DELINEATION OF YAKUTAT FORELAND COHO SALMON (*ONCORHYNCHUS KISUTCH*) STOCKS USING OTOLITH CHEMISTRY

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DELINEATION OF YAKUTAT FORELAND COHO SALMON (*ONCORHYNCHUS KISUTCH*) STOCKS USING OTOLITH CHEMISTRY

A

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By

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Abstract

Otolith chemistry was utilized to identify suspected coho salmon sub-stock populations on the Yakutat Foreland of southeast Alaska. In order to demonstrate that otolith chemistry might be successful in sub-stock differentiation, water samples were collected from four adjacent river systems and chemically segregated by collection site. Juvenile coho salmon and adult coho salmon were collected from the same four river systems and were subsequently analyzed for levels of select Ba, Ca, Mg, and Sr isotopes in all otolith edge and core regions using laser ablation-inductively coupled plasma-mass spectrometry. Otolith Sr^{87}/Ca^{48} and Mg^{24}/Ca^{48} were used to segregate collection sites, identify sub-stock populations, and infer straying rates for coho salmon on the Yakutat Foreland. Juvenile core and edge otolith chemistry returned moderate to high classification accuracy for three out of four collection sites (60%-92%) in statistical discriminant analyses. Adult core otolith chemistry could not segregate samples according to collection site in three out of four sites (7%-50%). Yakutat Foreland otolith chemistry analysis results allowed for (1) differentiation of adjacent freshwater systems, (2) a significant amount of coho salmon stock delineation, and (3) a higher suggested rate of straying from natal sites than coho salmon in other locations.

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Acknowledgements

This research will be submitted for publication in the Transactions of the American Fisheries Society. I will be senior author of the paper. I was responsible for collecting samples (water, fish otoliths) from the study site, obtaining supporting data, preparing and analyzing water and otolith samples in the laboratory, maintaining data, conducting statistical analyses, interpreting the results, and writing the paper. Dr. Nate Bickford provided invaluable otolith chemistry expertise, advice, and funding for this project. Matthew Keyse provided outstanding editing and figure help for this project. Thank you to the Fisheries Otolith Group laboratory personnel at the University of Alaska Fairbanks for assistance with otolith processing and to Vanessa Richie for help with water analyses. Thank you also to Alaska Department of Fish and Game, Juneau and Yakutat, AK and United States Forest Service, Yakutat, AK for providing samples, transportation, sampling equipment, and personnel to accomplish this project. Special thank you to Kevin Schaberg (ADF&G) for initial project development.

Introduction

The vast salmon fishery resources of Alaska are of tremendous importance to the economies of the state and the nation. In 2004, commercial fishermen landed over 167 million salmon worth over US$257 million (Eggers 2005). These resources are self-renewing if properly managed, but unknown stock information confounds management of many large Alaska fisheries. Survival of Pacific salmon today is a more difficult goal than ever with numerous factors presenting obstacles to the persistence of healthy anadromous fish populations. Maintaining healthy salmon populations through an understanding of salmon ecology and habitat use, such as natal stream sources, has become increasingly important due to the increased fishing and habitat pressures coupled with incomplete knowledge of fisheries.

Southeast Alaska is a major Pacific salmon producer, containing over 5200 anadromous salmon streams totaling 40,000 km in length (Halupka et al. 1999). The Yakutat Foreland of Southeast Alaska is home to some of the most productive salmonid populations in the world (Thedinga et al. 1993). Among the nine indigenous fish species found in the Situk River, coho salmon (*Oncorhynchus kisutch*) is one of the most abundant and the most important commercially caught salmon, accounting for a third to one half of the total Situk River salmon harvest (Bethers and Ingledue 1989). Juvenile coho salmon and Dolly Varden summer densities in the Situk River have been found at roughly 10 times the density of other Southeast Alaska streams in most channel types (Thedinga et al. 1993).

Management of Yakutat Foreland fisheries stocks occurs on a system-wide basis. That is, each fish species is managed as one stock regardless of spawning river, when in reality, each river system is a possible home to several sub-stocks or sub-populations that could have been hatched at different times. This type of management approach leaves various sub-stocks susceptible to unsustainable fishing pressures, where one sub-stock may be heavily fished at the peak of the commercial season, while another may see little to no fishing pressure due to a lack of overlap between fishing seasons and sub-stock run timing.

Although the Yakutat Foreland fisheries are of tremendous economic and cultural importance, little has been learned about the stock composition of coho salmon originating from systems of the Yakutat Foreland. Until recently, only tagging studies in conjunction with capture-recapture techniques have been employed to identify movements of fish among locations. Yet costly tagging studies generally have low recapture rates, such as a 1.9% rate reported by Hansen & Jacobsen (2003). A valuable and cost-effective tool that can be used to identify the stock composition of coho salmon is the use of otoliths, or ear stones, to discover previously unknown facets of fish life history. Nearly all fish have otoliths in their inner ear that are valuable sources of information, recording characteristics that elucidate stock information, migration histories, temperature and salinity histories, ages, proof of anadromy, food sources, and the water chemistry of the residing environment (Kalish 1990; Campana 1999). Three pairs of otoliths occur in teleosts: the sagittae, lapilli, and asterisci. Sagittae are generally the largest and most widely used in microstructure studies.

Sagittal otoliths are composed of 95% aragonite ($CaCO_3$) and a 5% protein-rich organic matrix and grow by the addition of concentric layers deposited on a daily basis (Bacon et al. 2004). Annular alternating organic dark and aragonitic opaque banding sequences are apparent in most otoliths. Although Pacific salmon have annual and daily growth rings, daily growth rings are less common (Campana and Thorrold 2001) and more evolved vertebrates usually do not produce daily growth patterns (Neville 1967). While there is a general belief that an endogenous circadian rhythm entrained by photoperiod drives daily otolith increment formation, caution should be exercised in any otolith analysis since variables including photoperiod, temperature, feeding, growth, and an endogenous circadian rhythm may change often and potentially influence otolith deposition (Campana and Neilson 1985).

Other structures used for aging include scales, fin spines, vertebrae, and eye lenses (Elsdon and Gillanders 2003). Although it is not clear exactly why aragonitic otoliths are much better recorders of age and habitat than other structures, the high specific gravity of aragonite likely aids its role as a gravity receptor in the ear structure of teleosts. Further, the very species specific shape of different otoliths suggests a tightly regulated biomineralization process is at work (Campana and Thorrold 2001). Thus, the otolith is highly valued for its superior chronological properties and its acellular nature, preventing the otolith from being metabolically reworked (Campana 1999).

Until recently, the goal of most otolith studies has been to determine the most accurate age possible for nearly any species. The most common methods employed to determine fish age are to count daily or annular bands in otoliths or to count circuli on

scales. Use of scales for the dating of fish has largely been replaced by the more reliable, and metabolically inert, otolith. Aging techniques associated with otoliths are preferred by most researchers due to a number problems associated with scales, including difficulty in reading scales of older fish due to decreased somatic growth and crowding of annuli, resorption problems, and lack of annuli formation (Chilton and Beamish 1982). Multiple studies have demonstrated inaccuracies in aging fish with scales (Boxrucker 1986; Beamish and McFarlane 1987; Lowerre-Barbieri et al. 1994). Many studies have proven the accuracy of dating a variety of species utilizing otoliths (Stuart and McKillup 2002; Ewing et al. 2003; Bujold et al. 2004; Howland et al. 2004).

Many elements, such as magnesium (Mg), barium (Ba), and strontium (Sr) substitute for calcium (Ca) in the aragonite of otoliths, but strontium and barium are the preferred elements of interest because they are found at levels that are similar to those of the aqueous environment and their binding characteristics cause them to be present in hundreds of parts per million (ppm) (Kalish 1990; Zimmerman and Reeves 2002). Thus, an analysis of strontium and barium in otoliths can be referenced to stream water strontium and barium content to determine fish habitat. Since strontium and barium substitute for calcium in the otolith matrix, the Sr/Ca and Ba/Ca ratio is often quantified for comparable reference with water samples and other otoliths. Otolith studies relating to habitat identification have examined not only Sr/Ca, Ba/Ca, Mg/Ca, and Mn/Ca ratios (Campana and Thorrold 2001), but also other elements that are found in measurable quantities of otoliths, including potassium, sodium, phosphorus, and sulfur (Begg et al.

1998). Otolith chemistry was employed here to study the stock composition and life history of coho salmon on the Yakutat Foreland.

Campana and Thorrold (2001) caution that otoliths may have different element proportions than habitat waters, pointing out that the three main interfaces that elements must pass through to be crystallized onto otoliths (brachial uptake, cellular transport, and crystallization) can either concentrate or dilute elements. That is, elements incorporated into an otolith must pass from water through the gills or intestine into the blood plasma, then into the endolymph, before crystallization in the otolith (Campana 1999). The pH of the endolymph, the inner ear fluid within which the otolith sits, is controlled by bicarbonate ion concentration and may be the main physical factor regulating otolith calcification (Romanek and Gauldie 1996).

Water-soluble proteins seem to form the structural framework of otoliths and also may be responsible for calcification regulation (Campana 1999), including the preferential formation of aragonite, as opposed to the crystallization of one of the other calcium carbonate polymorphs, calcite or vaterite. While water chemistry is believed to be the main source of elements that are incorporated into otoliths (the source of 80 to 90% of calcium and strontium), diet can also be a source of incorporated elements, adding the potential for more variance from habitat chemistry (Farrell and Campana 1996; Elsdon and Gillanders 2003). Habitat water, in turn, can have chemistry reflective of factors that include salinity, precipitation, evaporation, and bedrock chemistry (Palmer and Edmonds 1989).

The $^{87}Sr/^{86}Sr$ ratios recorded in salmonid otoliths are similar to Sr/Ca ratios in that both have been found to reflect the same ratio obtained from the environment. Some researchers have suggested, however, $^{87}Sr/^{86}Sr$ ratios may reflect environmental conditions with even greater certainty because isotopic composition is not affected by biological processes, is not susceptible to seasonal or annual fluctuations, and does not vary with the quantity of strontium and calcium of habitat water (Kennedy et al. 1997; Ingram and Weber 1999; Bacon et al. 2004). Campana (1999) has suggested strontium isotope analyses of otoliths are still not well understood and perhaps too costly for widespread application. However, Kennedy et al. (1997) successfully differentiated stocks of Atlantic salmon from the Connecticut River using strontium isotope compositions found in vertebrae. Recently, isotope analyses utilizing LA-ICP-MS (Laser ablation- Inductively Coupled Plasma- Mass Spectrometry) have achieved excellent sensitivity, precision, and accuracy without the need for costly TIMS (Thermal Ionizing Mass Spectrometry) analyses more common in the past (Becker and Dietze 2000).

Otolith analyses are quickly becoming a popular tool to determine teleost life history information. Researchers have utilized fish otolith, as well as cephalopod statolith, analyses to determine previously unknown life history facets of freshwater and marine species. Capitalizing on the ability of strontium to serve as a salinity barometer, several studies have differentiated strontium-enriched seawater regions of otoliths with strontium-depleted freshwater regions to track the timing and migration histories of anadromous species (Kalish 1990; Limburg 1998; Secor et al. 2001; Arai et al. 2002; Zimmerman and Reeves 2002; Bacon et al. 2004). Radtke et al. (1989) also

demonstrated the temperature sensitivity of strontium by experimentally rearing Atlantic herring in various temperatures.

Otolith chemistry techniques have been employed to accomplish intraspecific stock differentiation in marine environments (Begg et al. 1998, 2000; Goto and Arai 2003; Arai et al. 2003), in freshwater lentic environments (Quinn et al. 1999; Munro et al. 2005), and in freshwater lotic habitats (Ingram and Weber 1999; Kennedy et al. 2002; Wells et al. 2003; Bickford et al. 2003; Bickford and Hannigan 2005).

Prior to the advent of widespread otolith studies, genetics was the dominant methodology employed to identify sub-stocks using natural markers. DNA fingerprinting has also traditionally been how new species have been identified and 'proven' to be new species. Much more expensive than otolith chemistry analyses, genetics have also fallen short of stock differentiation where otoliths have succeeded. During a winter mixing period, Campana et al. (1999) identified four Atlantic cod stocks, but an analysis of the same fish using microsatellite DNA studies was less able to distinguish the four stocks likely due to occasional mixing of spawning sub-populations (Ruzzante et al. 1999). In general, genetic studies are well suited for questions involving parental or evolutionary linkages or to infer population structure, but are not very useful for localized stock identification or mixing issues, whereas otolith assays can infer environmental history but cannot characterize populations (Ferguson and Danzmann 1998; Campana and Thorrold 2001).

The primary objective of this investigation was to identify as many coho salmon sub-stocks as possible across four systems of the northern Yakutat Foreland, from the

Situk River and Old Situk River in the northwest, west to Tahwah Creek, and southeast to Sockeye Creek. To accomplish sub-stock identifications, I used the otolith chemistry of the core region, which is a fingerprint or chemical signature of natal waters. Otolith edge chemistry should reflect the chemistry of the river collection site and was used to identify any straying movement from natal waters through comparison with otolith core chemistry signatures. Similar elemental signatures in the core of the otoliths would indicate that coho salmon were from the same sub-stock, whereas groups with differing signatures would indicate that there were multiple sub-stocks.

The core otolith signatures of post-spawn adult coho salmon and young-of-the-year (YOY) were compared within juveniles, within adults, and between juveniles and adults. These comparisons should reveal (1) whether otolith core chemistry can differentiate among sites, (2) whether natal homing of adult spawners occurred, and (3) how much stock delineation of Yakutat Foreland coho salmon was possible. Due to different geology and landscape features (i.e. glaciers, lakes, and muskeg), it is likely that otolith core chemistry can differentiate natal grounds across the Yakutat Foreland. Natal homing is expected to occur with less than 4% straying, a common rate for coho salmon (Taft & Shapovalov 1938). Stock delineation is also anticipated due to variable landscape and geology among river systems, but within river system discrimination may be limited due to hydrologic, geologic, and landscape homogeneity.

Coho Salmon Ecology

Coho salmon (*Oncorhynchus kisutch*) are one of seven members recognized as species belonging to the genus *Oncorhynchus*. Although many introduced populations of coho exist in various cold temperate areas, endemic spawning populations are found in most of the North Pacific Ocean basin (reviewed by Sandercock, 1991). Coho salmon have historically played an important role in world fisheries, with a global average annual catch of 7.46 million coho from 1952 to 1976 and a peak of 10.6 million in 1986, while in Alaska, the average annual commercial catch of coho from 1920 to 1976 was 0.7 million in central Alaska and 1.5 million in southeastern Alaska (INPFC 1979). In the Situk River, total annual returns averaged 60,000 coho from 1980 to 1989 (Sandercock 1991).

In most of their range, coho salmon are anadromous and usually spend one winter in freshwater (but can spend up to four winters) before migrating to sea, then after two or three years at sea, they return to spawn and quickly thereafter die (Mecklenburg et al. 2002). Crone and Bond (1976) found that most coho in Sashin Creek from 1965-67 and Nakvassin Creek from 1966-1972 of southeast Alaska spent two years in freshwater and 18 months at sea. In contrast to coho salmon of other regions, Situk River coho usually spend one to two years in freshwater and only 18 months at sea, although many males return to freshwater as jacks after only a few months (M.A. Jones, personal observation). Alaska Department of Fish and Game reported the age composition of the Situk River commercial coho catch from 1985 to 1988 as averaging 40.2% wintering one year, 52.5% wintering two years, and 6.8% wintering three years in freshwater prior to seaward

migration. Complicating an assessment of Yakutat Foreland coho stock populations and defying the typical life history of other salmon, coho salmon can be found returning to Yakutat area freshwaters from August to March (Thedinga et al. 1993).

Coho freshwater habitat varies seasonally in response to changes in environmental conditions (Swales et al. 1986). In all seasons, juvenile coho salmon may congregate in quiet backwaters, side channels, and small shaded creeks (Bustard and Narver 1975). Habitat of the Yakutat Foreland is well suited for coho freshwater residency, with an abundance of flat drainages containing side channels and pools. In a study of juvenile coho movement and habitat use, Schaberg (2006) found that muskeg channel habitat connected to the Situk River may be responsible for up to 80% of coho production. Residence in main-channel habitat in usually confined to spring or summer, and as fall approaches and activity and temperatures decrease, coho seek pool habitats that are deeper with less water flow (Lister and Genoe 1970; Hartman 1965). Coho salmon require and may utilize more freshwater areas and habitat types within watersheds than any other salmonid species. Consequently, habitat destruction and loss of river side channels may have affected coho salmon populations across California and the Pacific Northwest more than other species.

Successful stocks of coho have thrived due in large part to their use of a variety of watershed habitat types, large temporal variation in spawning, and a highly variable diet. Healthy stocks are found in coastal streams a few miles from the open ocean, as well as thousands of river miles up the Yukon River. The nearly year-round advent of coho salmon spawning on the Yakutat Foreland allows stocks to survive potential physical and

climatic disasters that other species with smaller spawning periods cannot overcome. Coho salmon have also thrived from versatility in feeding, where aquatic and terrestrial invertebrate prey proportions vary drastically with season and abundance (Wipfli 1997) and piscivory becomes a proportionally larger strategy with size (Brodeur 1991).

Chapter 1:

Delineation of coho salmon stocks of the Yakutat Foreland in southeast Alaska using otolith chemistry[1]

Introduction

Maintaining healthy salmon populations through an understanding of salmon ecology and habitat use, such as natal stream sources, has become increasingly important due to increased fishing and habitat pressures coupled with an incomplete knowledge of fisheries. This approach is especially important in Alaska due to the vast salmon resources that are of tremendous importance to the economies of the state and nation. Though these resources can be self-renewing if properly managed, unknown stock information has confounded the management of many large Alaska fisheries. Thus, survival of Pacific salmon today is a more difficult goal than ever with numerous factors presenting obstacles to the persistence of healthy anadromous fish populations.

The Yakutat Foreland of southeast Alaska is home to some of the healthiest salmonid populations in the world. Among the nine indigenous fish species found in the Situk River, coho salmon (*Oncorhynchus kisutch*) is one of the most abundant and the most important commercially caught salmon, accounting for a third to one half of the total Situk River salmon harvest (Bethers and Ingledue 1989). Juvenile coho salmon and Dolly Varden summer densities in the Situk River (located on the Yakutat Foreland) have

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[1] Prepared for submission to Transactions of the American Fisheries Society:
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been found at roughly ten times the density of other Southeast Alaska streams in most channel types (Thedinga et al. 1993).

Alaska Department of Fish & Game (ADF&G) management of Foreland fisheries stocks occurs on a system-wide basis. That is, each fish species is managed as one stock regardless of spawning river, when in reality each river system is a possible home to several sub-stocks or sub-populations that could have been hatched at different times. This type of management approach leaves various sub-stocks susceptible to unsustainable fishing pressures, where one sub-stock may be heavily fished at the peak of the commercial season, while another may see little to no fishing pressure due to a lack of overlap between fishing seasons and sub-stock run timing.

Although the Yakutat Foreland fisheries are of tremendous economic and cultural importance, little is known about the stock composition of coho salmon. Recently, the use of otoliths, or ear stones, has proven to be a valuable and cost-effective tool to identify the stock composition of migratory fish. Nearly all teleosts have otoliths in their inner ear that are valuable sources of information, recording characteristics that elucidate growth rates, migration histories, food sources, and the water chemistry of the residing environment (Kalish 1990; Campana 1999). Three pairs of otoliths occur in teleosts: the sagittae, lapilli, and asterisci. Sagittae are generally the largest and most widely used in microstructure studies. Otoliths are composed of an aragonite (95%) and protein-rich organic matrix (5%), which grows by the addition of concentric layers deposited on a daily basis (Bacon et al. 2004).

The most widespread goal of otolith studies to date has been to determine the most accurate age possible for nearly any species. For most species, the preferred method employed to determine fish age is to count daily or annular bands in otoliths. Countless studies have proven the accuracy of dating a variety of species utilizing otoliths (Stuart and McKillup 2002; Ewing et al. 2003; Bujold et al. 2004; Howland et al. 2004).

In addition to the ability to age fish, otoliths also incorporate many trace elements into their structure that are linked to the environments that a fish may encounter. Many elements substitute for calcium (Ca) in the aragonite of otoliths, but magnesium (Mg), barium (Ba), and strontium (Sr) are some of the preferred elements of interest because they are found at levels that are directly related to those of the aqueous environment (Kalish 1990; Zimmerman and Reeves 2002). Since strontium, barium, and magnesium substitute for calcium in the otolith matrix, Sr/Ca, Ba/Ca, and Mg/Ca are often quantified for comparable references with that of water chemistries and other otoliths (Miller et al. 2005; Arai and Hirata 2006; Hobbs et al. 2007).

Otolith analyses are quickly becoming a popular tool to determine teleost life history information. Researchers have utilized fish otolith analyses to determine previously unknown life history facets of freshwater and marine species (Kennedy et al. 1997; Ingram and Weber 1999; Bickford et al. 2003; Feyrer et al. 2007). Capitalizing on the ability of strontium to serve as a salinity barometer, several studies have differentiated strontium-enriched seawater regions of otoliths with strontium-depleted freshwater regions to track the timing and migration histories of anadromous species (Kalish 1990;

Limburg 1998; Secor et al. 2001; Arai et al. 2002; Zimmerman and Reeves 2002; Bacon et al. 2004). Radtke et al. (1989) also demonstrated the temperature sensitivity of strontium by experimentally rearing Atlantic herring in various temperatures. Otolith chemistry techniques have been employed to accomplish intraspecific stock differentiation in marine environments (Begg et al. 1998, 2000; Goto and Arai 2003; Arai et al. 2003), in freshwater lentic environments (Quinn et al. 1999; Munro et al. 2005), and in freshwater lotic habitats (Ingram and Weber 1999; Kennedy et al. 2002; Wells et al. 2003; Bickford et al. 2003; Bickford and Hannigan 2005).

The primary objective of this coho salmon investigation was to identify as many sub-stocks as possible across four systems of the northern Yakutat Foreland, from the Situk River and Old Situk River in the northwest, west to Tahwah Creek, and southeast to Sockeye Creek. To accomplish sub-stock identifications, we used the otolith chemistry of the core region, which is a fingerprint or chemical signature of natal waters. Otolith edge chemistry should reflect the chemistry of the river collection site and was used to identify any straying movement from natal waters through comparison with otolith core chemistry signatures. Similar elemental signatures in the core of the otoliths would indicate that coho salmon were from the same sub-stock, whereas groups with differing signatures would indicate that there were multiple sub-stocks.

The core otolith signatures of post-spawn adult coho salmon and young-of-the-year (YOY) were compared within juveniles, within adults, and between juveniles and adults. These comparisons should reveal (1) whether otolith core chemistry can differentiate among sites, (2) whether natal homing of adult spawners occurred, and (3)

how much stock delineation of Foreland coho salmon was possible. Due to different geology and landscape features (i.e. glaciers, lakes, and muskeg), it is likely that (1) otolith core chemistry can differentiate natal grounds across the Foreland. Natal homing (2) is expected to occur with less than 4% straying, a common rate for coho salmon (Taft & Shapovalov 1938). A large amount of stock delineation (3) is also anticipated due to variable landscape and geology among river systems, but within river system differentiation may be limited due to hydrologic, geologic, and landscape homogeneity.

Study Site

The Yakutat Foreland is a glacial outwash plain and uplifted seabed located between the southeast panhandle and the main body of Alaska. The northern limit of the Tongass National Forest, the Yakutat Foreland lies between Glacier Bay National Park to the south and Wrangell-Saint Elias National Park to the north. Seaward of the Saint Elias Range, the Yakutat Foreland is 8 to 24 kilometers wide and is characterized by glacial detritus that is approximately 215 meters thick. The detritus of the Foreland gives rise to wetlands, shrublands, and dry forests. The region is characterized by low relief (less than 60 meters), with high points on glacial moraines adjacent to the mountains.

Sampled rivers were the Situk River, Old Situk River, Sockeye Creek, Tahwah Creek, and Ophir Creek (Figure 1). Near the northwestern extent of the Foreland, the Situk River is fed by three substantial lakes, imparting a laccustrine signature on the watershed. The Situk River is a clear, fourth-order stream with an average summer flow of 6 m^3/s (Clark and Paustian 1989). The low flow Old Situk River is fed by a very small

lake and exists as an upper watershed before flowing into the Situk River. Similarly, Tahwah Creek is also a lake fed system, but is unique in flowing mostly parallel to the coast and keeping within a few miles of marine waters. More geographically separated from these three watersheds and further south, Sockeye Creek is fed by adjacent spruce forest. All drainages of the Foreland are characterized by extensive pool and off-channel pond habitat that is often surrounded by peatland bogs and fens, shrub wetlands, and muskeg vegetation. The Situk River and a few adjacent watersheds empty into the highly productive Situk Estuary. Fish species found in the Situk Estuary include the indigenous species of the Situk River, as well as at least ten marine species.

Methods

To explore the feasibility of differentiating Foreland otolith signatures, water samples across the Foreland were collected for preliminary analysis. Known Situk River coho salmon spawning and rearing sites were identified from studies conducted by other researchers (Schaberg 2006; Thedinga et al. 1993), and suspected coho salmon spawning and rearing sites on other parts of the Yakutat Foreland were identified from recent USFS (United States Forest Service) digital GIS (Geographic Information Systems) drainage data. In July and August of 2005, water samples were collected for analysis. We collected samples in the Old Situk River (6), Situk River (9), Sockeye Creek (3), and Tahwah Creek (9). Latitude and longitude coordinates of each water sample location were recorded using a Garmin GPS unit. Water samples were collected in rinsed polyethylene bottles and immediately chilled in a dark cooler. Within 48 hours, samples

were transported to the University of Alaska Fairbanks and immediately preserved in a nitric acid (HNO_3) solution. The samples were then analyzed for 25 elements on the Agilent 7500c Inductively Coupled Plasma Mass Spectrometer (ICP-MS) at the University of Alaska Fairbanks. A regression analysis was subsequently performed.

Average peak spawning times for Yakutat Foreland coho salmon determined by Thedinga et al. (1993) were utilized to establish when to collect post-spawn carcasses for later otolith extraction. In October of 2005, post-spawn carcasses (N=87) were collected at all known and suspected spawning locations where carcasses could be found. Heads were immediately extracted with a fillet knife and the remaining carcass was left where it was found. Alaska Department of Fish and Game (ADF&G) Coded Wire Tag Lab in Juneau provided additional coho salmon heads (N=7) originating from known rivers of the Yakutat Foreland. Each coho salmon head was numbered, identified by latitude and longitude coordinates, and frozen. At the conclusion of sampling, frozen heads were transported to the Fisheries Otolith Group (FOG) Laboratory at the University of Alaska for later extraction and analysis.

Average peak emergence times for Yakutat Foreland coho salmon identified by Thedinga et al. (1993) were used to establish when to collect young of the year (YOY) individuals. Previously identified and currently suspected coho salmon rearing sites were revisited in April, May, and June of 2006. YOY (N=128), identified as individuals with a fork length ≤ 3 cm (Gribanov 1948), were trapped using fine mesh minnow traps and seine nets. Trapped coho salmon were immediately euthanized, while other trapped species were released right away. Individuals were numbered, identified by GPS

coordinates, frozen, and transported to the FOG Laboratory at the University of Alaska for later extraction and analysis.

Otolith Preparation

Frozen coho salmon were thawed and saggital otoliths were extracted. Each pair of otoliths were removed with forceps, cleaned, and stored dry in a separate, labeled polyethylene tube. One of each pair of otoliths was randomly selected and mounted sulcal side up in thermoplastic glue (Crystalbond). A Buehler Isomet Low Speed Saw equipped with a diamond blade was used to make two diameter cuts through the center region of each adult coho salmon otolith. Outside parent material was discarded and the remaining adult otolith, after reheating of the slide and Crystalbond, was rotated onto its side, forming a transverse section. Adult otoliths were wet polished using a sequence of 30, 15, and ≈13 micron (μm) waterproof sandpapers. Whole juvenile otoliths were polished using 9μm waterproof sandpaper. After polishing each sample, two digital photographs (25x and 80x magnification) were taking using the Motic BA400 Compound Light Microscope in conjunction with a Scion Corporation CFW1308C Color Digital Camera.

Otolith Chemical Analysis

Otoliths were analyzed for calcium, as well as the trace elements barium, magnesium, and strontium using a New Wave 213 nm Dry Laser Ablation System (LA) in conjunction with an Agilent 7500c Inductively Coupled Plasma Mass Spectrometer

(ICP-MS) at the University of Alaska Fairbanks. For each sample, a core, edge, and core-to-edge ablation transect was made. The laser used a scan speed of 35μm per second, a depth of 5μm, a spot size of 25μm, a repetition rate of 10hz, a 10 second warm-up time, and a 100% power output for all analyses. NIST 610 glass standard was utilized to correctly calibrate the ICP-MS data during post-processing. Integration and post processing of LA-ICP-MS data was performed using Geopro™ (Cetac Technologies v1.0 1999) software program.

Otolith Chemistry Statistical Analyses

To identify the most important individual element ratios in discriminating among water and otolith site groups, F-statistics were estimated using the 'proc stepdisc' procedure (SAS Institute Inc. v9.1 2006) for Mg^{24}/Ca^{48}, Sr^{86}/Ca^{48}, Sr^{87}/Ca^{48}, Sr^{88}/Ca^{48}, Ba^{137}/Ca^{48}, Ba^{138}/Ca^{48}, and Sr^{87}/Sr^{86}. The discriminant analysis ('proc discrim') procedure (SAS Institute Inc. v9.1 2006) was used to evaluate and assign water and otoliths to groups using element ratios previously identified by the stepdisc procedure. The discriminant analysis procedure constructs a linear discriminant model using specified parameters for the number of nearest neighbors. Each new observation is assigned into the group to which the majority of its *k* nearest neighbors belong. The created model was evaluated for accuracy by cross validation using Kernel Triweight Density Squared Distance Function. Cross validation adds validity to discriminant analysis results by removing samples and then assessing whether statistical groupings are reasonable for the same reassignment. This discriminant analysis allowed us (1) to

conclude whether core (natal site) and edge (capture site) chemistry was able to differentiate among sites and (2) to identify how much stock delineation was possible.

The discriminant analysis procedure model developed for the juvenile edge or 'known origin' data set was used to classify juvenile core data into site groupings. Strong juvenile edge and core site corroboration would validate the assumption that individuals had not moved far from their natal source. To evaluate juvenile straying by site, paired t-tests were used to assess agreement among juvenile core and juvenile edge data sets. For adults, corroboration of juvenile core chemistry with adult core chemistry would suggest no straying took place during spawning migrations, while disagreement between these data sets would suggest straying occurred.

Results

Significant differences (P=0.0001) were identified between individual stream water samples in a regression analysis. Elements identifying site differences included Mg^{24}, Sr^{86}, Sr^{87}, and Sr^{88}, reporting distinctive mean site values (Figure 2) for all locations. Results from the stepdisc procedure showed that Sr^{87}/Ca^{48} and Mg^{24}/Ca^{48} were the best ratios to be used with discriminant analyses for both otolith and water analyses. Discriminant analysis of water samples returned high rates of classification for the Situk River (66.67%), Old Situk River (100%), Sockeye Creek (100%), and Tahwah Creek (100%) (Table 1A, Figure 3).

Discriminant analysis of juvenile core and edge data using Sr^{87}/Ca^{48} and Mg^{24}/Ca^{48} returned high classification rates for three out of four site groups. Juvenile

core chemistry data classification accuracy was high in the Situk River (76.47%), Sockeye Creek (60%), and Tahwah Creek (89.29%), but classification accuracy was poor in the Old Situk River (4%) (Table 1B, Figure 4). Similarly, juvenile edge chemistry data classifications were high in the Situk River (88.24%), Sockeye Creek (62.50%), and Tahwah Creek (92.00%), while Old Situk River (18.18%) classified poorly (Table 1C, Figure 5).

To assess movement of juveniles, a paired t-test was performed for Sr^{87}/Ca^{48} by collection site. P values reported suggest little movement of Sockeye Creek individuals (0.7404) from emergence to capture, but other sites reported low P values, suggestive of juvenile movement from natal stream to capture sites (Table 2).

Adult core chemistry data returned high classification accuracy in only 1 of 4 collection site groups. Assuming 100% natal homing occurred for the purpose of discriminant analysis, adult core chemistry data classification accuracy was poor in the Situk River (28.57%), Old Situk River (6.90%), and Tahwah Creek (50.00%), while a high rate was found in Sockeye Creek fish (83.33%) (Table 1D, Figure 6). Discriminant analysis could not assign adult core data to juvenile core data classification site groups (Table 3), suggesting significant straying of adults from natal streams.

Discussion

Water sample analyses demonstrated that adjacent systems of the Foreland could be distinguished from one another using stream chemistry. Water sample chemistry was successful in collection site discrimination, returning strong classification accuracy in

discriminant analyses and encouraging the subsequent collection of Foreland coho salmon for otolith chemical analysis. However, due to the relationship of stream water chemistry with that of otoliths, comparisons between the two were limited. While, within species, fish otoliths incorporate the chemistry of stream water habitat in a predictable or consistent manner, the relationship between stream water and otolith chemistry is not necessarily 1:1. Given that isotopic signatures for many elements, such as strontium, are often stable over seasons and years (Kennedy et al. 2000) and given the substantial groundwater influx of most Foreland systems, water sampling serves as a good proxy to suggest whether an otolith study might be successful. In order to make water chemistry more useful in otolith studies and to allow for direct comparisons, more studies are needed on a species-specific basis to identify the relationship between stream water chemistry and otolith uptake of that chemistry.

Discriminant analysis was able to classify Foreland water samples and most juvenile Foreland coho salmon into chemically distinctive sub-stocks. Juvenile coho salmon revealed strong statistical site segregation in 3 out of 4 sites, but adult coho salmon core signatures were only able to successfully differentiate fish from 1 out of 4 collection sites, suggesting high straying rates. Coho salmon adult core chemistry and juvenile chemistry from the same collection sites failed to classify together, suggesting that adult otolith core chemistry data was representative of locations other than collection sites.

As expected, juvenile edge data was better than core data in discriminating between site groups. Since otolith growth is continuous (Campana and Thorrold 2001)

and YOY juveniles are unlikely to travel significant distances, it is believed that juvenile otolith edge material recorded chemistry representative of capture sites. Counts of otolith bands, inferred to be daily increments, confirm that most juveniles were less than 3 months of age at capture. Thus, it was not surprising that juvenile core data also showed good collection site discrimination. Juvenile otolith edge data returned slightly better group membership than core data, suggesting that some of these 2 to 3 cm YOY moved from natal stream sites between emergence and capture.

Adult otolith core chemistry disagreement with juvenile otolith core chemistry classifications, coupled with unique features of Foreland river systems, suggest substantial straying of adults from natal streams. Straying rates for coho salmon are historically less than 4% (Taft & Shapovalov 1938), yet the rate in the Yakutat area may be well over this level. As previously mentioned, the Foreland is a unique coho salmon system that is transformed when intensive fall and winter rains cause flooding events. During the Foreland coho salmon spawning season, these freshets often lead to coho salmon spawning in extensive flood habitat, such as road drainage ditches, muskegs, and rain puddles (Thedinga et al. 1993). The unpredictability of this flooding time and extent on the Foreland suggests that straying rates could be much higher than in a more stable and predictable system. Further, the extremely flat topography and close proximity of river systems to one another favors straying from natal systems more than other locations. By comparison, other locations with substantial coho salmon populations in Alaska or the Pacific Northwest, have river systems with substantially higher gradients and less stream density or interconnectivity.

While significant straying of adults and juveniles from natal sites is suggested by statistical analyses, geographic similarities may have contributed to some discriminant analysis classifications into adjacent site groups. The sampled site locations were all relatively close to each other, suggesting chemical differences may be small. Both juvenile and adult otoliths returned discriminant analysis results that suggest some geographic confusion among the chemistry. Tahwah Creek is unique among examined systems of the Foreland in running parallel to the coast and staying within a few miles of marine waters, but is also in close proximity to the Situk River, as well as similar to, the Situk River in being fed by a small lake. Difficulties in distinguishing between the Old Situk River and the Situk River are likely due to the fact that fish captured in the Old Situk River must first travel up the Situk River. These relationships and discriminant analyses suggest that, in addition to substantial straying, (1) these rivers have similar chemistry and that (2) there could be mixing signatures from fish inhabiting the interface of two examined rivers.

Juvenile otolith data was more useful than adult otolith data in site group discrimination. An analysis of juvenile otoliths benefits from representing chemistry from a small temporal and spatial scale. The core chemistry of both juvenile and adult otoliths captures maternal chemical signatures from yolk sacs that have the potential to confound analysis. Yet, with an extremely small size and in a shorter time period, a juvenile individual has inhabited a much smaller area compared to that of an adult otolith, narrowing the possible chemistry represented in the otolith.

The core analysis of all otoliths was performed just outside of the identified hatch mark, when coho salmon otoliths presumably begin to record the chemistry of their environment and maternal influence from the yolk sac has already been recorded. The yolk sac is believed to contain marine chemistry from the adult life stage of the parent, and if its presence is not considered or recognized, it can elevate strontium and other element levels, leading a researcher to falsely characterize the chemistry of natal waters. Adult otoliths have 1 (for jacks) to 7 more years of concentric rings than juvenile otoliths, sometimes including several sequences of annuli, that add to the challenge of locating the correct hatch mark outside the primordia (otolith center). The location of this hatch mark is critical because, in most teleosts, the 'core signature' representative of natal habitat begins to be recorded just after hatching, when the maternal yolk sac nutrients are thought to have been consumed. Additional uncertainty is created by the finding that hatch checks do not necessarily form on the day of hatching (Campana & Thorrold 2001). Thus, for both juvenile and adult otoliths, some shortcomings in regard to core signature discriminant analysis accuracy could be attributed to the challenges of avoiding parental marine signatures.

Elsdon & Gillanders (2003a) suggest that the elemental exposure time, potential interaction between available elements, temperature changes, and salinity may have confounding affects on the chemical make up of the otolith, which is still not well understood. While strontium and magnesium uptake in otoliths may change in predictable ways based on salinity, temperature, or other environmental factors, caution should be exercised when evaluating other elements whose uptake may be governed by

factors that are less understood (Elsdon & Gillanders 2003b). Not only should intra-species variation in otolith chemical uptake be expected, but additional caution should be exercised by the finding that older and faster growing fish of a species have a tendency towards lower Sr/Ca ratios (Sadovy & Severin 1994). The bias of older fish towards lower Sr/Ca ratios could have helped in hindering site group assignment of adults based on juvenile discriminant analysis, with some adult samples reporting lower ratio values.

The difficulties encountered in distinguishing Situk River and Sockeye Creek water and juvenile chemistries from each other, however, were not anticipated. These systems are further from each other than the other sites, establishing the bounds of the project study area, and were expected to be substantially different. Confusion in distinguishing these signatures suggests that geologic homogeneity resulting from massive glacial till deposits across the Foreland and similar and substantial volumes of groundwater upwelling may have played a role in this relationship and additional poor classifications in discriminant analyses.

Another factor that may have weakened adult otolith signature groupings is the potential for temporal variation in stream chemistry over several years. However, one of the main reasons that Yakutat Foreland fisheries are highly productive is due to stable and constant groundwater influx, and with the exception of fall freshets, a consistent base flow. Kennedy et al. (2002) pointed out that isotopic proportions of many elements are likely to stay constant for many years, yet every habitat may have unique geology and groundwater influencing water chemistry at different rates. The hydrologic stability and lack of surficial bedrock on the Foreland suggests that watersheds are likely to produce

similar chemistry over several years. Thus, long term temporal chemical variation in Foreland systems is unlikely a significant source of error in this analysis, but may be responsible for some of the inability of adult core signatures to be assigned to juvenile core signature site groups. Caution should always be exercised, however, to avoid seasonal variations in stream chemistry that are likely to occur in areas of autochthonous and allochthonous inputs alike.

Sampled locations on the Yakutat Foreland were limited by (1) a later and noticeably smaller spawning run than previous years (Bachman et al. 2005; Catterson, United States Forest Service - Yakutat Ranger District, personal communication), (2) the inability to find juvenile or adult coho in lower volume systems, and (3) the difficulties posed by sampling in glacial systems, such as the Ahrnklin River. Based on observations of a later than usual run, sampling of adult coho took place just after the typical Yakutat Foreland peak spawning time (Thedinga et al. 1993). Nonetheless, sampling of post-spawn adults still proved extremely challenging and collection of samples fell short of project goals in some locations. The Alaska Department of Fish and Game later reported a 83,000 coho salmon set gillnet fishery harvest for 2005, 60% below the prior 10-year average (Bachman et al. 2005). Initial sampling plans included obtaining fish from some smaller systems in the central region of the Yakutat Foreland, but we were unable to locate adult or juvenile fish in Seal Creek, Kunyosh Creek, or Rice Creek. Sampling plans also originally included the Ahrnklin River, a glacial, high volume system at the southern edge of the Yakutat Foreland with runs of all Pacific salmon, but difficulties associated with sampling in glacial waters prevented sample collection. Improved future

sampling efforts may be more successful in these locations, potentially contributing otolith chemistry from other Yakutat Foreland coho salmon systems that could indicate additional sub-stocks.

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Table 1. Discriminant analysis data after Kernel Triweight Density cross validation. All analyses utilized Sr87/Ca48 and Mg24/Ca48 for classifications. Water (A), juvenile core (B), juvenile edge (C), and adult core (D) chemistry data are shown below. Correct classifications of samples to their collection sites are shown in bold.

Collected		*% Classified to:*			
From	*n*	Situk River	Old Situk River	Sockeye Creek	Tahwah Creek
A					
Situk River	9	**66.7**	0.0	33.3	0.0
Old Situk River	6	0.0	**100.0**	0.0	0.0
Sockeye Creek	3	0.0	0.0	**100.0**	0.0
Tahwah Creek	9	0.0	0.0	0.0	**100.0**
B					
Situk River	17	**76.5**	0.0	17.7	5.9
Old Situk River	*25*	24.0	**4.0**	8.0	64.0
Sockeye Creek	25	24.0	4.0	**60.0**	12.0
Tahwah Creek	28	3.6	3.6	0.0	**89.3**
C					
Situk River	17	**88.2**	0.0	11.8	0.0
Old Situk River	25	4.6	**18.2**	0.0	77.3
Sockeye Creek	25	25.0	0.0	**62.5**	8.3
Tahwah Creek	*28*	0.0	8.0	0.0	**92.0**
D					
Situk River	14	**28.6**	21.4	28.6	21.4
Old Situk River	29	24.1	**6.9**	51.7	17.2
Sockeye Creek	30	6.7	0.0	**83.3**	10.0
Tahwah Creek	10	10.0	0.0	40.0	**50.0**

Table 2. Results of juvenile otolith core and juvenile otolith edge paired t-tests. All tests compared juvenile Sr87/Ca48 otolith core and juvenile Sr87/Ca48 otolith edge data by collection site.

Collected From	P Value Sr^{87}/Ca^{48}
Situk River	0.0020
Old Situk River	0.0002
Sockeye Creek	0.7404
Tahwah Creek	0.0001

Table 3. Results from adult core discriminant function analysis based on juvenile core chemistry data classifications after Kernel Triweight Density cross validation. All analyses utilized Sr^{87}/Ca^{48} and Mg^{24}/Ca^{48} for classifications. Agreement with juvenile edge classification groups are shown in bold.

Collected from	*n*	*% Classified to:* Situk River	Old Situk River	Sockeye Creek	Tahwah Creek
Situk River	17	**21.4%**	21.4%	42.9%	7.1%
Old Situk River	25	13.8%	**24.1%**	24.1%	24.1%
Sockeye Creek	25	10.0%	30.0%	**0.0%**	50.0%
Tahwah Creek	28	10.0%	30.0%	10.0%	**50.0%**

Figure 1. Map detail of the Yakutat Foreland study area. Studied river systems include Situk River, Old Situk River, Sockeye Creek, and Tahwah Creek.

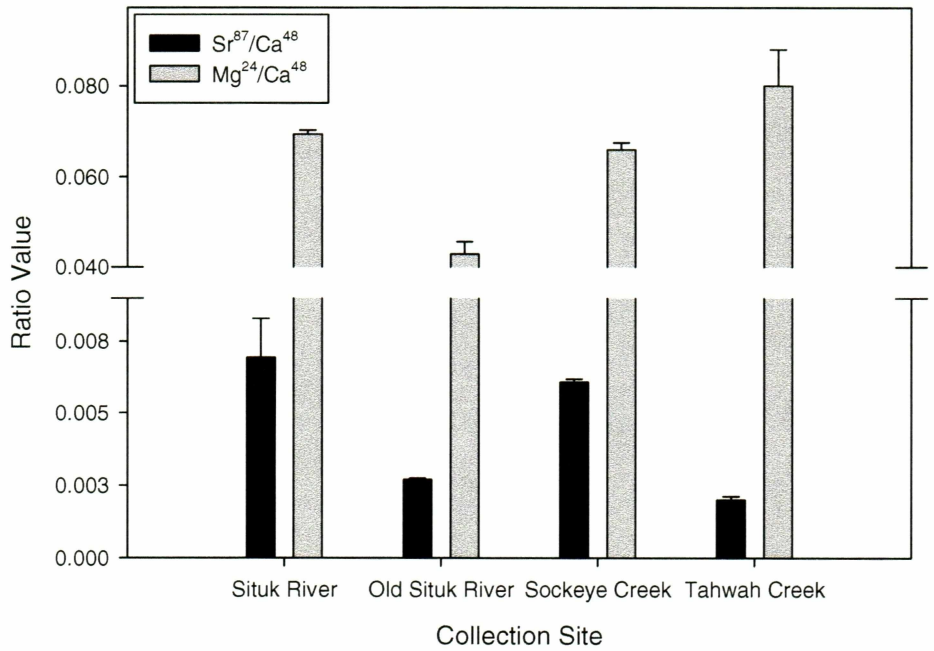

Figure 2. Bar graph of water sample Sr^{87}/Ca^{48} and Mg^{24}/Ca^{48} by collection site (±SD). Samples are plotted by collection site Sr^{87}/Ca^{48} and Mg^{24}/Ca^{48}.

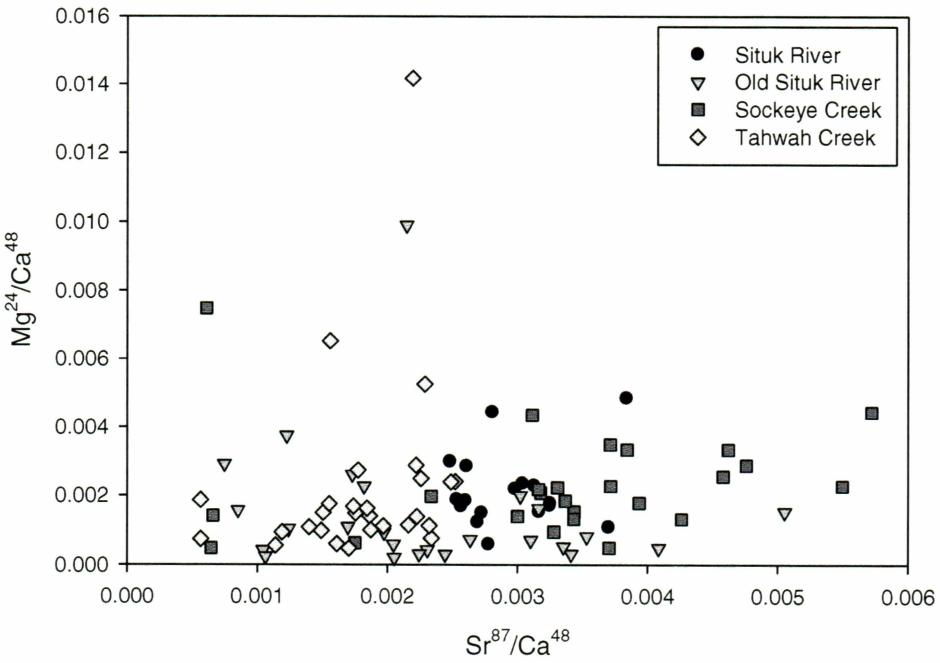

Figure 3. Scatter plot of juvenile otolith core chemistry by collection site. Samples are plotted by collection site Sr^{87}/Ca^{48} and Mg^{24}/Ca^{48}.

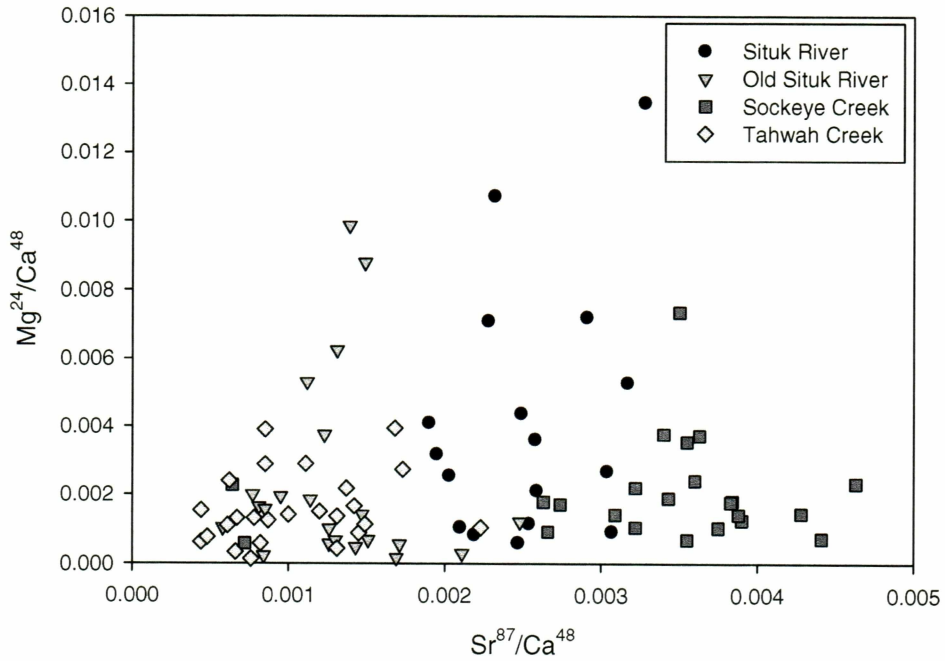

Figure 4. Scatter plot of juvenile otolith edge chemistry by collection site. Samples are plotted by collection site Sr^{87}/Ca^{48} and Mg^{24}/Ca^{48}.

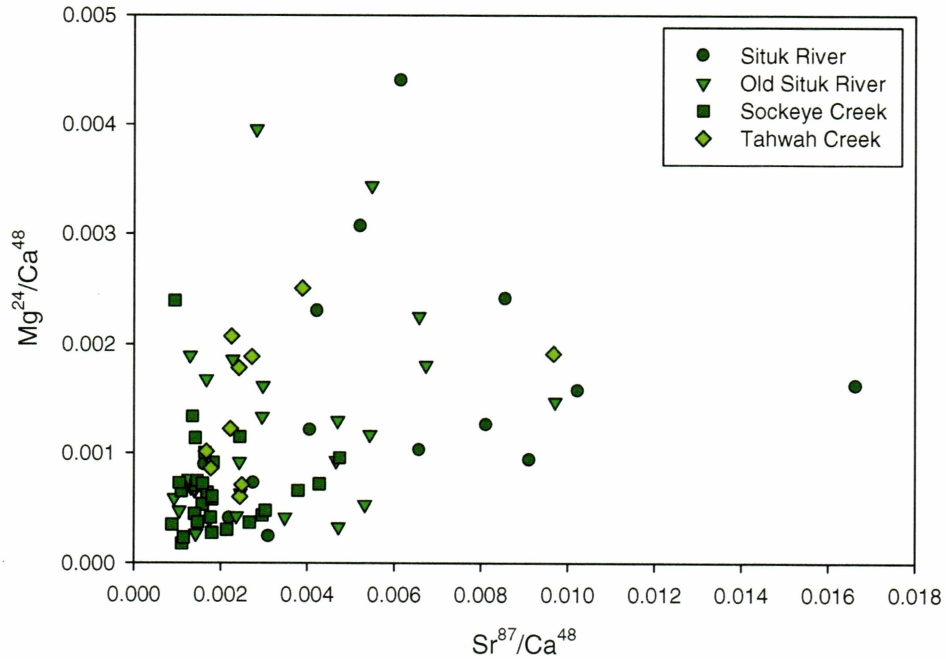

Figure 5. Scatter plot of adult otolith core chemistry by collection site. Samples are plotted by collection site Sr^{87}/Ca^{48} and Mg^{24}/Ca^{48}.

Conclusion

In this study, (1) otolith core chemistry was able to differentiate adjacent freshwater systems of the Yakutat Foreland, (2) a significant amount of stock delineation of coho salmon was possible on the Yakutat Foreland, and (3) adult otolith chemistry, coupled with Yakutat Foreland flat topography, closely space river systems, and seasonal freshets, suggested rates of straying from natal sites may be significantly higher than the coho salmon straying rates in other locations. The successful sub-stock identification of Situk River, Old Situk River, and Sockeye Creek contingents has important implications for the future management of Foreland coho salmon. A future study can use knowledge of these sub-stocks to identify outmigration and return migration run-timing. Identification of any 'run-partitioning' that exists among these sub-stocks could lead to the creation of multiple, shorter open fishing periods that target a specific sub-stock. Such a future management practice would allow for Alaska Department of Fish and Game to manage the harvested numbers of each sub-stock, rather than the Foreland harvest as a whole.

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