the elemental profile was constant for several years of growth in the otoliths of slimy sculpins. Canonical discriminant function analysis demonstrated that streams examined within the watershed were heterogeneous and that each river could be differentiated. Elemental signatures measured in the otoliths of sculpins sampled in the project were highly correlated to the stream chemistries where the fish were captured. The Incorporation Coefficient (IC), molar ratio of an element to Ca in the otolith over the molar ratio in the water (e.g. [Sr:Ca<sub>otolith</sub>] / [Sr:Ca<sub>water</sub>]) was

calculated: Sr:Ca = 0.21 (SE = 0.0041), Ba:Ca = 0.019 (SE = 0.0013), Mg:Ca = 0.00012 (SE = 5.9E-06), Mn:Ca = 0.31 (SE = 0.043). Both Sr:Ca and Ba:Ca ratios in otoliths were highly correlated to stream chemistries. Mg:Ca ratios were weakly correlated to stream chemistry, while Mn:Ca ratios showed a marginal correlation. Multivariate analysis of the chemical fingerprints in the otolith determined a significant relationship to water sample sites. Our study was successful in discriminating among slimy sculpin populations collected from different streams. Probabilities for correct classification of sculpins to their streams of capture were 100% for Bills Creek, Osilinka River, Manson River, and Davis River. Separation of Anzac and Table Rivers were classified correctly 93% of the time. The extension of the methodology used in this project to a migratory species in the Williston watershed appears reasonable. Future studies would benefit from the inclusion of both fin-rays and scales to enhance discrimination of habitat utilization. The inclusion of additional structures may allow this technique to be used without sacrificing the animals. This is of particular importance when life-history information is required on a threatened or endangered species.

#### INTRODUCTION

The headwaters of the Peace River in northern British Columbia offer an opportunity to examine how elemental signatures may be used to link habitat use of fish or identify stream of residence. Preliminary examination of the Williston Watershed shows that bedrock geology differs between the east and west sides of the Reservoir, as well as between the north and south (Rutter 1976). Bedrock formations in the Williston watershed were formed during different time periods, including the upper and lower Jurassic, Cretaceous, and Triassic, and are composed of materials that have variable chemical composition (Armstrong 1979).

A small fish species, the slimy sculpin (*Cottus cognatus*), was utilized in this study to determine if chemical ratios incorporated into bones are geographically distinct in tributaries of the Williston Reservoir. Slimy sculpins are abundant in the Peace River watershed and are considered to be a non-migratory species, making them a suitable candidate for this study. Very little research has been conducted on this species in British Columbia. It has been documented that slimy sculpins reach a maximum size of 12.1 cm and age of seven years (Morrow 1980). This small fish occupies a variety of habitats including fast-flowing cold streams and rocky areas of lakes; it is even tolerant of brackish water. Slimy sculpins are also noted to inhabit areas with a high amount of groundwater influence, as well as small springs (Page and Burr 1991).

In this study, we investigated the potential to discriminate locations of capture for fish within the Williston watershed using natural chemical tags. The objectives of our

study were to investigate the stability of the stream chemistry throughout the year within a group of Parsnip River tributaries, to investigate the heterogeneity of stream-specific chemical signatures and determine if we could discriminate among locations of rivers within the Parsnip River Watershed and tributaries throughout the Williston Reservoir Watershed, and to ascertain correlation in elemental signatures between stream of capture and the otolith of slimy sculpins.

#### METHODS

### Study Location

This study was conducted within tributaries of the Williston Reservoir located in north-central British Columbia (Figure 3.1). The Williston Reservoir is the largest body of freshwater in British Columbia and the reservoir was formed following completion of the WAC Bennet Dam on the Peace River in 1967. The Reservoir now drains an area representing 70,000 km<sup>2</sup>.

### Water Collection

The stability of the stream-specific chemical signatures was determined by collecting water samples, on six separate occasions from June 20, 2002 to November 15, 2002, from four locations within the Parsnip River mainstem and 11 Parsnip tributaries.

Access and icy river conditions prevented additional sampling during the remaining portion of the winter. To determine the heterogeneity of streams and rivers within the Williston Watershed, water samples were obtained from a total of 27 geographically distinct locations. These streams included the 15 sites sampled multiple times within the Parsnip watershed and 12 streams sampled a single time located throughout the reservoir watershed (Figure 3.1). The methods chosen for obtaining water samples followed the recommendations outlined by Shiller (2003) for sampling trace elements in remote locations, with some minor modifications. High density polyethylene bottles (50 ml) (Fisher Brand) were cleaned with ultra-pure water and filled with a solution of 2% high purity nitric acid and left for a minimum of two weeks. Bottles were then rinsed five times in ultra-pure water. Polyethylene/Polypropylene 50-ml syringes (Sigma Aldrich) were cleaned in the same manner as the 50 ml bottles. Nylon filters (25 mm x 0.45  $\mu$ m, Fisherbrand) were cleaned by passing 40 ml of a solution of 2% high purity nitric acid followed by a rinse of 20 ml of ultra-pure water. All filters were blown dry with high pressure clean air and left under a fume hood until use. For field sampling, all equipment used at each site was placed into an individual clean Ziploc bag (sampling kit). The kit contained two filters (replicate samples), two 50 ml bottles, one syringe, polyethylene gloves, and an extra Ziploc bag for collected water samples. Water samples were acidified to a solution containing 2% high purity nitric acid (600  $\mu$ l HNO<sub>3</sub>/30 ml water sample) in the field immediately after collection.



Figure 3.1 Map of the Williston Reservoir showing main tributaries. The numbered tributaries include: Table River (1), Tacheeda Creek (2), Bill's Creek (3), Hominka River (4), Misinka River (5), Wichika Creek (6), Swannell River (7), Davis River (8), Pelly Creek (9), Factor Ross Creek (10), Colbourne Creek (11), Reynolds Creek (12). The other tributaries sampled include the Parsnip River (4 locations), Anzac River, Wooyadilinka Creek, Misinchinka River, Nation River, Manson River, Osilinka River, Mesilinka River, Ingenika River, and the Finlay River.

## Water Analyses

Water analysis was completed with a PS 1000-UV Inductively-Coupled-Plasma-Optical-Emission-Spectrometer (ICP-OES) (Leeman Labs), at the University of Northern British Columbia. The elements measured included Ba, Ca, Sr, Mg, and Mn. Four calibration standards prepared from traceable (NIST) standards were run for every 10 samples analyzed. Laboratory blanks and field procedural blanks were also included in the analysis. Additionally, samples were analyzed at the University of Victoria with a solution based Inductively-Coupled-Plasma-Mass-Spectrometer (ICP-MS) as an additional quality control analysis.

### Fish Collection

To correlate chemical signatures present in the water with those in fish otoliths, several rivers were chosen to be representative of the different geographic regions of the watershed. We chose rivers on the east side of the reservoir that originate from the Rocky Mountain range, as well as rivers on the west side that flow from the Coast Mountain Range. The rivers on the west side of the reservoir included in this portion of the study were the Manson, Swannell, and the Osilinka . Rivers on the east side of the reservoir included the Table, Anzac, Davis, and Bill's Creek. Minnow traps were used to capture sculpins from the Swannell and Manson Rivers. Minnow traps proved to be

unreliable; therefore, only two sculpins from the Swannell River and one sculpin from the Manson River were collected. A Smith Root 12B backpack electrofisher was used to capture sculpins from the Table River, Anzac River, Bill's Creek, and Osilinka River during July 2002. One sculpin, found dead on the bank of the Davis River, was donated by the Ministry of Water, Land & Air Protection and included in the analysis. Fish and water samples were collected from the same area of each river system. Fork length (mm) and weight (g) were recorded for each fish (n=41) prior to removal of the sagittal otoliths. Sagittal otoliths were chosen, as they are the largest of the three pairs of otoliths found in teleosts.

### **Otolith Chemistry**

Otolith preparation, analysis, and data reduction were completed with the same protocol as Chapter 2. All otoliths were photographed in order to determine at what age chemical signatures were variable within the otolith.

### Cathodoluminescence Microscopy

Cathodoluminescent imaging was performed by focusing a high-energy beam of electrons (20 kV) onto a polished otolith placed in a chamber under vacuum. The incident electrons cause bound electrons to rise to higher energy levels. When the

electrons return to their original state, they release the energy and luminesce. The wavelength of the light emitted is specific to each element. Light emitted by the sample is collected with achromate lenses and guided to the entrance slit of the spectrometer. Images were acquired with a one-minute exposure onto a cooled colour CCD camera (Q-Imaging, Burnaby, BC, Canada).

#### Statistical Analyses

Linear regression was used to determine the relationship between otolith chemistry and water chemistry (SPSS v.11.5, Sep 2002, Chicago, IL). As well, the tracemetal chemistry in the otoliths was related to the trace-metal chemistry in the water using an 'incorporation coefficient' (Wells et al. 2000b). The coefficient was calculated as the molar ratio in the otolith over the molar ratio in the water (e.g. [Sr:Ca<sub>otolith</sub>] / [Sr:Ca<sub>water</sub>]).

Chemical signatures for both the rivers and the sculpins were differentiated using Canonical Discriminant Analysis (SPSS v.11.5, Sep 2002, Chicago, IL). Canonical discriminant analysis was utilized to provide a visualization of geographic separation using only water chemistry data. A multivariate combination of Sr:Ca, Ba:Ca, and Mn:Ca molar ratios was utilized to discriminate the sculpins in this study, while a combination of Sr:Ca, Ba:Ca, Mg:Ca, and Mn:Ca was utilized to discriminate tributaries of the Williston Reservoir. The elemental ratios were the factors loaded into the discriminant function (DCF) in order to produce DCF1 and DCF2. Manova (SPSS v.11.5, Sep 2002, Chicago,

IL ) was also used to test for significant differences in multi-element ratios between sample locations, under the hypothesis that there is no significant difference in chemical fingerprints between populations and locations.

## RESULTS

#### Stability of Water Chemistry

For the 15 sample sites where water samples were collected from the same locations six times from June 20, 2002 to November 15, 2002, the absolute concentrations of elements found within each system were observed to change over time, however, the ratios of the individual elements to the concentration of Ca (e.g. Sr:Ca) in the river systems remained stable over the sampling period from June 2003 until November 2003. Elemental ratios for the Parsnip River and tributaries are shown in Table 3.1. Small variations observed for each river reflect the low amount of deviation in elemental ratios measured over time. Most of the variation that was observed was due to lower than average values measured during high flow conditions associated with spring freshet. Figure 3.2 and 3.3 show the concentration of elements and the ratio of elements to Ca for the Table and Anzac Rivers, respectively. Fluctuations in mean Ba (ppm) and Sr (ppm) are apparent on a seasonal basis; however, ratios of these elements to Ca remain fairly stable over time.

Location	Sr:Ca	Ba:Ca	Mg:Ca	Mn:Ca
Parsnip Glacier	4.37	0.38	127	4.61
Parsnip Upper	$3.67\pm 0.08$	$0.37\pm0.01$	$163\pm2$	$2.15\pm0.35$
Parsnip H/M	$3.90\pm 0.06$	$0.49\pm0.01$	$181\pm4$	$1.07\pm0.13$
Parsnip A/T	$\textbf{3.42} \pm \textbf{0.10}$	$0.90\pm0.06$	$192\pm1$	$1.50\pm0.14$
Parsnip Lower	$3.39\pm0.06$	$0.64\pm0.04$	$178\pm2$	$0.41\pm0.04$
Hominka River	$4.24\pm0.11$	$0.32\pm0.01$	$287\pm6$	$1.75\pm0.07$
Misinka River	$2.43\pm0.09$	$\textbf{0.62} \pm \textbf{0.01}$	$307 \pm 4$	$1.56\pm0.27$
Wichika Creek	$4.01\pm 0.03$	$1.17\pm0.09$	97.5 ± 3	$0.08\pm0.01$
Bills Creek	$\textbf{3.08} \pm \textbf{0.03}$	$\textbf{3.44} \pm \textbf{0.11}$	$117 \pm 1$	$0.21\pm0.01$
Wooyadilinka Creek	$\textbf{3.72} \pm \textbf{0.05}$	$0.29\pm0.01$	$965\pm1$	$0.06\pm0.01$
Tacheeda Creek	$3.32\pm0.13$	$3.50 \pm 0.11$	$186 \pm 5$	$0.37\pm0.08$
Table River	$2.95\pm0.09$	$\textbf{0.42} \pm \textbf{0.01}$	$187\pm3$	$1.93\pm0.09$
Anzac River	$3.47\pm 0.07$	$0.33\pm0.02$	$151\pm2$	$0.66\pm0.03$
Reynolds Creek	$2.92\pm0.50$	$0.46 \pm 0.01$	$221\pm4$	$0.93\pm0.05$
Colbourne Creek	$4.04\pm0.12$	$0.45\pm0.02$	$226\pm7$	$1.41 \pm 0.10$
Misinchinka Creek	$\textbf{3.01} \pm \textbf{0.10}$	$0.25\pm0.01$	$186\pm3$	$1.01\pm0.13$
Davis River	2.23	0.33	316	0.05
Findlay River	4.18	1.28	253	0.08
Pelly River	7.13	0.54	334	0.03
Ingenika River	6.28	0.51	258	0.22
Swannel River	5.27	0.88	328	0.35
Factor Ross River	3.28	3.14	190	0.68
Mesilinka River	3.79	0.74	102	0.15
Omineca River	5.79	1.95	182	0.29
Osilinka River	7.13	3.15	190	0.68
Manson River	4.73	1.44	185	0.19
Nation River	4.64	1.63	182	0.14

Table 3.1. Average element ratios (mmol/mol  $\pm$  SE) measured by ICP-OES for each tributary over the sampling period from June - November 2002. Most tributaries of the Parsnip mainstem were sampled six times. Values for tributaries with no error associated were only sampled once.


**Table River** 

Figure 3.2 A comparison of Ba (ppm) and Sr (ppm) measured over the duration of the sampling period in the Table River (top) to Ba:Ca and Sr:Ca ratios (bottom). Chemical ratios were much more stable over the sampling period when compared to element concentrations.



**Anzac River** 

Figure 3.3 A comparison of Ba (ppm) and Sr (ppm) measured over the duration of the sampling period in the Anzac River (top) to Ba:Ca and Sr:Ca ratios (bottom). Chemical ratios were much more stable over the sampling period when compared to element concentrations.

### Heterogeneity of Water Chemistry

For the 16 rivers included in the discriminant function analysis, there was considerable separation for all, but with groupings that were related to distance between systems (Figure 3.4). Geographic separation formed three main groups: Parsnip tributaries on the right of the figure, northern Williston tributaries on the bottom left, and tributaries on the west side of the reservoir (grouping) in the middle left. The Davis River is included with the Parsnip tributaries even though it is near the top of Williston Lake; this is not surprising, as both areas are part of the Rocky Mountain range.

Solution-based ICP-MS analyzed 30 different elements. The results revealed variation in water chemistry for some elements that were not measured by the ICP-OES and were not measured in sculpin otoliths (Table 3.2). Elements of particular interest were Se and Li. Se was detectable in the Table River, Wooyadilinka Creek, and Bill's Creek. Li was only detected in the Parsnip River.

#### Otolith Chemistry

The majority of the line scans tracked across otoliths with the laser demonstrated a flat profile for Sr, Mn (not shown), and Ba (Figure 3.5). An area surrounding the otoliths from Bills Creek, Table River, and Anzac River sculpins, had a unique region surrounding the core with elevated levels of Ba and Mn. This area corresponded approximately to the first year of growth. Trace metal composition was very similar in





Element	L. Par	Misinch	Colb	Reyn	Anzac	Table	Wooy	Par A/T	Tach	Bills	U. Par	Wichika	Par H/M
7Li	0.054	0.077	nd	nd	nd	nd	nd	0.427	0.38	nd	nd	nd	nd
26Mg	2466	3158	1499	1990	2516	1879	884.6	2461	553.1	3645	1950	2700	2423
27Al	20.66	13.11	43.41	23.2	21.17	27.98	30.89	21.84	45.95	25.84	21.46	8.657	19.37
29Si	1340	1389	1547	1339	1156	1245	1317	1313	2262	2014	1231	1771	1415
31P	1.406	1.852	2.627	1.269	0.851	2.022	2.456	2.339	5.946	5.749	2.488	0.959	2.032
44Ca	15858	18174	6905	9149	17856	9995	8596	14733	39035	28336	13134	31026	15743
47Ti	0.389	0.409	0.48	0.33	0.409	0.479	0.457	0.644	0.922	0.973	0.553	0.493	0.518
51V	0.229	0.221	0.344	0.367	0.42	0.565	0.476	0.598	0.633	0.631	0.631	0.628	0.606
52Cr	0.244	0.284	0.331	0.245	0.32	0.287	0.31	0.28	0.343	0.713	0.341	0.271	0.332
55Mn	4.185	17.35	5.38	6.629	7.227	16.84	0.843	12.59	9.522	7.204	32.48	1.827	16.11
57Fe	69.93	148.8	90.61	60.4	105.5	120.1	69.96	111.3	166.6	258.9	162.8	114.8	160.4
59Co	nd	nd	nd	nd	nd	nd	nd	0.058	0.031	0.331	0.155	0.091	0.265
60Ni	nd	nd	nd	nd	nd	0.035	0.417	0.445	0.332	1.188	nd	nd	0.022
65Cu	0.427	0.288	0.475	0.4	0.298	0.476	0.409	0.739	2.293	0.5	0.531	0.18	0.528
66Zn	0.978	1.104	0.793	0.777	0.454	0.851	1.189	2.463	1.209	0.917	1.304	1.016	0.556
75As	0.22	0.138	0.129	0.14	0.124	0.12	0.05	0.126	0.19	0.086	0.163	0.043	0.088
77Se	nd	nd	nd	nd	nd	0.127	0.273	nd	nd	0.47	nd	nd	nd
85Rb	0.152	0.124	0.128	0.14	0.154	0.17	0.172	0.291	0.385	0.292	0.179	0.154	0.131
86Sr	44.37	46.41	23.29	27.53	52.56	24.41	27.28	40.65	107.3	84.71	33.66	104.5	49.56
95Mo	0.069	0.031	nd	nd	nd	nd	nd	nd	0.187	0.004	nd	nd	nd
107Ag	0.034	0.04	0.06	0.088	0.109	0.127	0.136	0.241	0.259	0.269	0.312	0.328	0.336
111Cd	0.086	0.017	0.018	0.06	0.066	0.064	0.049	0.103	0.091	0.076	0.104	0.092	0.072
121Sb	0.036	nd	0.003	0.025	0.045	0.039	0.033	0.055	0.03	0.005	0.003	nd	nd
133Cs	0.044	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
137Ba	9.167	4.634	3.393	4.057	4.913	4.15	2.831	9.06	146.9	106.5	4.18	27.84	7.481
139La	0.127	0.051	0.148	0.124	0.079	0.177	0.311	0.11	0.032	0.029	0.088	0.003	0.04
140Ce	0.105	0.068	0.152	0.127	0.115	0.213	0.259	0.189	0.126	0.113	0.182	0.076	0.14

Table 3.2 Element concentrations (ppb) measured by solution based ICPMS for tributaries of the Parsnip River. The table indicates some potential elements for discriminating fish and river systems within this watershed in future studies. A further examination is required in order to determine which elements are incorporated in measurable values into the bony structures of fish. Element concentrations below detection limits are labeled as non detectable (nd).



Figure 3.5. Cathodoluminescence image showing the elemental profile for Ba:Ca. Note the distinctive variation in the elemental concentration corresponding to the white luminescent region. The portion of the otolith coloured blue represents the mainstem river habitat which appears to have remained stable for more than three years. The yellow line corresponds to the location of the laser scan. The length of the line scan was 179 s long or 948.7  $\mu$ m (5.3  $\mu$ m/s).

both the core and the area in the core that represented the adult portion of the sculpins life. There was a unique region in each otolith composed of high levels of trace metals. The relatively stable composition of the otolith chemical signature confirms stream stability for two to five years depending on the age of the sculpin examined. Variations in stream chemistry over time would be reflected in variation of elements within an otolith, which was not seen. The elemental changes from the laser scan are overlain on the CL image of the otolith (Figure 3.5) to demonstrate that the chemical variations correlate with CL.

LA-ICP-MS revealed substantial differences in otolith chemistry between watersheds. Otolith (n=41) Sr:Ca and Ba:Ca chemical ratios were significantly correlated to the chemical ratios found in the water. The [Sr:Ca<sub>otolith</sub> / Sr:Ca<sub>water</sub>] coefficients for the linear equation were b(0) = 0.22, b(1) = 270.9, and  $r^2 = 0.93$ , p < 0.001; the coefficients for [Ba:Ca<sub>otolith</sub> / Ba:Ca<sub>water</sub>] were b(0) = 4.2, b(1) = 13.1, and  $r^2 = 0.84$ , p < 0.001 (Figure 3.6). Significant regressions did not exist for the Mg:Ca and Mn:Ca ratios measured in the otolith to the chemical signatures measured in the water. Both <sup>65</sup>Cu and <sup>66</sup>Zn were below the detection limits of the mass spectrometer for all of the sculpin otoliths sampled. These elements were not examined further in this study.



Figure 3.6 Relationship between measured Sr:Ca (top) and Ba:Ca (bottom) values in slimy sculpin otoliths compared to the measured values in the river where the fish was captured. The outlier is noted as the top value for Osilinka (O6) for each plot. The [Sr:Ca<sub>otolith</sub>/Sr:Ca<sub>water</sub>] coefficients for the linear equation were b(0) = 0.22, b(1) = 270.9, and  $r^2 = 0.93$ , p < 0.001; the coefficients for [Ba:Ca<sub>otolith</sub>/Ba:Ca<sub>water</sub>] were b(0) = 4.2, b(1) = 13.1, and  $r^2 = 0.84$ , p < 0.001.



Figure 3.7 No significant relationship exists between Mg:Ca (top) and Mn:Ca (bottom) measured in slimy sculpin otoliths compared to the values measured in the rivers the fish were captured in.

Incorporation coefficients for each element ratio were calculated: Sr:Ca = 0.21(SD = 0.0041), Ba:Ca = 0.019 (SD = 0.0013), Mg:Ca = 0.00012 (SD = 5.9E-06), and Mn:Ca = 0.31 (SD = 0.043). One fish from the Osilinka River had incorporation coefficients of 0.31 and 0.027 for Sr:Ca and Ba:Ca, respectively. This fish was also a noticeable outlier for the linear regression analysis for both Sr:Ca and Ba:Ca (Figure 3.6).

Canonical discriminant analysis using the multivariate combination of Sr:Ca and Ba:Ca as the linear combination of the predictor variables to produce DCF1 and DCF2 revealed good visual separation between sculpin populations, with the exception of the Davis River. Inclusion of Mn:Ca into the prediction equation resulted in better separation for all systems (Figure 3.8). The Davis River has a lower concentration of Mn than the other systems in the study (Table 3.1). Relative contribution of each element is shown by the variance accounted for with each of the three discriminant functions calculated. The first function accounted for 55.9% of variance, the second function 42.7%, and the third function 1.6%. Magnesium was excluded from this model as the within-population variance was greater than the variation among populations. Probabilities for correct classification of each stream were 100% for Bill's Creek, Osilinka River, Manson River, and the Davis River; 92.9% of the Anzac and Table River fish were classified separately.



Figure 3.8 Canonical discriminant function analysis showing how sculpins from individual populations clustered according to the multivariate combination of Sr:Ca, Ba:Ca, and Mn:Ca (discriminant factors) measured in each otolith. Sample sizes vary for each river system (Table n=8, Anzac n=10, Bill's n=11, Manson n=1, Osilinka n=8, Swannell n=2, Davis n=1).

Multivariate statistical examination revealed a significant difference between the natural-log-transformed molar ratios of Sr:Ca, Ba:Ca, and Mn:Ca (dependent variables) for the populations of sculpins, implying that fish from each stream incorporate a specific chemical signature. The following results were obtained: Pillai - Bartlett Trace = 2.3, df = 18, 105, F = 19.169, and P < 0.001. Independent factors include the Table River, Anzac River, Bill's Creek, Osilinka River, Manson River, Swannell River, and Davis River.

CL microscopy revealed an interesting visualization of trace element deposition in slimy sculpin otoliths (Figure 3.9). The Table River fish have a definite white luminescent region that corresponds to the first year of the fish's life. Anzac River fish and Bill's Creek fish are characterized by a similar pattern as the Table River; however, there appears to be more fluctuation in trace metal deposition early in life. Osilinka River fish have distinct luminescent regions that are likely correlated to seasonal or yearly movements, or possibly areas highly influenced by ground water. Swannell River sculpins appear to live in a much more chemically constant environment, as there is little to no change in luminescence throughout their lifehistory.



Figure 3.9 Representative cathodoluminescent images of otoliths from sculpin captured in the Table River (T7), Anzac River (A10), Bill's Creek (B5), Osilinka River (O5 & O10), and Swannell River (S1).



Figure 3.9 continued

#### DISCUSSION

Our study contributes to a growing field of research investigating the benefit of using natural chemical markers to examine life-history and movement patterns of fish in freshwater ecosystems. The streams sampled in this study maintained chemical stability over time and exhibited sufficiently different chemical signatures to allow differentiation based on geographic locations. Additionally, the elemental ratios measured in the fish otoliths were highly correlated to the stream ratios determined where sculpins were captured.

#### Stability of Elements

Tributaries of the Parsnip River remained chemically stable over the duration of sampling in this project. This was particularly true when examining the element to Ca ratio. Fluctuations were observed in the mean element concentration for each stream; however, element ratios remained remarkably constant. Element ratios were slightly lower during spring freshet (high flow conditions) for the tributaries examined, though this is of limited concern, as most growth for bony structures of fish has been documented to occur during base flow conditions (Kennedy et al. 2000). Water stability was an important component of this study, as a large amount of temporal variation in stream chemistry would ultimately confound classification of individuals to their natal stream. This consistency among rivers is well described by Taylor and Hamilton (1994) who examined 25 years of water chemistry data on the Saskatchewan River. Water was not sampled during the winter months in our study.

This should not be problematic as this period only represents a time of hydrograph draw-down. Therefore, element ratios should remain consistent for this period of time, even if element concentrations rise. Consistency in chemical signature is further supported by the line scans tracked across the otoliths of most individuals, suggesting stable water chemistry. Slimy sculpins are considered to be non-migratory, so if the water chemistry remains stable over extended periods of time, the chemical deposition in the otolith should also remain stable for elements that are incorporated proportionately. We found this to be true, as the chemical signature maintained a flat profile throughout the life of the individual. This period varied from two to five years, depending on the age of the fish. Variation in stream chemistries on an annual basis would have resulted in fluctuating elemental fingerprints within each otolith; therefore, it appears that chemical signatures have been stable over several years for the tributaries that we sampled in the Williston Watershed.

The region surrounding the core of the otolith for some of the populations examined, however, showed variation in chemical signatures. Surrounding the core was a noticeable increase in trace metal concentration for Ba:Ca (Figure 3.5) and Mn:Ca. The increase in Ba:Ca and Mn:Ca was seen in sculpins from Bill's Creek, Table River, and the Anzac River. CL microscopy also demonstrated that there was a significant change in trace metal concentration surrounding the core of the otolith; though, the core itself had a similar chemistry to that seen in the later stages of the sculpin's life for both methods. It is possible that we are observing a maternal signature incorporated into the core of the otolith of larval sculpin when they are still

dependent on the egg for nutrition. This is supported by the finding that adult salmonids pass on a maternal elemental signature to juveniles through the egg (Weber et al. 2002; Zimmerman and Reeves 2002). It appears that adult sculpins move into a distinct habitat such as a small stream or an area highly influenced by groundwater to spawn. The juvenile sculpins then rear in this habitat for one to two years until moving back into a separate habitat.

### Geographic Separation

Streams within the Williston watershed were sufficiently heterogeneous in water chemistry to discriminate their locations. Not only were the streams heterogeneous, they appear to form two main groupings for the east and west side (longitudinal) of the reservoir, as well as a latitudinal distribution from north to south (Figure 3.4). Our data support a recent study by Wells et al. (2003) that was also successful in discriminating geographic locations using chemical fingerprints measured in a freshwater environment. Variation in the freshwater environment appears to be common within watersheds, which is not surprising as the geology of most regions varies spatially due to differences in age and composition of bedrock; these differences result in variable stream chemistries.

The elemental composition of sculpin otoliths also differed significantly among sample locations. A combination of Sr:Ca, Ba:Ca, and Mn:Ca allowed adequate separation of all of the populations examined; but, the Table and Anzac Rivers show

marginally less separation. This is not surprising, as they are parallel streams that are only separated by a few kilometers. A potential element for inclusion into the analysis is Se, which was only detected in the Table River and is incorporated well into fish scales (Farrell et al. 2000); therefore Se may provide additional resolution between these two systems, as it is only present in detectable levels in the Table River.

Magnesium was not utilized to classify individuals because the incorporation into the otolith did not correspond well with the water from sample locations. As Mg does not show a definitive relationship with water chemistry, it may be of limited utility in determining life-history with otolith microchemistry. Magnesium has an upper limit for incorporation into the aragonitic matrix of the otolith; therefore the Mg levels in all the streams measured may be higher in concentration than the fish is able to incorporate, resulting in a poor relationship between stream Mg concentration and otolith Mg concentration. Wells et al. (2003) found a negative relationship between Mg:Ca in the otolith and Mg:Ca in the water while we found a slightly positive relationship. The small differences in the findings observed between the two studies may be due to species-specific incorporation of trace elements (Campana et al. 2000).

The methodology used in our study may be further improved with the inclusion of fin-rays and scales in the analysis. Mg has been demonstrated to be incorporated well into bull trout fin-rays (see Chapter 4), and Mn is incorporated well into scales (Wells et al. 2000b). The use of a complementary method may also

enhance resolution between streams. CL microscopy revealed differences in luminescence among streams. The results indicate that there is potential to discriminate sculpin stocks simply by examining the luminescent pattern created with CL emissions, particularly when combined with LA-ICP-MS. CL microscopy is a particularly valuable tool for analysis, as it is extremely cost effective.

Not all of the studies conducted to date have agreed with either our results or with those obtained by Wells et al. (2003). A study examining barramundi (*Lates calcarifer*) was unsuccessful in discriminating movement patterns using Cu, Mn, Zn, Cd, and Pb within the Fly River, Papua New Guinea (Milton et al. 2000). Possibly, the elements chosen for the study were not suitable as some elemental species form colloids in the water, limiting their bio-availability (Benes 1979; Gaillardet et al. 2003; Peltier et al. 2003) or are incorporated into the matrix of the otolith as some elements attach to Ca binding proteins (Milton et al. 2000; Campana and Thorrold 2001). Sr and Ba move into the otolith through Ca channels and are more likely to be incorporated based on their concentration in the environment (Kalish 1991).

Incorporation coefficients we observed were 0.21 for Sr:Ca and 0.019 for Ba:Ca. The coefficient obtained for Sr:Ca was substantially lower than that obtained for Westslope cutthroat trout in the Couer d'Alene River basin, where the value was 0.40 (Wells et al. 2003). The value we obtained, however, is similar to values measured in two experiments looking at the chemical uptake in juvenile spot (*Leistomus xanthuras*) (Bath et al. 2000; Wells et al. 2000a). The Ba:Ca incorporation coefficient determined by our study was lower than values obtained for both cutthroat trout

(Wells et al. 2003) and juvenile spot (Bath et al. 2000; Wells et al. 2000a). Wells et al. (2003) hypothesized that the differences observed between freshwater and marine species were likely a result of the different elemental uptake mechanisms between these two groups of fish. Our data suggests that differences are more likely to be attributed to species rather than environmental effects, as our values for Sr:Ca reflect the values measured in a saltwater fish.

One fish in this study, captured in the Osilinka River, was a notable outlier (Figure 3.7). This fish had a higher incorporation coefficient for both Sr:Ca and Ba:Ca when compared to the other fish in this study. Both the left and right otoliths were examined in this fish on separate occasions to ensure that sample preparation did not influence the results. It is likely that this fish was associated with a different habitat before it was captured in the Osilinka River. Potentially, this fish could have moved into the area from another stream or been associated with a unique groundwater source. Slimy sculpins are a benthic species (Scott and Crossman 1973) living within the substrate of the river system (Clarke AD, *personal observation*), so they are more likely to be in direct contact with groundwater percolating into the river system; salmonids reside higher in the water column where there should be more mixing of water.

The present study demonstrated that otolith microchemistry is a valid method to discriminate the geographic location of fish within a number of tributaries of the Williston watershed. We believe this approach would be suitable for discriminating movements of migratory fish, however, there is the potential for increasing the

resolution of the technique used. Classification accuracy of sculpins to their natal stream was excellent as indicated by the high predicted probabilities for each river system (ranging from 92.9% to 100%). Most rivers are clearly distinct, but there is the potential for some overlap in the Table and Anzac Rivers. As indicated earlier, the addition of lithium and selenium may increase the resolution of this application for these two systems. Lithium is known to be incorporated into the otoliths of teleost fish in proportion to the environment (Campana et al. 2000). Lithium would be useful as it is only detectable in the Parsnip River. Both the Table and Anzac Rivers flow into the Parsnip making it a corridor for fish movement between these two systems. The utility of this technique could also be increased by analyzing otoliths, fin-rays, and scales for each individual fish. Scales and fin-rays incorporate some elements differently than otoliths. Farrell et al. (2000) determined that Ca, Mg, Zn, Hg, and Pb in Arctic grayling scales could be sufficiently resolved with LA-ICP-MS. Their study determined that these elements could potentially be utilized to rebuild past exposure to metals in the environment. Additionally, selenium was only present in the Table River at detectable levels. Farrell et al. (2000) demonstrated that when Arctic grayling are present in a stream that contains selenium, it is incorporated into the scales.

# Chapter 4: Movement Patterns of Bull Trout (Salvelinus confluentus) in the Morice Watershed using Chemical Signatures Deposited Spatially in Fin-rays

#### ABSTRACT

The potential to discriminate between fish that show large movements within a watershed versus fish that show no or few migrations using natural chemical markers deposited in bony structures was examined within the Morice River Watershed. Finrays were collected from 10 bull trout. The chemical make-up of streams known to contain bull trout in the Morice River watershed were measured once during this project, determining that the water chemistry of most of the streams was similar and not useful for discerning movement patterns. The results of our study suggest that Sr, Ba, Mg, and Mn are deposited in fin-rays proportionately to the concentrations measured in the water where the fish were captured. Incorporation coefficients were determined for the fin-rays: Ba:Ca = 0.020 (SD = 0.003), Mg:Ca = 0.36 (SD = 0.060), Mn:Ca = 0.19 (SD = 0.037), and Sr:Ca = 0.15 (SD = 0.014). Finding that trace elements are deposited in proportion in fin-rays to what is present in the fishes aquatic environment is important because it strengthens the assumption that fin-rays can be used to rebuild environmental life histories in a sufficiently chemically distinct aquatic environment. Seasonal oscillations in Zn:Ca deposition in fin-rays were also observed in this study. We determined that these oscillations most likely correspond to winter and summer growth and, thus, represent a method to approximate age using chemical signatures deposited spatially in fin-rays. The application of using natural chemical tags deposited spatially in fin-rays to rebuild environmental life

histories of fish, therefore, has the capability to complement or replace otoliths in future studies. Otoliths are preferred in chemical studies examining life-history, as they offer much higher spatial resolution; however, where populations of fish are either threatened or endangered, fin-rays may provide an acceptable non-lethal alternative for life-history investigations.

#### INTRODUCTION

Bull trout are currently declining in British Columbia and have been designated as blue-listed (endangered) by the provincial government. They are sensitive to habitat degradation and are considered to be an indicator of ecosystem health (Cannings and Ptolemy 1998). Bull trout are a cold-water species, generally found in streams with temperatures less than 12°C. This species is sensitive to human activities that change temperature, habitat, substrate composition, or migration patterns. Determining movement patterns of bull trout will provide information to assess critical habitat requirements and, potentially, population structure.

Bull trout movement patterns, population structure, and habitat utilization were assessed in a previous study using radio telemetry and genetic analysis (Bahr 2002). This work showed that bull trout could be spatially clustered by geographic region in the Morice River Watershed. Additionally, fish from each spatial cluster showed variable patterns of movement, some moving extensive distances (>100 km) and some not moving beyond the stream reach they inhabited for the two-year study. The long distances and variability in movement shown by some bull trout within the Morice River watershed offers an opportunity to examine if differences in movement can be detected using chemical markers.

Fin-rays record a spatial and temporal scale similar to otoliths; however, the fin-ray matrix is made up of  $Ca_3(PO_4)_2$  whereas the otolith is mainly composed of

CaCO<sub>3</sub>. The use of fin-rays for rebuilding environmental life histories may be problematic, as these structures have the potential for resorption and mobilization of trace elements after deposition (Veinott and Evans 1999). Veinott and Evans (1999) determined that K is not stable in fin-rays of white sturgeon (*Acipenser transmontanus*); however, Sr, Mg, Pb, Br, Zn, Ba, Sn, Mn, and Na remained stable for at least six years . Additionally, fin-rays have been used successfully to determine anadromous movements of white sturgeon from the Fraser River using Sr:Ca ratios spatially deposited in fin-rays to differentiate between freshwater and saltwater, two very distinct chemical environments (Veinott et al. 1999).

A closely related species to the bull trout, the Arctic char (*Salvelinus alpinus*) has been examined extensively with otolith microchemistry to determine between migratory char moving between freshwater and marine environments and non-migratory freshwater char (Halden et al. 1996; Babaluk et al. 1997). Both studies were successful in differentiating between anadromous and non-anadromous fish by examining Sr concentrations measured across the otolith. Arctic char otoliths also demonstrate well-defined seasonal fluctuations in Zn that correlate to the annular structure of the otolith, thus enabling an age estimate (Halden et al. 2000). Zn uptake in Arctic char was shown to be higher in younger fish than older members of the population. Halden et al. (2000) hypothesized that Zn uptake was higher during the summer due to elevated water temperatures that resulted in increased nutrient production and intake.

In this study we examined the chemical signatures deposited in the pectoral fin-rays of bull trout. We compared results of the chemical analyses to the telemetry data gathered by Bahr (2002) to compare the different techniques for assessing movement and population structure within the watershed. Fin-ray chemical analysis has the potential to provide information over a much longer time scale than the telemetry data, as this technique should indicate movement patterns and habitat utilization over the entire life of the individual, potentially including their stream of origin. The objectives of our study were to investigate the heterogeneity of stream-specific signatures in the Morice River watershed to determine if geographical locations within the watershed were distinct, to examine fin-ray chemistry to determine if the elemental deposition in the outer edge of the structure correlated to the water chemistry at the capture location, and to correlate seasonal fluctuations in Zn deposition to annuli deposited in the fin-ray to see if age could be assessed using chemical signatures.

### METHODS

#### Study Location

This project was conducted within the Morice River watershed located in northwestern British Columbia (Figure 4.1). The Morice River watershed represents a drainage basin of 4300 km<sup>2</sup>. Morice Lake is fed by two large river systems: the Atna and Nanika Rivers. The Morice River flows out of the northeastern portion of Morice

Lake. Main tributaries to the Morice River are the Thautil River, Gosnell River, Crystal Creek, Lamprey Creek, Owen Creek, Houston Tommy River, and Gold Creek.

## Water Collection

Water samples were collected from 15 geographically distinct locations (Figure 4.1) in August 2003 along the mainstem of the Morice River, and from major tributaries and areas where bull trout are known to inhabit. The methods for water collection and analysis are outlined in Chapter 3.

## Fish Collection

To correlate fin-ray chemistry to water chemistry, 10 fish were used where capture location was known (i.e. upper Morice River (n=6), lower Morice River (n=3) and the Nanika River (n=1). Fin-rays were obtained from a radio-telemetry study conducted earlier on the Morice River (Bahr 2002). The leading pectoral fin-ray of each fish was surgically removed prior to release of the animal.

## Fin-ray Chemistry

Fin-rays were sectioned and sonicated in ultra-pure water for five minutes, embedded in epoxy resin, polished with 1200  $\mu$ m silicon carbide paper, and



Figure 4.1. Map of the Morice River watershed showing water sampling locations. Water was collected from Gold Creek, Houston Tommy Creek, Owen Creek, Lamprey Creek, Thautil River, Denys Creek, Gosnell Creek, Crystal Creek, Redslide Creek, Nanika River, Morice Lake, and three locations in Morice River.

sequentially polished with 6  $\mu$ m, 1  $\mu$ m, and 0.05  $\mu$ m diamond suspension polish to obtain an adequate surface for ablation with the laser. LA-ICP-MS analysis, collection and reduction, and statistical analyses were all completed using the same protocol outlined in Chapter 2 and 3.

#### RESULTS

Water chemistry values (mmol/mol) for the 15 sampling sites located throughout the Morice River watershed are shown in Table 4.1. The water chemistries for Ba:Ca, Sr:Ca, and Mg:Ca were similar throughout the watershed. Greater differences in Mn:Ca were found throughout the watershed, particularly in tributaries when compared to the mainstem river. The two tributaries that showed distinct differences from the rest of the watershed were Lamprey Creek and Owen Creek. Assigning a unique chemical signature to a particular stream, however, was difficult, as there was considerable overlap in signatures among many of the streams (Table 4.1). Canonical discriminant function analysis was used to assess separation of the various sampling locations using the factor loadings of Sr:Ca, Ba:Ca, and Mn:Ca (Figure 4.2). These data clearly show that geographical separation using the elemental ratios measured in this study for the Morice River watershed are not adequate to distinguish movement patterns within Morice Lake and both the Morice and Nanika Rivers, where adult bull trout reside for the majority of their life-history in this watershed.

Map #	Location	Ba:Ca	Mg:Ca	Mn:Ca	Sr:Ca		
1	Lower Morice	2.35	87.1	0.181	458.4	-	
2	Middle Morice	2.09	87.0	0.395	439.4		
3	Upper Morice	2.23	75.1	0.098	445.4		
4	Morice Lake	2.24	74.6	0.134	464.0		
5	Gold Creek	2.41	126.0	0.101	295.3		
6	Houston Tommy	3.69	91.0	0.033	292.0	292.0	
	Creek						
7	Denys Creek	1.23	106.0	0.008	325.3		
8	Crystal Creek	3.26	70.1	0.015	278.3		
9	Gosnell River	1.31	108.0	0.622	383.0		
10	Thautil River	2.33	146.0	0.074	400.0		
11	Owen Creek	1.65	234.0	1.001	900.0		
12	Lamprey Creek	2.64	219.5	1.637	909.0		
13	Lower Nanika	1.93	83.4	0.361	490.0		
14	Upper Nanika	1.83	87.5	0.506	501.0		
15	Redslide Creek	2.51	49.5	0.427	366.6	366.6	

Table 4.1. Water chemical ratios measured in the Morice River watershed. All measured values are expressed as mmol/mol.





Incorporation coefficients for each element ratio were calculated as well as the sample standard deviations (SD) (Microsoft Excel 2002, Microsoft Corporation) Ba:Ca = 0.020 (SD = 0.003), Mg:Ca = 0.36 (SD = 0.060), Mn:Ca = 0.19 (SD = 0.037), and Sr:Ca = 0.15 (SD = 0.014) (Table 4.2).

Figures 4.3 and 4.4 show representative line scans for two bull trout. In Figure 4.4, there is little change in the line scans for any of the elemental ratios for bull trout 10, except near the core for both Ba:Ca and Mn:Ca ratios. In contrast, the line scans for bull trout #7 in Figure 4.3 show much more variation over the entire region of the pectoral fin-ray. One fish depicts migratory behaviour, while the other fish appears to be non-migratory, as determined by chemical ratios measured across all life-history stages. Due to the similarity in chemical make-up of both the Morice River and the Nanika River, it is difficult to determine if the non-migratory fish show movement. The fish that show high variability in chemical signatures over their life-history appear to be moving into chemically distinct environments. On the other hand, fish that show relatively constant line scans may be either non-migratory or just moving among river systems where elemental ratios are similar. Figures 4.5 and 4.6 also demonstrate with Canonical Discriminant Function analysis that the Morice River watershed is not suited for an examination of movement patterns using the factors (water chemistry) Ba:Ca, Sr:Ca, and Mn:Ca. Representative bull trout showing both variation in chemical signatures and a relatively consistent line scan across the fin-ray, bull trout #7 and #10 respectively, are illustrated (Figures 4.3 and 4.4). Nine different areas were utilized for bull trout #7 across the line scan, where variability in

Fish #	Ba/Ca (mmol/mol)			Mg/Ca (mmol/mol)			Mn/Ca (mmol/mol)			Sr/Ca (mmol/mol)		
	Water	Fish	IC	Water	Fish	IC	Water	Fish	IC	Water	Fish	Í IC
1	2.2	0.043	0.019	74.9	26	0.347	0.10	0.022	0.224	4.5	0.750	0.168
2	2.2	0.047	0.021	74.9	33	0.440	0.10	0.020	0.204	4.5	0.730	0.164
3	2.2	0.045	0.020	74.9	28	0.374	0.10	0.019	0.193	4.5	0.600	0.135
4	2.2	0.050	0.022	74.9	32	0.427	0.10	0.021	0.214	4.5	0.770	0.173
5	2.2	0.041	0.018	74.9	33	0.440	0.10	0.024	0.244	4.5	0.680	0.153
6	2.2	0.042	0.019	74.9	30	0.400	0.10	0.018	0.183	4.5	0.650	0.146
7	2.4	0.040	0.017	87.1	25	0.287	0.18	0.030	0.165	4.6	0.700	0.153
8	2.4	0.060	0.025	87.1	26	0.299	0.18	0.020	0.110	4.6	0.700	0.153
9	2.4	0.040	0.017	87.1	26	0.299	0.18	0.036	0.199	4.6	0.600	0.131
10	1.8	0.030	0.016	87.6	30	0.342	0.51	0.083	0.164	5.0	0.700	0.140

Table 4.2. Relationship between fin-ray chemistry measured at the outer edge and water chemistry for 10 bull trout captured in the Morice watershed. Incorporation coefficients were calculated for each fish and the sample standard deviation (SD) was calculated.



Figure 4.3. Representative line scans for a bull trout (bull trout #7) that had chemical signatures which were indicative of movements throughout its life-history.



Figure 4.4. Representative line scans for a bull trout (bull trout #10) that had chemical signatures which showed little movement throughout its life-history.



Figure 4.5. Canonical discriminant function analysis for bull trout # 10. The letters indicate locations throughout the life of each individual with (a) representing the capture location and (i) representing the approximate first year of growth.




the chemical signature was noted. The same nine areas were analyzed for bull trout #10 to maintain consistency in the analysis. Letters (a-i) were assigned where (a) represents the capture location and (i) represents what was estimated to be the first year of growth. Each letter corresponds to a unique chemical signature (Ba:Ca, Sr:Ca, Mn:Ca) that were input into the Canonical Discriminant function. Bull trout #7 showed very little variation according to the discriminant functions. Most of the variation in the line scan for bull trout #7 was attributed to Mn:Ca ratios. Where changes are noted, there was no corresponding river system suggesting that the fish might have moved into an area where water chemistries were unknown. Interestingly, elemental ratios suggest that bull trout #10 remained in the Morice and Nanika Rivers, with one migration to the Gosnell Creek watershed.

Measured values of Zn showed an interesting pattern of deposition across the finray. Oscillations were apparent and seemed to correlate to annulus formation in the finray. Bull trout #10 demonstrated six full oscillations, while bull trout #7 demonstrated seven full oscillations (Figure 4.7). Table 4.3 provides the length, weight, and age of the bull trout utilized for the analysis. Conventional ageing was accomplished for the 10 bull trout in this study by North/South consultants who counted the opaque and translucent zones formed in the fin-ray. The zones typically represent winter and summer growth, respectively. Ages were also assessed by counting the number of full oscillations shown by Zn:Ca ratios. Zn:Ca oscillations were counted by two readers who did not know the ages of the fish prior to examination of the data.



Figure 4.7. Zn profile for bull trout #2. (top) and bull trout #7 (bottom) There are six clear oscillations that correspond to the six annuli present in the fin-ray for bull trout #2 and seven clear oscillations that correspond to the seven annuli present in the fin-ray in bull trout #7.

Fish #	Length (mm)	# of Annuli (age)	Seasonal Zn fluctuations (age)
1	540	6	6, 5.5
2	420	6	6.5, 6.5
3	515	9	8, 9
4	545	7	7,7
5	510	8	unreadable, 8
6	465	7	6.5, 6
7	540	7	7,7
8	560	8	7,8
9	455	6	5.5, 6
10	520	6	6, 6

Table 4.3. Length and age for the 10 bull trout sampled in this investigation. Age assessments (annuli) were performed independently by North/South consultants. Seasonal fluctuation in Zn were determined by the # of peaks of Zn:Ca measured in the fin-ray. Values separated by comma were conducted by two different readers.

#### DISCUSSION

Our research is important to the growing knowledge on using natural chemical tags in fish as determinants of life-history. Elemental ratios fluctuate over the life of the fish, as we observed differences among the 10 fish examined in this study. Changes in Ba:Ca and Mn:Ca ratios were evident, but little change was observed for the Sr:Ca and Mg:Ca ratios. It would appear the elemental signatures do not differ sufficiently in all elements to detect movements within the mainstem of the Morice River, however, we did determine that fin-ray chemistries measured at the outer edge were proportional to water chemistries measured at the same location. Additionally it was shown that Zn incorporation into the fin-ray appears to follow a seasonal oscillation with more incorporation occurring in the summer.

#### Geographic Separation

The mainstem Morice River showed a remarkably consistent chemical signature throughout its length. Tributaries, however, were sufficiently different to discriminate water sample locations with Canonical Discriminant Function analysis. Two other tributaries, Owen and Lamprey Creeks, had unique water chemistries; however, Bustard and Schell (2002) note that only Dolly Varden are present in these creeks. Owen and Lamprey Creeks are both lake fed tributaries characterized by seasonally warmer temperatures likely unsuitable for bull trout. Movement patterns of bull trout were

difficult to discriminate as it appears most movement is confined to the chemically similar systems including: Morice Lake and both the Morice and Nanika Rivers. Bahr (2002) observed both small movements and large scale movements of bull trout within these same systems with radio telemetry data. Our study could not resolve the differences between these systems; thus, we could only come to the conclusion that most movements were restricted to the larger systems in this watershed causing discrimination between individuals to be difficult. We were also unable to classify movements into spawning tributaries, even though water chemistries were distinctive within these systems. Many bull trout in the Morice watershed spend less than a week in these spawning tributaries (Bahr 2002), possibly limiting the amount of material incorporated into the fin-ray during these migrations. The amount of time spent spawning for the 10 fish examined was probably insufficient for us to spatially resolve with probe-based analysis. An examination using otoliths may have revealed these migrations, as otoliths provide much more spatial resolution due the larger size of the structure. Otolith growth is also continual and, in bull trout, provides a greater surface area for ablation than the fin-ray. Another possible explanation is that the bull trout investigated had not yet made their first spawning migration. The latter scenario is unlikely, as bull trout of the same size class have been observed spawning within the Morice River tributaries (Bahr 2002). It appears that in a chemically similar environment (such as the Morice River watershed), that radio-telemetry is a better tool for resolving fish movements.

# Fin-ray Chemistry

To date, there has been very little research conducted using natural chemical markers with fin-rays. For this reason, the process of incorporation of elements is poorly understood. Interestingly, the coefficients obtained for Ba:Ca and Sr:Ca were very close to the coefficients determined in slimy sculpin otoliths: 0.019 and 0.21 respectively (Chapter 3). On the other hand, the coefficients determined for Mg:Ca and Mn:Ca were much higher in fin-rays than the coefficients measured for the same elements in otoliths. These data suggest that fin-rays incorporate both Ba:Ca and Sr:Ca in similiar proportion as otoliths; however fin-rays show a much higher affinity for the incorporation of Mg and Mn. Using fin-rays to resolve movements in chemically unique watersheds, therefore, is very promising. Resolution of stream of origin for slimy sculpins in Chapter 3 would have been much higher if both Mg and Mn could have been used to help differentiate populations; however, bull trout movement patterns were very difficult to resolve in the Morice River watershed, due to the similarity of the water in this geographical region. In fact, we were unable to discriminate between fish that were expected to show large movements from fish where small migrations were anticipated. Some of the fish in this study showed variation in measured fin-ray chemical ratios, suggesting movements into chemically distinct habitats. On the other hand, fish that had relatively flat line scans may have exhibited highly migratory behaviour restricted to Morice Lake, and both the Morice and Nanika Rivers. Bahr (2002) supports this with telemetry data where many

Morice bull trout made extensive migrations from the lower Morice River to the Upper Nanika River, (a distance greater than 100 km).

Our results support the findings of Halden et al. (2000), who determined that seasonal deposition of Zn in Arctic char otoliths correlates to annulus formation. Milner (1982), as well as, Bradley and Sprague (1985), suggest that metabolic rate influences Zn deposition in fish. Seasonal summer temperatures likely influence the uptake and production of Zn, while colder winter temperatures would represent times when lower levels of Zn production and uptake occur (Halden et al. 2000). Bull trout fin-rays also show seasonal fluctuations in the distribution of Zn. For nine of the 10 bull trout examined in our study, we were able to interpret the fluctuations of Zn present in finrays as yearly increments because ages corresponded well to independent age estimates provided by North/South Consulting for the same nine fish. Halden et al. (2000) noted that the incorporation of Zn into Arctic char otoliths decreases with age. The results of Halden et al. (2000) are also consistent with other studies examining Zn uptake by fish (Milner 1982; Bradley and Sprague 1985; Campbell and Stokes 1985). Our results, however, did not show Zn uptake decreasing with age over the lifetime of the bull trout in our study. The seasonal fluctuations in Zn for bull trout fin-rays determined by our study appear to vary in magnitude along the line scan; however, there is no trend showing an increase or decrease in overall Zn content.

An examination of fin-ray chemistry determined that these structures incorporate elements in proportion to the recent ambient water chemistry. The chemical ratios measured at the outside edge of the fin-rays were representative of the water chemical ratios measured at the capture location. Measuring chemical ratios in bony structures at the outer edge and comparing the values to those of water is not the most effective way of obtaining an incorporation coefficient. Fish in a non-migratory life-history stage should be captured from an area where it has been determined that water chemistry is stable over time periods long enough to obtain a stable signature from the bony structure. Alternately, fish can be raised in an artificial environment where water chemistries are known. For these reasons, our incorporation coefficients may not reflect the exact physiological relationship between the magnitude of elemental concentration in the water to the uptake of trace elements by the fish; however, we feel that the values are representative.

The findings of our study are important, as they show that fin-rays have the potential to replace or compliment otoliths in investigations examining fish life histories with chemical markers and can offer a non-lethal alternative, however, water chemical ratios in the Morice River watershed were very similar, so movements of bull trout could not be determined. Results we obtained in Chapter 3 for the Williston Reservoir determined that the streams and rivers showed distinctively different elemental signatures. Conflicting results for the two studies show that not all watersheds are

suitable for an examination of fish movement patterns or stock identification with natural chemical markers. This determination is important, as researchers must be aware of the limitations that are associated with this technique. The potential to resolve freshwater migrations using natural chemical tags deposited spatially in bony structures is high, provided the streams and rivers in the study area are both chemically stable over time and distinct. We found in Chapter 3 that the Williston Reservoir meets both of these requirements and is likely a very good candidate for examining movement patterns of migratory fish using natural chemical tags in the future.

# **Chapter 5: Epilogue**

This thesis examined the life-history of three different species using natural chemical markers deposited spatially in bony structures. We were successful in resolving the movements of the anadromous eulachon and matching the specific geographic location of slimy sculpins to the water chemistries where they were captured. Unfortunately, we were unable to reveal the movements of migratory bull trout within the Morice River watershed. Nevertheless, bull trout fin-rays appear to proportionately incorporate some trace elements from the ambient water; this result will provide researchers with another avenue to explore the developing field of using natural elemental markers as determinants of life-history.

Differences in chemical signatures among aquatic media were measured to assess habitat use and movement patterns in an attempt to understand life-history of these three species. Substantial differences exist in chemical signatures between both freshwater and saltwater environments. The specific element examined in Chapter 2 to infer the life-history of eulachon moving between the two environments was Sr. Movements should have been easy to assess as the difference in the magnitude of Sr concentration is high between fresh and saltwater; nevertheless short residency time in freshwater preand post-spawning limited our ability to detect a freshwater Sr signal for eulachon. Seasonal differences in Ba:Ca uptake were also observed and we felt that the most plausible explanation was variable ocean temperatures. The fluctuations in Ba:Ca uptake were considered a proxy for winter and summer growth and were subsequently used to

assign ages to the individual eulachon. Geochemical differences in freshwater chemical ratios were utilized in Chapter 3 to infer the stream of residency for slimy sculpins. Large differences between streams were observed which allowed good separation of habitats based on the chemical geology. Thus, we were successful in assigning the capture locations to the stream chemistry using otolith microchemistry. On the other hand, the geochemical differences in the Morice River watershed were not sufficiently distinct to resolve movements using fin-rays. Results from the Williston and Morice watersheds suggest that natural chemical markers are only suitable for areas where large difference in chemical signatures exists among tributaries and throughout the mainstem river. Where there are distinct differences the use of this technique will offer a new tool in order to understand the complex life histories of many fish species.

Fisheries managers are always looking to find new ways to assess fish population status and structure, habitat utilization, and movement patterns. Many fish species in northern British Columbia are difficult to track and locate due to many factors including: river turbidity, lake depth, and remote locations. The ability to reveal previously unknown life-history characteristics without physically tracking the animals will undoubtedly help provide information to make informed management decisions. Population (stock) structure has previously been determined for many species of fish using either, or, a combination of, genetics, radio telemetry, and conventional tagging techniques. The results of genetic analyses for determining population structure are sometimes confounded by migration as fish often move in and out of a specific area.

Even a very small amount of juvenile or adult mixing between populations will limit the capabilities of genetic analyses for inferring stock structure (Hartl and Clark 1989). Genetic analyses are better suited to pedigree analysis (family structure) and determining phenotypic evolutionary linkages (Wilson and Ferguson 2002). Genetic analyses are also not suitable for an examination of fish movement patterns and habitat utilization due to the high dispersal rates and mixing of populations common to many fish species (Campana and Thorrold 2001).

Habitat utilization is a key life-history characteristic that fisheries managers need to understand. Radio-telemetry, and to a lesser extent conventional tagging techniques, provide information on both movement patterns and habitat utilization of fish large enough to tag; however, these techniques offer little insight into stock structure as only a small portion of the population can be examined. Additionally, physical tags only allow for an understanding of a small period of the animals life as it is prohibitive to track an animal from the juvenile stage until senescence. In fact, there is likely a bias in study designs where larger fish are utilized (Steingrimsson and Grant 2003). An inherent problem with physically tagging the animal is that the normal physiology and behaviour of the animal may be affected through the application of the external tag. Bridger and Booth (2003) point out that researchers' must be keenly aware of the effects that the application of a physical tag (radio transmitter) has on fish and how various attachment locations affect fish differently.

Elemental analysis of bony structures provides fisheries managers with a tool that is indicative of fish population structure over a very recent time period (the lifetime of individuals within a stock) so periodic migrations are not an issue; genetic analysis examines population structure over a much longer time period. The combination of these two techniques may provide the best option for examining stock structure in fish species, as natural tags may provide information that is more relevant to recent changes that populations encounter; genetic analyses provides information towards parental and evolutionary linkages (Ferguson and Danzmann 1998). Additionally, we believe that chemical markers have the potential to resolve both movement patterns and habitat utilization over the life of individual fish, regardless of their size or life-history characteristics. In a recent study using a non-invasive tagging and recovery technique (tagged subcutaneously with dye and monitored with snorkeling) 320 Atlantic salmon (Salmo salar) young of the year were monitored successfully for 28-74 days (Steingrimsson and Grant 2003). That study determined that most of the young of the year Atlantic salmon moved less than 120 m from their original location. One hundred and nineteen tagged young of the year fish were lost due to either emigration or mortality over the monitoring period. Those authors suggest qualitatively that the fish not recovered, disappeared as a result of mortality and not emigration from the study site. Although Steingrimsson and Grant (2003) did an exceptional job in ensuring that their tagging protocol did not influence the behaviour of the fish, their study outlines one of the main issues surrounding tagging; the location and fate of the missing fish is unknown.

Elemental analysis of bony structures has a definite advantage in that the natural lifehistory of the animal is not affected by tagging, the entire life-history can be examined, and fish cannot be lost due to emigration or mortality.

Using chemical signatures, we were able to determine a previously undetermined life-history characteristic, semelparity for eulachon, and confirm age at maturity. Additionally, our study was able to use otolith microchemistry to correlate the specific capture location of slimy sculpins to the water chemistry at each location within the Williston watershed. Moreover, using natural chemical markers to track fish in sufficiently chemically distinct watersheds appears to be a reasonable next step in this rapidly developing field.

### REFERENCES

- Akielazek, J.J, Moring, J.R., and Chapman, S.R. 1985. Experimental culture of young rainbow smelt Osmerus mordax. Trans. Am. Fish. Soc. **114:** 596-603.
- Armstrong, A.T. 1979. Report on exploration activities on North Carbon Creek Coal property Supplementary Geological Investigations. Private Utah Mines Ltd. report. 32 pp.
- Babaluk, J.A., Halden, N.M., Reist, J.D., Kristofferson, A.H., Campbell, J.L., and Teesdale, W.J. 1997. Evidence for non-anadromous behaviour of arctic charr (Salvelinus alpinus) from Lake Hazen, Ellesmere Island, northwest Territories, Canada, based on scanning microprobe analysis of otolith strontium distribution. Arctic 50: 224-233.
- Bahr M.A. 2002. Movement patterns, timing of migration and genetic population structure of bull trout (Salvelinus confluentus) in the Morice River Watershed, British Columbia, MSc thesis, University of Northern British Columbia. 99 pp.
- Bailey, M. 2000. Eulachon Research Council, May 2000. Minutes summarizing meetings in New Westminister, Terrace, and Bella Coola, B.C.. Informal joint report prepared jointly by BC Forests and Fisheries and Oceans, Canada. 24 pp.
- Barraclough, W.E. 1964. Contribution to the marine life-history of the eulachon Thaleichthys pacificus. J. Fish. Res. Bd. Can. **21**: 1333-1337.
- Bath, G.E., Thorrold, S.R., Jones, C.M., Campana, S.E., McLaren, J.W., and Lam, J.H.W.
  2000. Strontium and barium uptake in aragonitic otoliths of marine fish.
  Geochemica et Cosmochimica Acta 64: 1705-1714.
- Bath-Martin, G.E., Thorrold, S.R., and Jones, C.M. 2004. Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). *Can. J. Fish. Aquat. Sci.* **61**: 34-42.
- Benes, P. 1979. Semicontinuous monitoring of truly dissolved forms of trace elements in streams using dialysis *in situ*. Principles and conditions. *Water Research* **14**: 511-513.
- Bilton, H.T. and Robbins, G.L. 1971. Effects of starvation, feeding, and light period on circulus formation on scales of young sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board. Can. 28: 1749-1755.

- Bustard, D., and Schell, C. 2002. Conserving Morice watershed fish populations and their habitat. Stage II Biophysical profile. Technical Report Submitted to Community Futures Development Corporation of Nadina, Smithers BC. 97pp.
- Bradley R.W., and Sprague J.B. 1985. The influence of pH,water hardness, and alkalinity on the acute lethalityof zinc to rainbow trout (Salmo gairdneri). *Can. J.Fish. Aquat. Sci.* **42**: 731-736.
- Bridger, C.J., and Booth, R.K. 2003. The effects of biotelemetry transmitter presence and attachment procedures on fish physiology and behaviour. *Reviews in Fisheries Science* **11**: 13-34.
- Campana, S.E., Chouinard, G.A., Hanson, J.M., Frechet, A., and Brattey, J. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. *Fisheries Research* **46**: 343-357.
- Campana, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation models. *Journal of Fish Biology* **59**: 197-242.
- Campana, S.E., and Thorrold, S.R. 2001. Otoliths, increments, and elements: key to a comprehensive understanding of fish populations? *Can. J. Fish. Aquat. Sci.* **58**: 30-38.
- Campana,, S.E., Thorrold, S.R., Jones, C.M., Gunther, D., Tubrett, M., Longerich, H., Jackson, S., Halden, N.M., Kalish, J.M. Piccoli, P., de Pontual, H., Troadec, H., Panfili, J., Secor, D.H., Severin, K.P., Sie, S.H., Thresher, R., Teesdale, W.J., and Campbell, J.L. 1997. Comparison of accuracy, precision and sensitivity in elemental assays of fish otoliths using electron microprobe, proton-induced X-ray emission, and laser ablation inductively coupled mass spectrometry. *Can. J. Fish. Aquat. Sci.* 54: 2068-2079.
- Campana, S.E., Fowler, A.J., and Jones, C.M. 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. *Can. J. Fish. Aquat. Sci.* **51**: 1942-1950.
- Campana, S. E. and J.D. Nielson. 1985. Microstructure of fish otoliths. *Can. J. Fish. Aquat. Sci.* **42**:1014-1032.
- Campbell, P. C. G., and Stokes, P. 1985. Acidification and toxicity of metals to aquactic biota. *Can J. Fish. Aquat. Sci.* **42**: 2034–3049.

- Cannings, S.G., and Ptolemy, J. 1998. Rare freshwater fish of British Columbia. British Columbia Ministry of Environment, Lands, and Parks, Victoria, BC. 214 pp.
- Chan, L. H., Drummond, D., Edmond, J. M, and Grant, B. 1977. On the barium data from the Atlantic GEOSECS Expedition, *Deep-Sea Res.* **24**: 613-649.
- Charnov, E.L., and Schaffer, W.M. 1973. Life-history consequences of natural selection: Cole's result revisted. *Am. Nat.* **107**: 791-793.
- Cooper, J.E. 1978. Identification of eggs, larvae and juveniles of the rainbow smelt, Osmerus mordax, with comparison to larval alewife, Alosa pseudoharengus and gizzard shad, Dorosoma cepedianum. Trans. Am. Fish. Soc. **107**: 56-62.
- Copeman, D.G. 1977. Population differences in rainbow smelt, Osmerus mordax: multivariate analysis of mensural and meristic data. J. Fish. Res. Bd. Can. 34: 1220-1229.
- Coutant, C.C., and Chen, C.H. 1993. Strontium microstructure in scales of freshwater and estuarine striped bass (*Morone saxatilis*) detected by laser ablation mass spectrometry. *Can. J. Fish. Aquat. Sci.* **50**: 1318-1323.
- Delacy, A.C., and Batts, B.S. 1963. Possible population heterogeneity in the Columbia River smelt. Circular No. 198. Fisheries Research Institute, College of Fisheries, University of Washington, Seattle.
- Denoyer, E.R., Fredeen, K.J., and Hager, J.W. 1991. Laser solid sampling for inductively coupled plasma mass spectrometry. *Anal. Chem.* **63**: 445-457.
- DFO 2003. 2002 Pacific Region State of the Ocean. DFO Science Ocean Status Report, Nanaimo British Columbia 56 pp.
- Eek, D. and Bohlin, T. 1997. Strontium in scales verifies that sympatric sea-run and stream-resident brown trout can be distinguished by coloration. *J. Fish Biol.* **51**: 659-661.
- Elsdon, T.S., and Gillanders, B.M. 2002. Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. *Can. J. Fish. Aquat. Sci.* **59**: 1796-1808.

- Farara, D. 2000. Eulachon Research Council, May 2000. Minutes summarizing meetings in New Westminister, Terrace, and Bella Coola, B.C.. Informal joint report prepared jointly by BC Forests and Fisheries and Oceans, Nanaimo BC, Canada. 24 pp.
- Farrell, A.P., Hodaly, A.H., and Wang, S. 2000. Metal analysis of scales taken from Arctic grayling. Arch. Environ. Contam. Toxicol. **39**: 515-522.
- Fenwick, J.C. 1989. Calcium exchange across fish gills. Vertebrate Endocrinology: Fundamentals and Biomedical implications (eds. P.K.T. Pang and M.P. Schreibman). Academic Press, San Diego, CA. Volume 3: 319-342.
- Ferguson, M.M. and Danzmann, R.G. 1998. The role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? Can. J. Fish. Aquat. Sci. 55: 1553-1563
- Fowler, A.J., Campana, S.E., Jones, C.M., and Thorrold, S.R. 1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using laser ablation ICPMS. *Can. J. Fish. Aquat. Sci.* **52**: 1431-1441.
- Frie, R.V. and G.R. Spangler. 1985. Dynamics of rainbow smelt during and after exploitation in South Bay, Lake Huron. *Trans. Am. Fish. Soc.* **114**: 713-724.
- Friedland, K.D., Reddin, D.G., Nobumichi, S., Hass, R.E., and Youngson, A.F. 1998. Strontium: Calcium ratios in Atlantic salmon (*Salmo salar*) otoliths and observations on growth and maturity. *Can. J. Fish. Aquat. Sci.* **55**: 1158-1168.
- Gaillardet J., Viers J., and Dupre, B. 2003 Trace Elements in River Waters. . Surface and Ground Water, Weathering, and Soils (ed. J.I. Drever) vol 5 Treatise on Geochemistry (eds. H.D. Holland and K.K. Turekian), Elsevier-Pergamon, Oxford. pp. 225-272
- Gowan, C., Young, M.K., Fausch, K.D, and Riley, S.C. 1994. Restricted movement in resident stream salmonids a paradigm lost? *Can. J. Fish. Aquat. Sci.* **51:** 626-2637.
- Guay, C. K., and Falkner, K.K. 1998. A survey of dissolved barium in the estuaries of major Arctic rivers and adjacent seas. *Cont. Shelf. Res.* **18**: 859-882.
- Halden, N.M. 2001. Coloured fish ears: Cathodoluminescence as a guide to variation in aqueous environments. Newsletter of the Mineralogical Association of Canada 64: 1-16.

- Halden, N.M., Babaluk, J.A., Kristofferson, A.H., Campbell, J.L., Teesdale, W.J., and Reist, J.D. 1996. Micro-Pixe studies of strontium zoning in Arctic charr otoliths: migratory behaviour and stock discrimination. *Nucl. Instrum. Meth. B.* 189: 190-195.
- Hartl, D.L., and Clark, A.G. 1989. Principles of Population Genetics. Sinauer Associates, Sunderland, MA. 450 pp.
- Haldorson, L. and Craig, P. 1984. Life-history and ecology of a Pacific-Arctic population of rainbow smelt in coastal waters of the Beaufort Sea. *Trans. Am. Fish. Soc.* **113**: 33-38.
- Hart, J.L. 1974. Pacific Fishes of Canada. Fish. Res. Bd. Canada Bull. 180: 737 pp.
- Hart, J.L. and McHugh, J.L. 1944. The smelts (Osmeridae) of British Columbia. *Res. Bd. Can. Bull.* **64:** 27 pp.
- Hay, D.E., Harbo, R., Southey, C.E., Clarke, J.R., Parker, G., and McCarter, P.B. 1998.
   Catch composition of British Columbia shrimp trawls and preliminary estimation of bycatch – with emphasis on eulachons. Canadian Stock Assessment Secretariat Research Document. Fisheries and Oceans Canada 1999, Nanaimo, BC. 111 pp.
- Hay, D.E. and McCarter, P.B. 2000. Status of the eulachon *Thaleicthys pacificus* in Canada. Canadian Stock Assessment Secretariat Research Document. Fisheries and Oceans Canada 2000, Nanaimo, BC. 50 pp.
- Howland, K.L., and Tonn, W.M. 2001. Identification of freshwater and anadromous inconnu in the Mackenzie River system by analysis of otoliths strontium. *Trans. Amer. Fish. Soc.* **130**: 725-731.
- Kalish, J.M. 1990. Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. *Fish. Bull.* **88**: 657-666.
- Kalish, J.M. 1991. Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus*. *Mar. Ecol. Prog. Ser.* **74**: 137-159.
- Karnacky, K.J. Jr. 1998. Osmotic and Ionic Regulation. The Physiology of Fishes Second Edition (ed. David H. Evans). CRC Press, Washington DC. 519 pp.

- Katano, O. and Iguchi, K. 1996. Individual differences in territory and growth of ayu, *Plecoglossus altivelis* (Osmeridae). *Can. J. Zool.* **74**: 2170-2177.
- Kelson, J. 2000. Eulachon Research Council, May 2000. Minutes summarizing meetings in New Westminister, Terrace, and Bella Coola, B.C.. Informal joint report prepared jointly by BC Forests and Fisheries and Oceans, Nanaimo, BC. 24 pp.
- Kennedy, B.P., Folt, C.L., Blum, J.D., and Nislow, K.H. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. *Can. J. Fish. Aquat. Sci.* **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. *Can. J. Fish. Aquat. Sci.* **59**: 925-929.
- Kennedy, B.P., Blum, J.D., and Folt, C.L. 1997. Natural isotope markers in salmon. *Nature*. **387:** 766-767.
- Li, Y.-H., and Chan, L. H. 1979. Desorption of Ba and <sup>226</sup>Ra from river borne sediments in the Hudson Estuary. *Earth Planet. Sci. Lett.* **43**: 343-350.
- Limburg, K.E. 1998. Anomalous migrations of anadromous herrings revealed with natural chemical tracers. *Can. J. Fish. Aquat. Sci.* **55**: 431-437.
- Marshall, D.J. 1988. Cathodoluminescence of Geological Materials:, Unwin Hyman, Boston, MA. 146 pp.
- McAllister, D.E. 1963. A revision of the smelt family, Osmeridae. Bull. 191. Biological Series 71. National Museum of Canada, Ottawa, ON. 53 pp.
- McDowall, R.M. 1992. Diadromy: origins and definitions of terminology. *Copeia* **1992**: 248-251.
- McDowall, R.M. 1987. The occurrence and distribution of diadromy among fishes. American Fisheries Society Symposium 1: 1-13.
- McDowall, R.M. 1972. The taxonomy of estuarine and brackish-lake Retropinna from New Zealand (Galaxioidei: Retropinnidae). J. R. Soc. N.Z. 2: 501-531.
- McLean, J.E., Hay, D.E., and Taylor, E.B. 1999. Marine population structure in an anadromous fish: life-history influences patterns of mitochondrial DNA variation in the eulachon, *Thaleicthys pacificus*. *Molecular Ecology* **8**: S143-S158.

- McPhail, J.D. and Lindsey, C.C. 1970. Freshwater fishes of north-western Canada and Alaska. *Fish. Res. Bd. Can. Bull.* **173**.
- Milner, N. J. 1982. The accumulation of zinc by O-group plaice, *Pleuronectes platessa* (L.), from high concentrations in seawater and food. *J. Fish. Biol.* **21**: 325 -336.
- Milton, D.A., Tenakanai, C.D., and Chenery, S.R. 2000. Can the movements of barramundi in the Fly River, Papua New Guinea be traced in their otoliths? *Estuarine, Coastal, and Shelf Science* **50**: 855-868.
- Morrow, J.E. 1980. The freshwater fishes of Alaska. Alaska Northwest Publishing Company, Anchorage, AK. 248 pp.
- Moyle, P.B., Herbold, B., Stevens, D.E., and Miller, L.W. 1992. Life-history of delta smelt in the Sacremento-San Joaquin Estuary, California. *Trans. Am. Fish. Soc.* **121:** 67-77.
- Moyle, P.B. 1976. Inland fishes of California. University of California Press, Berkely. 405 pp.
- Moyle, P.B. and Cech Jr, J.J. 2004. Fishes: An introduction to ichthyology. 5<sup>th</sup> edition. Prentice-Hall Inc. Upper Saddle River, NJ. 612 pp.
- Page, L.M. and Burr, B.M. 1991. A field guide to freshwater fishes of North America north of Mexico. Houghton Mifflin Company, Boston. 432 pp.
- Pannella, G. 1971. Fish otoliths daily growth layers and periodical patterns. *Science* **173**: 1124.
- Payne, S.A., Johnson, B.A., and Otto, R.S. 1999. Proximate composition of some northeastern Pacific forage fish species. *Fish Oceanogr.* **83**: 159-177.
- Peltier, E.F., Webb, S.M., and Gaillard, J.F. 2003. Zinc and lead sequestration in an impacted wetland system. Advances in Environmental Research **8**: 103-113.
- Pootlace, A. and Siwallace, A. 2000. Eulachon Research Council, May 2000. Minutes summarizing meetings in New Westminister, Terrace, and Bella Coola, B.C.. In formal joint report prepared jointly by BC Forests and Fisheries and Oceans, Nanaimo, BC, Canada. 24 pp.
- Ricker, W.E., Manzer, D.F., and Neave, E.A. 1954. The Fraser River eulachon fishery, 1941-1953. Fish. Res. Bd. Canada. No. 583: 35 pp.

- Rutter, N.W. 1976. Multiple glaciations in the area of Williston Lake, British Columbia. Geological Survey of Canada. Bulletin 273. 31 pp.
- Sanborn, M., and Telmer, K. 2003. The spatial resolution of LA-ICP-MS line scans across heterogeneous materials such as fish otoliths: an experiment on a sandwich of NIST glasses 611, 613, and 615. *JAAS* **18**: 1231-1238.
- Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. *Fish. Res. Bd. Canada Bull.* **184:** 1-966.
- Shepherd, B.G., and Vroom, P.R. 1977. Biology of the Nass River eulachon. Technical Report: PAC, /T-, 77-10. Department of fisheries and the environment, Pacific Region.
- Shiller, A.M. 2003. Syringe filtration methods for examining dissolved and colloidal trace element distributions in remote field locations. *Environmental Science & Technology* **37**: 3953–3957.
- Steingrimsson, S.O., and Grant, J.W.A. 2003. Patterns and correlates of movement and site fidelity in individually tagged young-of-the-year Atlantic salmon (Salmo salar). Can. J. Fish. Aquat. Sci. 60: 193-202.
- Swanson, C., Young, P.S., and Cech Jr., J.J. 1998. Swimming performance of delta smelt: Maximum performance, and behavioral and kinematic limitations on swimming at submaximal velocities. J. Exp. Biol. 201: 333-345.
- Taylor, E.B. and Bentzen, P. 1993. Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (*Osmerus*) in North-Eastern North America. *Evolution* **47**: 813-832.
- Taylor B.R. and Hamilton H.R. 1994. Comparison of methods for determination of total solutes in flowing waters. *Journal of Hydrology* **154**: 291-300.
- Thorrold, S.R., and Shuttleworth, S. 2000. In situ analysis of trace elements and isotope ratios in fish otoliths using laser ablation sector field inductively coupled mass spectrometry. *Can. J. Fish. Aquat. Sci.* **57**: 1232-1242.
- Townsend, D.W., Radtke, R.L., Malone, D.P., and Wallinga, J.P. 1995. Use of otolith strontium: calcium ratios for hindcasting larval cod distributions relative to water masses on Georges Bank. *Mar. Ecol. Prog. Ser.* **119**: 37-44.
- Townsend, D.W., Radtke, R.L., Morrison, M.A., and Folsom, S.C. 1989. Recruitment implications of larval herring over wintering distributions in the Gulf of Maine, inferred using a new otolith technique. *Mar. Ecol. Prog. Ser.* **55**: 1-13.

- Veinott, G., and Evans, R.D. 1999. An examination of elemental stability in the fin-ray of the white sturgeon with laser ablation sampling inductively coupled plasma mass spectrometry (LAS-ICP-MS). *Trans. Amer. Fish. Soc.* **128**: 352-361.
- Veinott, G., Northcote, T., Rosenau, M. and Evans, R.D. 1999. Concentrations of strontium in the pectoral fin-rays of the white sturgeon (*Acipenser transmontanus*) by laser ablation sampling – inductively coupled plasma- mass spectrometry as an indicator of marine migrations. *Can. J. Fish. Aquat. Sci.* 56: 1981-1990.
- Wang, S., Brown, R., and Gray, D.J. 1994. Application of Laser Ablation-ICPMS to the spatially resolved micro-analysis of biological tissue. *Applied Spectroscopy* **48**: 1321-1325.
- Ware, D.M., and Thompson, R.E. 1991. Link between long-term variability in upwelling and fish production in the northeast Pacific Ocean. *Can J. Fish. Aquat. Sci.* **48**: 2296-2306.
- Weber, P.K., Hutcheon, I.D., McKeegan, K.D., and Ingram, B.L. 2002. Otolith sulfur isotope method to reconstruct salmon (*Oncorhynchus tshawytscha*) life-history. *Can. J. Fish. Aquat. Sci.* **59**: 587-591.
- Wells, B.K., Rieman, B.E., Clayton, J.L., Horan, D.L., and Jones, C.M. 2003.
  Relationship between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: The potential application of hard-part chemistry to describe movements in freshwater. *Trans. Amer. Fish. Soc.* 132: 409-424.
- Wells, B.K., Bath, G.E., Thorrold, S.R., and Jones, C.M. 2000(a). Incorporation of strontium, cadmium, and barium in juvenile spot (*Leiostomus xanthurus*) scales reflect water chemistry. *Can. J. Fish. Aquat. Sci.* **57**: 2122-2129.
- Wells, B.K., Thorrold, S.R., and Jones, C.M. 2000(b). Geographic variation in trace element composition of weakfish scales. *Trans. Am. Fish. Soc.* **129:** 889-900.
- Wilson, A.J. and Ferguson, M.M. 2002. Molecular pedigree analysis in natural populations of fishes: approaches, applications and practical considerations. *Can. J. Fish. Aquat. Sci.* **59**: 1696-1707
- Yamada, S.B., and Mulligan, T.J. 1982. Strontium marking of hatchery-reared coho salmon (*Oncorhynchus-kisutch* Walbaum). J. Fish. Biol. 14: 267-275.

Zimmerman, C. and Reeves, G.H. 2000. Population structure of sympatric anadromous and nonadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. *Can. J. Fish. Aquat. Sci.* **57**: 2152-2162.