AN ABSTRACT OF THE THESIS OF

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Title: Natal Source Contributions of Pacific Cod (*Gadus macrocephalus*) Recruits in the Southeastern Bering Sea.

Abstract approved:

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Effective and sustainable fisheries management not only depends on identifying and defining stocks (fundamental unit of management), but also on having knowledge of factors influencing the abundance, distribution and connectivity of stocks. Population structure of Pacific cod (*Gadus macrocephalus*) in the southeastern Bering Sea (EBS) remains unresolved although genetic data indicate isolation-by-distance throughout the species range across the North Pacific Ocean. Pacific cod are fished on their spawning grounds, therefore, it is important to evaluate whether particular spawning sources are more critical than others in sustaining the productivity of fished populations. Chemical analysis of otoliths can provide information on mixing among groups of fish on ecological time scales and at finer spatial scales than genetic analyses. Therefore, I completed laboratory and field research on the otolith elemental composition of larval and juvenile cod to: (1) investigate temperature and growth-rate effects on otolith elemental incorporation to aid interpretations of field data; (2) evaluate the potential to detect larval exchange between the Gulf of Alaska

(GOA) and the EBS; (3) quantify spatial scales at which there was significant variation in otolith elemental composition; and (4) quantify the number of larval sources (chemically distinct groups) contributing to juvenile recruits in the EBS. In the laboratory study, I observed higher rates of incorporation at lower temperatures for both Sr and Ba but similar ranges of Mg incorporation at 2°C, 6°C and 8°C. There was no significant effect of somatic growth or otolith precipitation rate on elemental incorporation detected, indicating that variation in individual growth rates should not confound interpretations of field data. In two years of field collections (2006 & 2008), otolith elemental signatures (Mg:Ca, Mn:Ca, Zn:Ca, Sr:Ca, and Ba:Ca) differed between larvae collected in the EBS and GOA, and over 70% of the larvae were correctly classified to source. Furthermore, I examined the chemical signatures of the otolith edge (representing approximately the last 10 d of life) for juveniles collected at six locations throughout the EBS. Seventy-nine and 71% of the juveniles were correctly classified to their collection location in 2006 and 2008, respectively. These results demonstrate that there is sufficient spatial variation in otolith composition to examine spatial structure in Pacific cod within the EBS and to identify GOA larval contribution to the EBS population. Finally, larval source signatures, represented by otolith core chemistry, of juveniles collected throughout the EBS were classified into chemically distinct groups using hierarchical cluster analysis. In both years we identified two dominant sources representing 56% and 30% of the cohort in 2006, and 53% and 40% in 2008. Most collection sites contained a mixture of larval sources although one or two sites per year were comprised of a single source. These

chemically unique sources likely represent different spawning locations with distinct environmental characteristics, although the exact locations are unknown. These sources potentially represent contributions from known spawning areas, which include the Alaskan Peninsula, Aleutian Islands, and Pribilof Islands. Our results highlight the potential of otolith chemical analyses to identify source contributions of Pacific cod in the EBS but larval collections throughout the spawning season are needed to provide additional detail on the spatial distribution of those sources.

Natal Source Contributions of Pacific Cod (*Gadus macrocephalus*) Recruits in the Southeastern Bering Sea

by
Ruth A. DiMaria

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Ruth A. DiMaria, Author

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CONTRIBUTION OF AUTHORS

Dr. Thomas P. Hurst assisted with data analysis and was involved with the design and writing of Chapter 2.

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DEDICATION

I dedicate this thesis to my parents, James and Janet, my siblings, Mark and Emma, and to my best friend, Trevor, who have always encouraged and supported me in fulfilling my ambitions and pursuing my dreams, wherever they may take me.

Natal Source Contributions of Pacific Cod (*Gadus macrocephalus*) Recruits in the Southeastern Bering Sea

CHAPTER 1 – GENERAL INTRODUCTION

Pacific cod, *Gadus macrocephalus*, is a commercially and ecologically important groundfish species that inhabits the northern Pacific Ocean and supports major fisheries in the United States. Second only to walleye pollock (*Theragra chalcogramma*) in catch and product value for the Bering Sea and Gulf of Alaska, Pacific cod accounted for 228,670 t or 15% of the total groundfish catch in 2009 (see http://www.afsc.noaa.gov/species/catch_value.htm). Cod are a critical component in the trophodynamics of the North Pacific as they consume walleye pollock and crabs, and are forage for other fishes, such as Pacific halibut (*Hippoglossus stenolepis*), and several marine mammals and birds. Despite their value, few studies focus on the ecology and life history of Pacific cod (e.g., Dean et al. 2000, Abookire et al. 2007, Laurel et al. 2007) although such information is valuable for sustainable management of the fishery.

The gap in studies focused on the ecology and life history of Pacific cod may be because harvests of Alaskan fishes, including cod, are dominated by walleye pollock, and the economic value of Pacific cod is less than that for salmonids which also have a strong cultural significance in the Pacific Northwest. Additionally, as posited by Laurel et al. (2007), the research gap might be due to assumed similarities between the better-studied congener, Atlantic cod, *Gadus morhua*. While the two

species share similar life history characteristics, such as aggregate spawning and high fecundities (Matarese et al. 2003), there are key differences which might affect the patterns of population structure of Pacific cod in the Bering Sea region. For example, while both species aggregate in large numbers, Atlantic cod are mid-water batch spawners that release pelagic eggs whereas Pacific cod spawn once a season near bottom and release semi-adhesive, demersal eggs (Matarese et al. 2003, Laurel et al. 2007). Additionally, differences in geology and physical oceanography between the North Atlantic and North Pacific oceans likely influenced the development of life history strategies for Atlantic and Pacific cod. Further, Atlantic cod and Pacific cod are less taxonomically and genetically similar to each other when compared to other species in the family Gadidae (Coulson et al. 2006, Evseenko et al. 2006).

As with many marine species, questions of stock structure (i.e., the presence of spatially discrete groups of fish) persist, and the existence of stock structure would have important implications for the management of Pacific cod. The cod fishery in the United States is currently managed as two separate components: the Gulf of Alaska stock (GOA) and the larger Bering Sea – Aleutian Island stock (BSAI). Genetic analyses indicate that stock structure is present across the species' range throughout the North Pacific, but not within the smaller geographic scale of the southeastern Bering Sea (EBS) and GOA fisheries management region (Grant et al. 1987, Cunningham et al. 2009). Movement of tagged Pacific cod from EBS spawning

grounds into the GOA supports exchange between the current management regions (Shimada & Kimura 1994).

Pacific cod are harvested on their spawning grounds; therefore it is important to understand the relative contribution of spawning regions supporting the EBS population. Spawning grounds of cod have yet to be fully characterized; however, general spawning regions are located along the Aleutian Islands, Pribilof Islands, shelf break, and throughout the Unimak Pass-Alaska Peninsula (UP-AP) region in both the EBS and GOA (Figure 1.1a). The UP-AP region within the EBS is assumed to support the majority of spawning activity for southeastern Bering Sea Pacific cod, although larvae are potentially transported into the EBS via major water currents from any of the spawning regions. This assumption is supported by adult tagging data (Shimada & Kimura 1994), distribution of spawning females throughout the reproductive season (Ormseth et al. 2008, Logerwell & Neidetcher 2009), and higher densities of larvae collected throughout the GOA and EBS (Matarese et al. 2003). Additionally, Unimak Pass is the major conduit connecting the North Pacific Ocean and the eastern Bering Sea, with flow generally northward from the GOA into the EBS via a portion of the Alaska Coastal Current (Stabeno et al. 2002). Therefore, cod hatched within the western GOA along the Alaska Peninsula that are transported northward through Unimak Pass potentially recruit to the EBS cod population. However, the degree that different spawning sources contribute to the EBS cod population is unknown, particularly for fish spawned in the GOA.

Cod hatched within the UP-AP spawning region, either within the Bering Sea or transported through Unimak Pass from the GOA, are expected to be entrained within the Bering Coastal Current and carried along the Alaska Peninsula into Bristol Bay, with potential transport northward within the Bering Slope Current (Lanksbury et al. 2007; Figure 1.1b). Observations of age-0 Pacific cod in the Gulf of Alaska suggest that fish settle within shallow, coastal embayments and move to deeper waters as they increase in size and age (Abookire et al. 2007, Laurel et al. 2007, Laurel et al. 2009). However, in the EBS, age-0 cod are commonly captured across the shelf in both benthic and pelagic trawl surveys, indicating that they are not confined to near-shore habitat (Hurst et al. in review).

Analysis of otolith chemical composition is a promising tool for reconstructing connectivity and dispersal patterns within teleost fish populations on ecologically relevant time scales (Campana 1999). Otoliths are paired calcium carbonate ($CaCO_3$) structures of the inner ear used for balance and hearing and have been used successfully as natural tags (Campana et al. 1994, Jónsdóttir et al. 2006). Unlike bones and scales, otoliths are acellular and metabolically inert, so any elements or compounds incorporated into the growing crystal are permanent and not resorbed (Campana 1999). Some elements incorporated into the growing otolith surface reflect the environmental and chemical characteristics of the ambient water; however, physiology can influence otolith elemental incorporation (Farrell & Campana 1996, Elsdon & Gillanders 2004). Therefore, an understanding of the species-specific factors

that influence elemental incorporation is important for accurate interpretation of field data.

In regions with distinct water chemistries, mixed groups of fish can be differentiated based on unique otolith elemental signatures (Campana 1999, Elsdon et al. 2008). Significant variation in otolith elemental composition has been observed in fishes throughout the North Pacific. For example, Gao et al. (2005) observed significant differences in otolith stable isotope ratios ($\delta^{18}O$ and $\delta^{13}C$) and elemental concentrations (Sr, Mg, and Na) of Pacific Cod between two regions of Puget Sound, supporting the presence of two recognized spawning stocks. When characterizing natal sources of walleye pollock, Fitzgerald et al. (2004) observed significant differences in otolith elemental ratios (Mn:Ca, Sr:Ca, and Ba:Ca) in the natal signatures of juveniles throughout the Bering Sea and between the Bering Sea and northern Gulf of Alaska, suggesting separate spawning sources contributed to these locations. These studies indicate that spatial variation in otolith chemistry occurs within and between ocean basins; therefore it is likely that natal sources of EBS Pacific cod and the relative contribution of Gulf of Alaska larvae to the EBS can be quantified using otolith chemistry and structure.

A better understanding of the mechanism controlling otolith elemental incorporation is important in interpreting patterns observed in field collected fish. Therefore, in this study I first conducted a controlled laboratory experiment to determine the effects of temperate and growth rate on elemental incorporation into the

otoliths of larval cod. I then completed field research on the otolith elemental composition of larval and juvenile cod from two cohorts (2006 and 2008) to: (1) evaluate the potential to detect larval exchange between the Gulf of Alaska and the southeastern Bering Sea; (2) quantify spatial scales at which there was significant variation in otolith elemental composition; and (3) quantify the number of larval sources (chemically distinct groups) contributing to juvenile recruits in the southeastern Bering Sea.

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FIGURE LEGEND

Figure 1.1: Pacific cod spawning regions (a.) and major water currents (b.) in the southeastern Bering Sea region. General spawning regions (shaded) are located along the Aleutian Islands (1.), Pribilof Islands (2.), and throughout the Unimak Pass - Alaska Peninsula in the Bering Sea (3.) and Gulf of Alaska (4.). Major water currents: Aleutian North Slope Current (ANSC); Bering Coastal Current (BCC); Bering Slope Current (BSC); and Alaska Coastal Current (ACC).

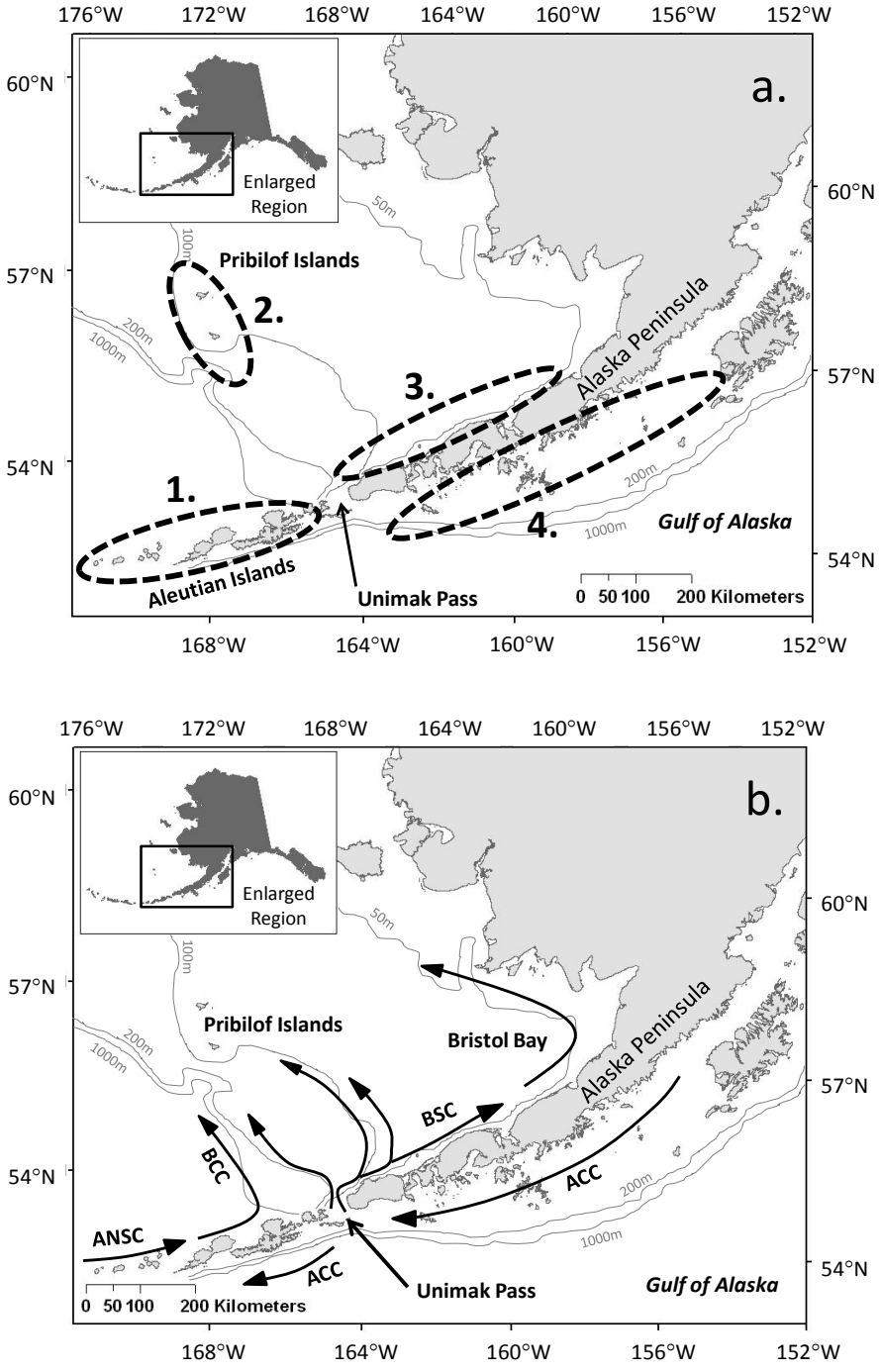
a.
b.
Enlarged Region
Pribilof Islands
Alaska Peninsula
Aleutian Islands
Unimak Pass
Gulf of Alaska
Bristol Bay
1.
2.
3.
4.
BSC
BCC
ACC
ANSC
50m
100m
200m
1000m
0 50 100 200 Kilometers
176°W
172°W
168°W
164°W
160°W
156°W
152°W
60°N
57°N
54°N

Figure 1.1

TEMPERATURE AND GROWTH EFFECTS ON OTOLITH ELEMENTAL CHEMISTRY OF LARVAL PACIFIC COD, *GADUS MACROCEPHALUS*

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CHAPTER 2 – TEMPERATURE AND GROWTH EFFECTS ON OTOLITH ELEMENTAL CHEMISTRY OF LARVAL PACIFIC COD, *GADUS MACROCEPHALUS*

Abstract

Variation in otolith elemental composition is used to investigate movements of teleost fishes based on observations that otolith elemental composition reflects variation in water chemistry. Various environmental (e.g., temperature, salinity, and water concentration) and biological (e.g., growth, diet, and ontogeny) factors can influence otolith elemental incorporation although the relative influence of these factors remains poorly understood for most species. Therefore, we examined the effects of temperature and growth rate on the otolith elemental composition of larval Pacific cod, *Gadus macrocephalus*. The larvae were progeny of wild Pacific cod collected from spawning grounds near Kodiak Island, Alaska. Immediately after hatching, larvae were acclimated to 2°C, 5°C, and 8°C and reared for 38-51 days. Otolith concentrations of Li, Mg, Ca, Mn, Zn, Sr, and Ba were measured using laser ablation-inductively coupled plasma mass spectrometry. Li and Zn otolith concentrations were near detection limits and excluded from subsequent analyses. The effects of temperature on otolith partition coefficients (D_{Me}) varied among elements. D_{Mg} showed no relationship with temperature whereas D_{Sr} and D_{Ba} decreased with increasing temperature. It is possible that, for larval Pacific cod, kinetic effects are

more important in the incorporation of Sr and Ba whereas metabolic effects may play a larger role in the incorporation of Mg. There was no evidence for a direct effect of somatic growth rate or otolith precipitation rate on D_{Me} for any of the elements, which indicates that individual growth variation is unlikely to lead to misinterpretation of field-collected data. Understanding variable relationships among otolith elemental signatures, environmental conditions, and fish physiology can improve the accuracy of interpretations of field data, particularly in marine systems where spatial variation in element concentrations are typically lower than freshwater environments.

Introduction

Analysis of patterns in otolith elemental composition provides information applicable to ecology and fisheries management including insights into population structure and individual movements (Campana 1999; Campana and Thorrold 2001; Elsdon et al. 2008). These analyses are based on the principle that variation in otolith chemical composition is primarily driven by environmental factors that differ across the species' range or among discrete habitats. Laboratory and field studies have confirmed positive relationships between water and otolith composition for certain element:calcium ratios, such as Ba:Ca and Sr:Ca (e.g., Bath et al. 2000; Elsdon and Gillanders 2003; Miller 2009). However, experimental studies have demonstrated that otolith chemical composition can be influenced by other factors such as temperature

and growth rate (see reviews in Campana 1999; Elsdon et al. 2008). Quantifying the magnitude of these abiotic and biotic influences on otolith composition will aid applications of this technique to understand spatial and temporal movements of fishes.

Temperature variation occurs across a variety of spatial and temporal scales, potentially affecting all aspects of physiology including elemental incorporation into otoliths (Bath et al. 2000; Elsdon and Gillanders 2003; Miller 2009). Otolith variation in four ratios, Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca, is commonly used to discriminate among groups of fish, and temperature effects on the otolith incorporation of these elements have been documented (Thresher 1999; Elsdon et al. 2008). In large marine systems where water chemistry is expected to be more homogeneous than in freshwater systems, the thermal effects on otolith composition may be greater than those due to spatial variation in water concentrations. Quantifying the magnitude of thermal influence on otolith composition across a range of species and life history stages can be used to evaluate the potential for applying otolith microchemistry to questions of stock structure and dispersal in large marine ecosystems.

In addition to temperature effects on otolith chemistry, several studies have demonstrated effects of biotic factors such as growth rate and diet (Buckel et al. 2004; Martin et al. 2004). Growth rate and diet can differ among individuals inhabiting the same water mass, potentially confounding interpretations of field data. Laboratory studies have demonstrated that elevated elemental concentrations in the diet can lead to elevated otolith Sr:Ca and Ba:Ca (Gallahar and Kingsford 1996; but see Walther

and Thorrold 2006). Furthermore, significant effects of somatic and otolith growth rate on otolith incorporation of Mg, Sr, and Ba have been documented in some species (e.g., Sadovy and Severin 1992, 1994) but mixed or no effects were observed in others (e.g., Martin et al. 2004; Martin and Thorrold 2005; Miller 2009). Given these variable results and the potential for growth rate effects to confound efforts to group fishes geographically based on variation in otolith composition, potential effects of growth rate warrant further attention. Partition coefficients (D_{Me}) provide a comparable metric to evaluate the effects of water concentration, temperature, and growth rate on the elemental composition of otoliths (Morse and Bender 1990). For example, the partition coefficient of Sr (ratioed to calcium) in otoliths is calculated as: $D_{Sr} = [Sr:Ca]_{otolith} / [Sr:Ca]_{water}$ (Eq. 1).

In this study, we examined the effect of temperature on otolith elemental composition in larval Pacific cod, *Gadus macrocephalus* reared in the laboratory at 2, 5, and 8^{o}C. Natural variation in body and otolith size at the end of the experiment was used to examine potential effects of growth rate. Pacific cod is a widespread marine fish supporting important fisheries in both the eastern and western Pacific. The fishery in the United States is managed as two components, the Gulf of Alaska stock and the Bering Sea – Aleutian Island stock. Although genetic analyses have not identified stock structure relevant to these management units (Cunningham et al. 2009), there is minimal information on connectivity among these stocks on ecologically-relevant time scales. Pacific cod spawn demersal eggs with larvae rising to the surface waters

immediately after hatch (Doyle et al. 2009; Hurst et al. 2009). Larvae are potentially transported from discrete spawning grounds by prevailing water currents, but the degree of mixing among regions remains unknown. An improved understanding of the stock structure, dispersal, and movement patterns of Pacific cod has been identified as a critical knowledge gap (NPFMC 2006). Otolith chemical analysis of field-collected larval and juvenile Pacific cod is being used to characterize larval sources and examine the contributions of those sources to the Gulf of Alaska, Bering Sea and Aleutian Island regions. The results presented here will aid interpretation of those field data.

Methods

Larval rearing

Larvae used in this experiment were the progeny of wild adult Pacific cod (two females and five males). Spawning adults were collected with commercial jigging gear in Chiniak Bay, Kodiak Island, Alaska. Gametes were stripped, mixed, and incubated at 4°C for 24 hours. Fertilized eggs were shipped in 4°C chilled seawater in insulated containers to the Alaska Fisheries Science Center laboratory in Newport, Oregon. Eggs were placed into 4 L plastic trays with flow-through seawater and incubated at 4°C. Upon hatching (19-22 days post-fertilization), larvae were transferred into 100 L cylindrical upwelling tanks and acclimated to 2°C, 5°C and 8°C. Three hundred larvae

were added to each of three replicate tanks for each temperature treatment (*n*=9 tanks). Larvae were fed a combination of rotifers (*Brachionus plicatilis*) and microparticulate dry food (Otohime A). Rotifers were supplied at densities of 4 prey·ml^{-1} twice daily and dry food was provided 2-3 times per day. Due to slower growth at lower temperatures, larvae were reared longer prior to sampling in the 2°C and 5°C treatments than the 8°C treatment to ensure a sufficient amount of otolith material for chemical analysis. Thirty larvae were sacrificed from each of the 2°C, 5°C and 8°C tanks after 51, 50, and 38 days, respectively (Table 1.1). Due to low larval survival at lower temperatures, only two tanks were available for the 2°C treatment. Larvae were weighed (wet weight to 0.001 g), photographed under a dissecting microscope, and measured (standard length, SL, to 0.01 μm) using the analytical imaging software, ImagePro® (Media Cybernetics).

Water samples were collected to determine if elemental ratios were homogeneous throughout the experiment and across temperature treatments and to calculate partition coefficients (Eq. 1). To determine if elemental ratios were homogenous across time, one water sample was collected from each temperature treatment (*n*=3) approximately weekly throughout the rearing experiment. To determine if elemental ratios were homogenous across temperature treatments, water samples were drawn from each of the nine experimental tanks on days 25 and 36 of the experiment. Samples were filtered (0.2 μm) in a Class 100 laminar flow bench, acidified to <2 pH with UltraPure™ HNO_3, and stored at 4°C until analysis. Elemental

concentrations were measured with a Leeman-Teledyne inductively coupled plasma-optical emission spectrometer (Mg at 279.1 nm, Ca at 317.9 nm, Sr at 421.5 nm, and Ba at 493.4 nm). Filtered, acidified samples were diluted (100x) and standard curves were generated with SPEX Certiprep® Group certified reference materials. National Institute of Standards and Technology (NIST) standard (1643e) was used to assess accuracy. Measured Mg, Ca, Sr, and Ba concentrations were within 5%, 6%, 1%, and 1%, respectively, of certified values. Samples of known concentration were introduced throughout the analysis to estimate precision. Repeated measurements varied by 3% for Mg (n=4), 1% for Ca (n=4), <1% for Sr (n=2), and 2% for Ba (n=2).

Variation in water elemental ratios over time and among treatments was evaluated with two-way analysis of variance (ANOVA) with temperature treatment and sampling date as main, fixed effects. Additionally, differences in water elemental ratios among tanks were examined with two-way ANOVA using the data collected on the two days when concentrations were measured in all nine rearing tanks. Finally, mean elemental water ratios were calculated for each tank by averaging ratios over the entire rearing period (2°C n=6; 5°C n=6; 8°C n=4) and used to calculate partition coefficients (Eq. 1).

Otolith analysis

Both sagittal otoliths were removed for elemental analysis using standard methods to minimize contamination (e.g., Miller 2009). Otolith diameter (0.5 µm) was measured at the widest position using ImagePro®. All otoliths were ultrasonically cleaned in NANOpure® (18M Ohm) water for 15 minutes, dried in Class 100 conditions, and stored dry in acid-washed plastic trays. Larval otoliths were then fixed in a random manner to acid-washed slides with double-stick tape (Scotch™) under Class 100 conditions.

Otolith elemental composition (Mg, Ca, Mn, Zn, Sr, and Ba) was quantified using a VG PQ ExCell inductively coupled plasma mass spectrometer with a New Wave DUV193 excimer laser at Oregon State University's WM Keck Collaboratory for Plasma Spectrometry. The laser was set at a pulse rate of 3 Hz and a 50-µm stationary spot ablation was used to collect data through the otoliths. Background levels of all analytes were measured before ablation and subtracted from measurements during ablation. Analytes were ratioed to ^{43}Ca and converted to molar ratios based on measurements of NIST 612 standard glass (Miller 2009). Only Mg, Ca, Sr, and Ba were consistently above detection limits in the otoliths and used for subsequent analyses. The mean percent relative standard deviations (%RSD) for NIST 612 standard glass during analyses were: ^{24}Mg = 3.8%, ^{43}Ca = 3.6%, ^{86}Sr = 4.4%, and ^{138}Ba = 4.9%. A calcium carbonate standard (USGS MACS-1) was used to assess

accuracy: measured ratios were within 3%, 1%, and 6% of known values for Mg:Ca, Sr:Ca, and Ba:Ca, respectively.

Otolith primordia contain elevated concentrations of Mn (Brophy et al. 2004; Ruttenberg et al. 2005). In larval Pacific cod otoliths, the average otolith Mn concentration within the primordial region was 20−100x greater than the average Mn concentration outside of this region. Therefore, we averaged otolith elemental ratios from the region outside of the Mn-rich zone to isolate deposition that occurred during the experiment. Average ablation depth (mean±SD) for each treatment was 4.6±0.7µm for the 8°C treatment, 4.7±0.9µm for the 5°C treatment, and 4.7±0.9µm for the 2°C treatment.

A paired *t*-test was used to determine if there were differences in otolith elemental composition between otolith pairs of individual fish. As there were no detectable differences in elemental ratios between otolith pairs (df=79, $p>0.31$), one otolith was chosen randomly from each individual for subsequent analyses. Partition coefficients (D_{Me}) were then calculated for Mg, Sr and Ba using individual otolith elemental ratios ($[Me:Ca]_{otolith}$) and mean tank water ratios. Outliers, defined as values >3 SD from the mean value, were excluded from analyses ($n=4$). The effect of temperature on D_{Me} was examined using ANOVA with temperature as a fixed factor. D_{Me} were examined for normality and homogeneity of variance and, if necessary, transformed.

To evaluate potential growth rate effects on otolith elemental incorporation, we determined somatic growth and otolith precipitation rates during the experiment. Mean size at hatch of Pacific cod used in these experiments was 5.16 mm SL. A mean otolith diameter at hatch of 8.25 µm (Narimatsu et al. 2007) was applied to all treatments. As the experiments were initiated upon hatching, these values were subtracted from final fish size (SL) and otolith diameter to estimate growth during the experiment. Individual somatic growth and otolith precipitation rates were determined by dividing the change in each metric by the number of experimental days (2°C=51 days; 5°C=50 days; 8°C=38 days). Tank means were calculated and compared among temperature treatments using ANOVA with temperature as a fixed factor. The direct effect of temperature on growth and the minimal overlap in observed growth rates between temperature treatments precluded incorporating growth rate as a covariate in the analysis of partition coefficients. Therefore, potential growth effects on partition coefficients were evaluated by determining the relationship between D_{Me} and somatic growth and otolith precipitation rate within temperature treatments using regression analysis. Correction for multiple comparisons was not performed given the probability of finding a significant result declines dramatically as the number of statistical comparisons increases thus inflating Type II error (Moran 2003). Data were examined for normality and homogeneity of variance and, if necessary, transformed.

Results

Rearing water

Water temperatures (mean±SD) during the experiment were 2.39±0.67°C, 4.67±0.48°C, and 7.92±0.42°C, hereafter referred to as 2°C, 5°C, and 8°C treatments. Water elemental ratios (mean±SD) were 4709±18.0 mmol·mol^{-1}, 8.01±0.03 mmol·mol^{-1}, and 4.44±0.33 µmol·mol^{-1}, for Mg:Ca, Sr:Ca and Ba:Ca, respectively (Table 2.2). There were no significant differences in elemental ratios of Mg:Ca, Sr:Ca, and Ba:Ca among rearing tanks (p>0.20) or temperature treatments (Table 2.3). There was a gradual increase in Sr:Ca and Ba:Ca over the course of the experiment, possibly in conjunction with seasonal coastal upwelling patterns, which resulted in a significant effect of date. There was no significant effect of date on Mg:Ca in rearing waters. Average values for water Mg:Ca, Sr:Ca, and Ba:Ca were used to calculate partition coefficients.

Otolith partition coefficients

Overall, partition coefficients (mean±SD) during the experiment were 0.001±0.001, 0.431±0.074, and 0.090±0.104 for D_{Mg}, D_{Sr} and D_{Ba}, respectively (Table 2.1). For statistical analyses, partition coefficients were transformed ($\log_{10}(x + 1)$) to obtain normality and stabilize the variance. Transformations were effective for the

temperature treatments (F-ratio <3.4, Tabachnick and Fidell 2001) but inadequate to stabilize the variance among tanks for D_{Mg} and D_{Ba}.

There was a significant effect of temperature on partition coefficients for Sr and Ba (Table 2.4) with partition coefficients for both elements being higher at low temperatures (Figure 2.1). For both elements, mean partition coefficients in the 2°C treatments were significantly higher than in the 5°C and 8°C treatments (LSD post-hoc test, $p<0.05$). The 5°C treatment was slightly, but not significantly, higher than the 8°C treatment ($p>0.05$). In addition, there was significant variation among tanks for D_{Sr} and D_{Ba}, primarily the result of Tank 3 > Tank 2 at 2°C for D_{Sr} ($F_{(5, 136)}=3.64$, $p<0.001$), and Tank 1 > Tank 3 at 5°C for D_{Ba} ($F_{(5, 136)}=3.75$, $p=0.003$). There was no effect of tank or temperature treatment on D_{Mg}.

As expected, mean somatic growth and otolith precipitation rates varied among temperature treatments ($F_{(2,136)}=113.5$, $p<0.001$, Figure 2.2) and there was considerable variation among individual tanks ($F_{(5,136)}=2.83$, $p=0.002$, Figure 2.2). Somatic growth (mean±SD) was 0.05±0.02, 0.09±0.01, and 0.12±0.02mm·day^{-1} in the 2°C, 5°C, and 8°C treatments, respectively. Otolith precipitation rate (mean±SD) was 0.70±0.13, 1.65±0.30, and 2.60±0.56µm·day^{-1} in the 2°C, 5°C, and 8°C treatments, respectively. There was minimal overlap in the range of growth and precipitation rates among temperatures (Figure 2.2). The regression analysis indicated that D_{Me} was not directly related to somatic growth or otolith precipitation within temperature treatments (Table 2.5). In general, only 1 out of 18 cases showed a significant

relationship (p<0.05; Table 2.5) and all explained less than 10% of the variance in D_{Me}.

Discussion

In this study, we evaluated the effects of temperature and growth rate on the otolith elemental incorporation of larval Pacific cod while limiting the potentially confounding effects of ontogeny, genetics, diet, and water chemistry. Larval life history stages are under-represented in controlled laboratory studies that examine factors regulating otolith elemental incorporation. Larvae may exhibit patterns of elemental incorporation distinct from juvenile or adult life stages due to rapid growth and development. Larvae display marked physiological differences from later life stages, including the use of the integument for osmoregulatory, respiratory and excretory functions, which may contribute to ontogenetic changes in patterns of elemental discrimination (see review by Varsamos et al. 2005). Therefore, we exposed larvae to a representative temperature range to quantify the effect of temperature on incorporation of Mg, Sr, and Ba and determine if somatic growth and otolith precipitation rate effects could confound interpretations of variation in elemental signatures of field-collected individuals.

Our observation of a negative effect of temperature on D_{Sr} is similar to findings from other studies focused on larvae of cold-adapted species (Radtke et al.

1990; Townsend et al. 1989, 1992, 1995). However, studies focused on juvenile stages of temperate species whose natural range encompass warmer temperatures than that of Pacific cod documented positive (e.g., Secor et al. 1995; Bath et al. 2000) or no effects of temperature (e.g., Gallahar and Kingsford 1996; Elsdon and Gillanders 2004) on otolith incorporation of Sr. Negative temperature effects on the otolith incorporation of Sr were observed in larval Atlantic cod, *Gadus morhua* (Townsend et al. 1995) and larval Atlantic herring, *Clupea harengus* (Townsend et al. 1989, 1992; Radtke et al. 1990). As all of these negative temperature effects involve larval stages of cold-adapted species, it is possible that reduced metabolic and/or osmoregulatory activity at lower temperatures contribute to the observed patterns. Additionally, negative temperature effects on the incorporation of Sr have been documented in inorganic aragonite precipitation studies (e.g., Gaetani and Cohen 2006). The concentration of certain elements in the otoliths of larvae, which have less developed osmoregulatory capabilities than juveniles and adults, may reflect kinetic process regulating crystal growth more than older life history stages.

The patterns of otolith Ba:Ca observed in larval Pacific cod differ from previous observations. The range in D_{Ba} in this study (0.05–0.20) is lower than previously reported (0.10–0.80) (Bath et al. 2000; Martin and Thorrold 2005; Martin and Wuenschel 2006; Miller 2009). We do not have an explanation for the lower values of D_{Ba} but it is worth noting that this is the first study to quantify partition coefficients in larvae of a cold-adapted species. Furthermore, there was a negative

effect of temperature on D_{Ba}, whereas most other studies on eury- or stenohaline fishes documented a positive or no effect of temperature on Ba incorporation (see review in Miller 2009). Given that negative temperature effects on the incorporation of Ba have been documented in controlled precipitation experiments of inorganic aragonite (Gaetani and Cohen 2006), the otolith concentrations of both Sr and Ba in larval Pacific cod may be more related to direct effects of temperature on aragonite composition than metabolic or osmoregulatory effects. Additional research is required to determine if the negative temperature effects on otolith incorporation of Ba and Sr observed here are common to larvae of cold-water species.

The range in D_{Mg} observed in this study (0.0001–0.007) overlaps previously reported values (Martin and Thorrold 2005; Martin and Wuenschel 2006). Also similar to our results, most previous studies on the otolith incorporation of Mg:Ca in larval or juvenile marine fishes report no significant effect of temperature (Hoff and Fuiman 1995; Martin and Thorrold 2005; Martin and Wuenschel 2006). However, there is some evidence for a negative temperature effect on the otolith incorporation of Mg in larval Atlantic croaker *Micropogonus undulatus* (Fowler et al. 1995a, b). Interestingly, Gaetani and Cohen (2006) also observed a negative temperature effect on the incorporation of Mg in abiotic aragonite. The lack of a temperature effect on the otolith incorporation of Mg is in contrast to our findings for Sr and Ba. These contrasting patterns indicate that Mg, which is abundant in the marine environment

and important for a suite of physiological process such as proper cell function and bone development, may be more tightly regulated than Sr or Ba in larval Pacific cod.

The presence of somatic growth or otolith precipitation rate effects on otolith elemental incorporation could lead to misinterpretations of field observations in some applications. For example, individuals that experienced different growth rates could have distinct otolith elemental signatures even though they resided in the same water mass. Previous laboratory studies documented negative and no relationships between otolith elemental concentrations and growth (Chesney et al. 1998; Bath et al. 2000; Martin et al. 2004; Martin and Thorrold 2005; Miller 2009). In this study, there were no detectable effects of somatic growth or otolith precipitation rate on the incorporation of Mg:Ca, Sr:Ca, or Ba:Ca within temperature treatments. The variation in growth rates observed within temperature treatments was similar to that observed in other experimental cultures of larval gadids (Puvanendran and Brown 2002). Furthermore, individual size variation within our experiment is only slightly less than that observed in wild collections of similarly aged gadids, likely because some factors that contribute to growth variability were controlled in the experiment. We calculated a range:mean size ratio within temperature treatments of 32%, which is comparable to the 40% range:mean size ratio observed for a wild cohort of walleye pollock at similar known ages by Brown and Bailey (1992). Given the comparable growth rate variability observed in this study and in wild cohorts, our observations of growth

effects on otolith elemental incorporation is a realistic representation of what may occur in wild populations.

As temperature and growth rates are tightly coupled, it is difficult to unequivocally isolate and evaluate the independent effects of each factor on otolith composition, especially over wide temperature ranges. In this study the growth effect was evaluated by examining the relationship between D_{Me} and natural variation in growth rate within temperature treatments. Other laboratory studies that have investigated growth effects generally found weak or non-significant effects on otolith chemistry (Bath et al. 2000; Martin et al. 2004; Miller 2009; but see Martin and Thorrold 2005). We also observed no significant effects of somatic growth or otolith precipitation rate on otolith composition within temperature treatments, thus it appears unlikely that rate effects will confound interpretations of field data for larval Pacific cod. However, future studies should explore these issues further through independent manipulation of temperature and feeding regime to increase growth variation within temperature treatments and overlap between temperature treatments to the maximum practicable degree.

The results of this study can be used to determine the amount of observed variation in otolith composition that is potentially due to temperature variation. As Pacific cod larvae can be exposed to temperatures ranging from 2–8°C in the Gulf of Alaska and Bering Sea (L. Ciannelli, unpublished data), we examined the effect of temperature across the same range in the laboratory. However, within a year, spatial

variation in temperature is unlikely to be more than 3°C. Based on our observed D_{Me}, otolith Sr:Ca and Ba:Ca could vary by 18% and 64%, respectively, given a 3°C difference in temperature. Preliminary data on the larval otolith signatures of juvenile Pacific cod collected from the Pribilof Islands in the Bering Sea and Gulf of Alaska varied by ~10% for Sr:Ca and ~30% for Ba:Ca (Miller et al., unpublished data). Our findings underscore the point that temperature alone could account for at least some of the observed spatial variation in otolith elemental composition. Furthermore, given that temporal variation in temperature at a particular location may vary by 1–3°C during a spawning season, it is important that individuals of known, and preferably similar, hatch dates are examined when making inferences regarding larval sources based on otolith elemental composition.

In this study, we demonstrated that there are significant negative effects of temperature on otolith incorporation of Sr and Ba that may account for observed variation in field studies in Pacific cod. In contrast, we observed no effect of temperature on Mg otolith incorporation. It is possible that, for larval Pacific cod, kinetic effects are more important in the incorporation of Sr and Ba whereas metabolic effects may play a larger role in the incorporation of Mg. Additionally, the lack of evidence for significant effects of somatic growth or otolith precipitation rate on elemental composition indicates that individual growth variation is unlikely to lead to misinterpretation of field-collected data on larval source signatures although additional research is needed.

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FIGURE LEGEND

Figure 2.1: Mean (±1 SD) partition coefficients (D_{Me}) for (a) Mg, (b) Sr, and (c) Ba. Values for each temperature treatment were staggered to distinguish replicates.

Figure 2.2: Individual and mean (±1 SD) partition coefficients (D_{Me}) for (a) Mg, (b) Sr, and (c) Ba, plotted against otolith precipitation rate for each temperature treatment (2°C, ●; 5°C, □; and 8°C, ▲). Data were $\log_{10}(x+1)$-transformed prior to analysis.

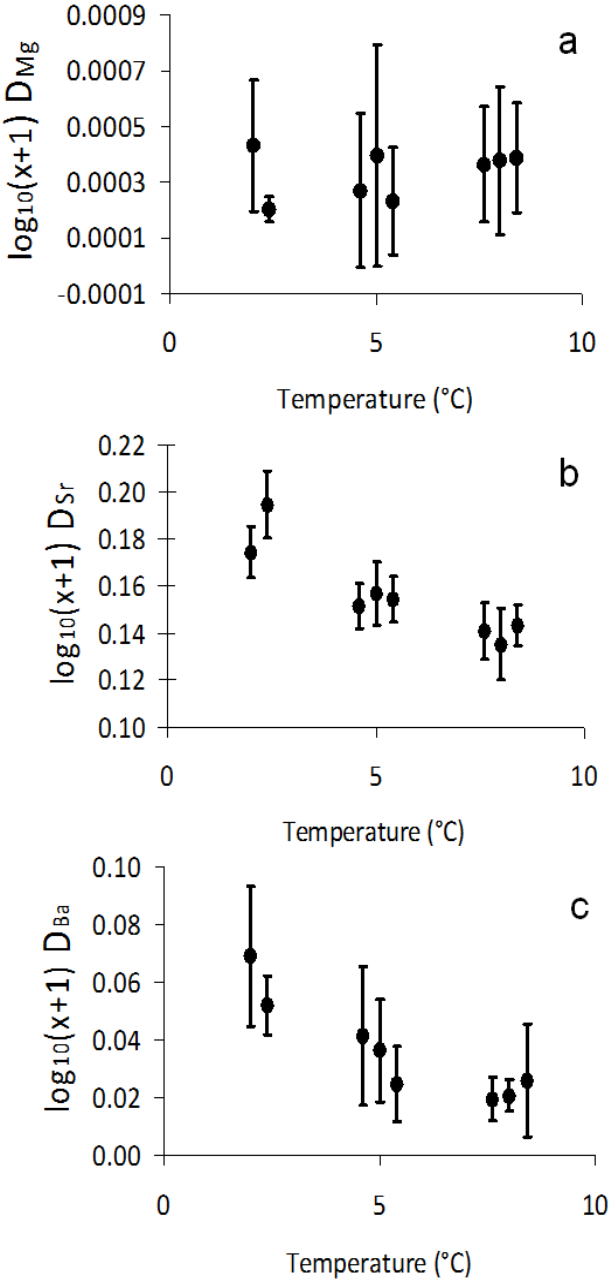
a
0.0009
0.0007
0.0005
0.0003
0.0001
-0.0001
log10(x+1) DMg
0
5
10
Temperature (°C)
b
0.22
0.20
0.18
0.16
0.14
0.12
0.10
log10(x+1) DSr
0
5
10
Temperature (°C)
c
0.10
0.08
0.06
0.04
0.02
0.00
log10(x+1) DBa
0
5
10
Temperature (°C)

Figure 2.1

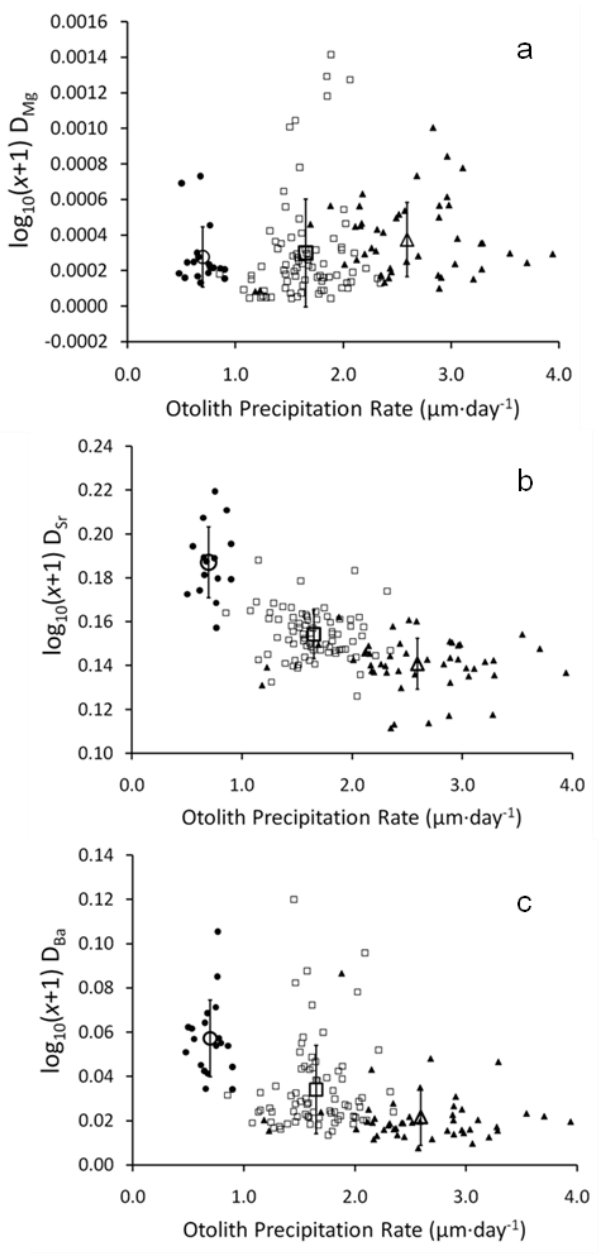
a
$\log_{10}(x+1)$ D_{Mg}
0.0016
0.0014
0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
-0.0002
0.0
1.0
2.0
3.0
4.0
Otolith Precipitation Rate (µm·day^{-1})
b
$\log_{10}(x+1)$ D_{Sr}
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.0
1.0
2.0
3.0
4.0
Otolith Precipitation Rate (µm·day^{-1})
c
$\log_{10}(x+1)$ D_{Ba}
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0.0
1.0
2.0
3.0
4.0
Otolith Precipitation Rate (µm·day^{-1})

Figure 2.2

TABLE LEGEND

Table 2.1: Experimental summary, including temperature, fish age, number of otoliths analyzed (*n*), mean standard length (SL±SD, mm), and mean otolith diameter OD±SD, µm) for each tank. Mean (±SD) otolith elemental ratios ($[Me:Ca]_{otolith}$) and partition coefficients (D_{Me}) for Mg, Sr and Ba are included. Otolith elemental ratios are reported in $mmol \cdot mol^{-1}$ for Mg and Sr, and $\mu mol \cdot mol^{-1}$ for Ba.

Table 2.2: Mean (±SD) water ratios for each tank (*n*=2) from the two days in which all tanks were sampled for each temperature treatment (2°C, 5°C and 8°C). Water elemental ratios ($[Me:Ca]_{water}$) are reported in $mmol \cdot mol^{-1}$ for Mg:Ca and Sr:Ca and $\mu mol \cdot mol^{-1}$ for Ba:Ca.

Table 2.3: Results of ANOVA to evaluate the effect of temperature (2°C, 5°C and 8°C) and day on water Mg:Ca, Sr:Ca and Ba:Ca.

Table 2.4: Results of ANOVA to evaluate the effect of temperature (2°C, 5°C and 8°C) on mean partition coefficients (D_{Me}). Data were $\log_{10}(x+1)$ -transformed prior to analysis.

Table 2.5: Results of linear regressions for partition coefficients (D_{Me}) against somatic growth rate and otolith precipitation rate. Data were $\log_{10}(x+1)$-transformed prior to analysis. Correction for multiple comparisons was not applied.

Table 2.1

Treatment	Age (d)	Tank	*n*	SL	OD	$[Mg:Ca]_{otolith}$	$[Sr:Ca]_{otolith}$	$[Ba:Ca]_{otolith}$	D_{Mg}	D_{Sr}	D_{Ba}
2°C	51	1	0	NA	NA	NA	NA	NA	NA	NA	NA
		2	6	8.25 (0.37)	43.5 (5.39)	4.68 (2.55)	3.97 (0.30)	0.87 (0.51)	0.0009 (0.0005)	0.49 (0.04)	0.20 (0.11)
		3	13	7.66 (0.95)	44.0 (7.09)	2.21 (0.50)	4.83 (0.68)	0.57 (0.12)	0.0004 (0.0001)	0.60 (0.09)	0.13 (0.03)
5°C	50	1	24	9.82 (0.72)	90.0 (15.6)	2.93 (3.00)	3.34 (0.25)	0.60 (0.86)	0.0006 (0.0006)	0.42 (0.03)	0.14 (0.19)
		2	24	9.80 (0.63)	95.2 (13.9)	4.28 (4.30)	3.49 (0.36)	0.39 (0.20)	0.0009 (0.0009)	0.44 (0.05)	0.09 (0.05)
		3	25	9.72 (0.43)	87.5 (14.9)	2.51 (2.08)	3.42 (0.25)	0.26 (0.15)	0.0005 (0.0004)	0.43 (0.03)	0.06 (0.03)
8°C	30	1	24	10.0 (0.63)	112 (21.1)	3.96 (2.25)	3.07 (0.30)	0.19 (0.08)	0.0008 (0.0005)	0.38 (0.04)	0.05 (0.02)
		2	8	9.49 (0.60)	108 (20.4)	4.10 (2.88)	2.93 (0.38)	0.20 (0.06)	0.0009 (0.0006)	0.37 (0.05)	0.05 (0.01)
		3	15	9.24 (0.87)	98.3 (20.7)	6.18 (8.10)	3.13 (0.22)	0.35 (0.46)	0.0013 (0.0017)	0.39 (0.03)	0.08 (0.11)

Table 2.2

Treatment	Tank	$[Mg:Ca]_{water}$	$[Sr:Ca]_{water}$	$[Ba:Ca]_{water}$
2°C	1	4716 (39.1)	8.04 (0.02)	4.64 (0.29)
	2	4707 (13.8)	7.99 (0.05)	4.50 (0.01)
	3	4710 (5.86)	8.00 (0.02)	4.38 (0.27)
5°C	1	4721 (18.7)	8.01 (0.02)	4.50 (0.02)
	2	4689 (12.2)	8.00 (0.01)	4.24 (0.04)
	3	4726 (18.9)	8.02 (0.02)	4.56 (0.09)
8°C	1	4698 (2.93)	7.97 (0.01)	4.51 (0.04)
	2	4716 (26.6)	8.02 (0.01)	4.38 (0.32)
	3	4725 (5.06)	8.03 (0.01)	4.38 (0.28)

Table 2.3

Me:Ca	Effect	df	MS	F	*p*
Mg:Ca	Temperature	2	118.0	0.368	0.696
	Day	5	440.8	1.375	0.278
	Error	19	320.3		
Sr:Ca	Temperature	2	<0.001	0.432	0.692
	Day	5	0.002	3.109	0.029
	Error	19	0.001		
Ba:Ca	Temperature	2	0.012	0.394	0.677
	Day	5	0.414	13.77	<0.001
	Error	19	0.030		

Table 2.4

D_{Me}	Effect	df	MS	F	*p*
D_{Mg}	Temperature	2	<0.000001	0.658	0.558
	Error	5	<0.000001		
D_{Sr}	Temperature	2	0.001207	23.74	<0.003
	Error	5	0.000051		
D_{Ba}	Temperature	2	0.000898	14.36	0.008
	Error	5	0.000063		

Table 2.5

Temp	D_{Me}	Growth rate (mm·day^{-1})		Precipitation rate (µm·day^{-1})	
		R^2	p	R^2	p
2	Mg	0.002	0.856	0.062	0.303
	Sr	0.021	0.594	0.010	0.603
	Ba	0.007	0.732	<0.001	0.982
5	Mg	<0.001	0.976	0.025	0.166
	Sr	0.087	0.009	0.035	0.105
	Ba	0.006	0.520	0.002	0.682
8	Mg	0.002	0.740	0.012	0.463
	Sr	0.039	0.177	0.002	0.779
	Ba	0.023	0.300	0.010	0.501

CHAPTER 3 – NATAL SOURCE CONTRIBUTIONS OF PACIFIC COD RECRUITS IN THE SOUTHEASTERN BERING SEA

Introduction

A "stock" is the fundamental unit of fisheries management; therefore, identifying and defining stocks is a central component of sustainable fisheries management (Cadrin et al. 2005). The stock structure of Pacific cod, *Gadus macrocephalus*, across the North Pacific Ocean remains unclear, although it has been a topic of investigation for many years (e.g., Ketchen 1961, Grant et al. 1987, Cunningham et al. 2009). The Pacific cod fishery in the United States is currently managed as two components, the Gulf of Alaska (GOA) stock and the larger Bering Sea - Aleutian Island (BSAI) stock. Early work by Wilimovsky et al. (1967) reported geographic differences among Pacific cod using meristic measures, suggesting the potential for distinct stocks in southern British Columbia, southeast Alaska, and the Bering Sea; however, small sample sizes may have prevented clearer delineations. Additionally, several genetic studies (Grant et al. 1987, Cunningham et al. 2009) have observed isolation by distance across the species range throughout the North Pacific Ocean but not between the current management regions.

Pacific cod annually aggregate in large numbers at discrete spawning locations throughout the Aleutian Islands, Pribilof Islands, and the Unimak Pass- Alaska Peninsula regions. However, the extent to which each of these spawning regions

contribute to the Bering Sea population and the degree of spawning site fidelity of adult cod to these locations remains unknown. Tagging studies of adult fish indicate that the Unimak Pass – Alaska Peninsula region may support the majority of spawning activity for Being Sea Pacific cod (Shimada & Kimura 1994). In addition to accurate delineation of stock structure, it is important to know what influences the abundance, distribution, and connectivity of stocks. Given that Pacific cod are fished on their spawning grounds, it is important to evaluate whether particular spawning sources are more critical than others in sustaining the productivity of fished populations within the Bering Sea.

Tracking the dispersal of larvae from spawning to settlement is challenging, particularly within the vast and complex fluid marine environment. Small size and high rates of mortality during the larval stage make external tagging techniques impractical due to the large number of tagged individuals needed to ensure sufficient numbers are recovered (but see Jones et al. 1999). Similarly, the ability of traditional population genetic techniques is limited due to the low level of exchange required to maintain genetic homogeneity over ecologically relevant time scales (e.g., Slatkin 1993). Isotopic and elemental analysis of otoliths has shown great promise as a means to investigate spatial structure in fishes on ecological time scales and has been used to examine natal sources (e.g., Thorrold et al. 2001, Barbee & Swearer 2007) and dispersal histories of marine fishes (e.g., Swearer et al. 1999).

Otoliths are paired calcium carbonate structures in the inner ear of teleost fishes and are used for orientation and hearing. Formed during the embryonic stage, the otoliths grow daily and the accreted layers form a pattern of concentric rings around a central nucleus (i.e., core). When deposited daily, the growth increments can be used to age fish. Otoliths can be used as natural geochemical tags due to two key attributes. First, unlike other bony structures such as skeletal bones and scales, otoliths are metabolically inert: once deposited, otolith material is neither reabsorbed nor reworked (Campana 1999). Second, the chemical composition of otoliths partly reflects the physical and chemical properties of the ambient water. Some elements (e.g., Sr and Ba) are incorporated into the growing otolith in proportion to their water concentration (i.e., elemental signature). If water masses have distinct physiochemical properties, then the elemental signature incorporated into the otoliths of individuals residing in those masses should also differ.

In this study, I investigated larval source contributions of Pacific cod in the southeastern Bering Sea using otolith chemistry and structure. The objectives of this study were: (1) to characterize the otolith elemental signatures of recently hatched larvae collected from the Unimak Pass – Alaska Peninsula region, considered the primary spawning source of Pacific cod in the Bering Sea; (2) determine whether juvenile cod collected throughout the Bering Sea could be distinguished using distinct otolith elemental signatures; and (3) quantify the number and spatial distribution of putative natal sources contributing to juvenile population.

Methods

Sample collection

Pacific cod larvae were collected north and south of Unimak Pass in the Bering Sea and Gulf of Alaska, respectively, to characterize potential natal sources (Figure 3.1). Larvae were collected along a fixed grid of stations during May 2006 and 2008 as part of an annual survey conducted by the Alaska Fisheries Science Center's (AFSC) Ecosystems and Fisheries Oceanography Coordinated Investigations (Eco-FOCI) program. Larvae were collected with a 60-cm bongo net (505-µm mesh) fitted with flow meters and towed from 10 m off-bottom (or 300 m) to the surface (see Lanksbury et al. 2007 for additional details). Larval Pacific cod were preserved in ethanol for subsequent analysis.

Juvenile Pacific cod were collected throughout the southeastern Bering Sea to evaluate the dispersal and spatial distribution of contributing natal sources (Figure 3.1). Juveniles were collected from August through September 2006 and 2008 by the AFSC's annual Bering Aleutian Salmon International Survey (BASIS) using a 38-m chartered commercial fishing vessel. Across the continental shelf, juveniles were collected with a 198-m mid-water rope trawl modified to sample the top 15 m of the water column and composed of hexagonal mesh wings and a body fitted with a 1.2-cm mesh cod-end liner. For further details on sampling, refer to Farley et al. (2005). Additional samples in both years were collected in the Bristol Bay region with a 3-m

beam trawl. Juveniles were preserved frozen after capture and transported to the AFSC's Fisheries Behavioral Ecology laboratory at the Hatfield Marine Science Center in Newport, Oregon for analysis.

Determination of larval and juvenile age

Larval and juvenile cod were aged using otoliths. Fish were aged to enable the comparison of otolith elemental chemistry from similar points in time and to ensure that observed patterns reflected spatial (not temporal) differences in water chemistry and environmental conditions experienced by the fish. Narimatsu et al. (2007) validated daily increment deposition in the otoliths of laboratory-reared Pacific cod. The age of fish in this study was estimated by counting the number of daily otolith increments from the hatch check to the otolith edge. Fish were aged independently at least twice until the precision between consecutive counts was <10%. The average of the multiple counts for each fish was then used for subsequent analysis.

Larvae were weighed (wet weight, 0.001 g), photographed under a dissecting microscope, and measured (standard length, SL, 0.01 μm) using the analytical imaging software ImagePro® (Media Cybernetics). The size (SL) of the larvae was corrected for shrinkage due to preservation using a best-fit model relating size of larval Pacific cod before and after preservation in ethanol (Hurst and Lögers, National Oceanic and Atmospheric Administration, unpublished data). Both sagittal otoliths were removed

from the larvae using standard methods to minimize contamination (Miller 2009). Otoliths were photographed under a dissecting microscope and their diameter measured across the longest axis using ImagePro®. The right otolith, which was used to age the fish, was embedded and ground to expose the core. Otoliths were ground with 3 M™ tri-mite Wetordry paper (3.0 μm) and polished with Buehler AlO_2 powder (0.3 μm). Polished otoliths were photographed and increments enumerated using ImagePro® and a compound microscope at 1,000 x magnification. All larvae were aged except for those fish where only one otolith was available (used for chemical analysis); in these cases, age of the larvae was estimated from best-fit models relating fish age to otolith width (2006: $r^2 = 0.73$, $P < 0.001$, $n = 56$; 2008: $r^2 = 0.78$, $P < 0.001$, $n = 112$). Once aged, hatch date and average somatic growth rate were calculated for each fish. Hatch dates were calculated by subtracting the age (d) of the larvae from the capture date; the first otolith increment appears to form at hatch (Narimatsu et al. 2007). Average somatic growth rate ($mm \cdot d^{-1}$) was calculated by subtracting the average size at hatch (4.6 mm SL; Laurel et al. 2008) from size at capture (SL) and dividing this value by the age (d).

Juveniles were weighed (0.01 mg) and measured (SL, 1.0 mm). Both sagittal otoliths were removed from the juveniles and prepared as described above for the larvae except that: (1) otolith length as well as width was measured (0.01 μm); and (2) otoliths were embedded in resin (Polytranspar™), sectioned on the transverse plane using an IsoMet® low speed diamond blade saw (BUEHLER®), and ground to

expose the core using 3 M™ tri-mite Wetordry paper (240-1200 grit). Polished otoliths were then mounted onto glass slides with the order of otoliths randomized.

A subsample of juveniles was aged for each year. Juveniles were pooled across sampling sites, divided into size bins, and then 15% of fish from each size bin were aged. A composite image (core to edge) of the polished otolith was acquired and used to age each fish using a compound microscope at 400 x magnification and ImagePro®. The average age of each fish (independent reads over several days) was used for analysis. Ages of all other fish in each year were estimated from best-fit, polynomial models relating average age of the subsampled fish (SL < 85 mm) to otolith width (2006; $r^2 = 0.52$, $P < 0.001$, $n = 29$) and SL (2008; $r^2 = 0.48$, $P < 0.001$, $n = 28$). As the age of juveniles > 85 mm (SL) was being underestimated by the best-fit model, all fish ≥ 85 mm were excluded from the model and aged separately. Once aged, hatch date and average somatic growth rate were calculated for all juveniles as previously described for the larval cod.

Otolith elemental composition

Otolith composition (Li, Mg, Ca, Mn, Cu, Zn, Sr, Ba, and Pb) of larval and juvenile Pacific cod was quantified using a VG PQ ExCell inductively coupled plasma mass spectrometer with a New Wave DUV193 excimer laser at Oregon State University's WM Keck Collaboratory for Plasma Spectrometry. Background levels of

all analytes were measured before ablation and subtracted from measurements during ablation. Analytes with measures below background levels were excluded from analysis. Normalized ion rations were converted to concentration based on measurements of National Institute of Standards and Technology (NIST) 612 standard glass and presented as molar ratios (metal to calcium, Me:Ca) (Miller 2009). The mean percent relative standard deviations (%RSD) for NIST 612 standard glass during analyses were: ^{7}Li = 5.3%, ^{24}Mg = 4.3%, ^{43}Ca = 2.9%, ^{55}Mn = 4.3%, ^{65}Cu = 6.4%, ^{66}Zn = 6.3%, ^{86}Sr = 3.7, and ^{138}Ba = 4.8%. A calcium carbonate standard (USGS MACS-1) was used to assess accuracy. Measured ratios were within 8%, 7%, 4%, 5%, and 6% of known values for Mg, Mn, Zn, Sr and Ba, respectively.

Sagittal otoliths of larvae were used for chemical analysis. The left otolith (whole, not polished) from each larvae was cleaned with a 5.0% dilution of H_2O_2, rinsed with NANOpure® water (18 M Ohm), and then fixed to an acid-washed slide (randomized by collection site) with double-stick tape (Scotch™) under Class 100 conditions. When the left otolith was not available or broken, the right otolith was used. The laser was set at a pulse rate of 3 Hz and a 50-µm stationary spot ablation was used to collect data through the otoliths. The average depth ablated was 5.13 µm (± 0.97 SD). Elevated concentrations of Mn have been observed in the otolith primordia of Pacific cod (DiMaria et al. 2010) and other species (Brophy et al. 2004, Ruttenberg et al. 2005). Therefore, otolith elemental ratios were averaged from the

region outside of the Mn-rich zone to isolate the most recent deposition that occurred prior to fish capture.

For juveniles, the left sagittal otoliths, previously mounted and polished for aging, were also used for chemical analysis. The otoliths were cleaned ultrasonically in NANOpure® water (18 M Ohm) and dried in a Class 100 clean bench prior to elemental analysis. To remove any surface contamination, each otolith was pre-ablated along a transect from the core to anterior-dorsal edge (Figure 3.2); the laser was set at a pulse rate of 2 Hz and a 100-µm spot size moving at 100 $\mu m \cdot sec^{-1}$. To collect otolith elemental data, the laser followed the pre-ablated transect and was set at a pulse rate of 7 Hz and a spot size of 50 µm moving at 2 $\mu m \cdot sec^{-1}$. To characterize the natal source signature, data were collected from a 20-µm section post hatch, approximately representing the first 10 d of life. The primordial region was identified by elevated Mn (DiMaria et al. 2010). When elevated Mn was not detected (51% of juveniles), elemental data were collected from a 20-µm section immediately outside of the hatch-check. To characterize the last 10 d of life before capture, elemental data were averaged across the final 30-µm of the otolith edge. Daily increments at the core were consistently narrower (mean width 1.92 µm ± 0.05 SE) than increments deposited at the edge (2.81 ± 0.06 µm). Therefore, although the size of the section analyzed at the edge was larger than for the natal source signature, both sections represented approximately 10 d.

Statistical analyses

To examine spatial variation in otolith elemental composition of larvae and juveniles, Multivariate Analysis of Variance (MANOVA) was used. MANOVA was also used to evaluate group differences in the biological characteristics of the fish (size at capture, age, hatch date, and average somatic growth rate). Variables were transformed when needed to meet assumptions of normality and homogeneity of variance. Non-parametric tests were used to evaluate differences between (Mann-Whitney U test) or among (Kruskal-Wallis ANOVA by ranks) groups when transformed data failed to meet assumptions.

Comparison of larval Pacific cod from the Bering Sea and Gulf of Alaska

Pacific cod within the BSAI and GOA management units are believed to be connected through the dispersal of GOA larvae through Unimak Pass, although the degree of exchange is unclear. We determined whether otolith elemental signatures could be used to differentiate between collection locations within the Bering Sea and Gulf of Alaska using Sequential Discriminant Function Analysis (DFA; TIBCO Spotfire S+ Program). Due to low catch numbers of larvae per tow, fish from multiple tows in each basin were pooled based on geographic proximity (within 40 km and 100

km in 2006 and 2008, respectively) resulting in one larval source group in each basin (Figure 3.3).

Spatial variation in juvenile Pacific cod

I quantified the spatial scale at which juveniles from known locations could be distinguished throughout the Bering Sea using otolith chemistry. In both years juveniles were selected from six trawl sites that covered the distribution of juveniles collected by BASIS. The general locations of 5 collection sites were consistent between years: Pribilof Islands East (PE); mid-continental shelf (MS); Bristol Bay (BB); Port Moller (PM); and Unimak Island (UI) (Figure 3.4). The general location of the sixth site differed between years: a northwestern site in 2006 (Pribilof Islands West, PW) and a northeastern site in 2008 (Cape Newenham, CN). DFA was used to evaluate how accurately juveniles were assigned to their collection site based on the elemental signature collected from the otolith edge. The data were randomly split and otolith elemental signatures from half of the fish were used to develop the discriminant function. The efficacy of the function was then evaluated by its ability to correctly assign fish from the second group to collection locations based on their individual otolith elemental signatures.

To further evaluate spatial variation across sites within years, and temporal stability between years, variation in otolith elemental ratios across sites was examined

through residual analysis. Residuals were calculated for the five geographically comparable locations in both years and compared between years:

$$Residual_{site} = (Site\ Mean - Grand\ Mean) / Grand\ Mean \quad \text{(Eq. 1)}$$

A positive (negative) site residual indicates that the otolith elemental ratio (e.g., Sr:Ca) is greater (lower) than the mean value across all sites. A consistent positive (negative) site residual value between years suggests temporal stability in otolith elemental signatures.

Natal sources of juvenile Pacific cod recruits

The natal source signatures of the juvenile otoliths were used to estimate the number and distribution of potential sources contributing to the recruits in the southeastern Bering Sea. Although the original goal was to assign recruits to characterized larval sources, there was inadequate overlap in hatch dates between the two life stages, precluding direct comparisons of otolith natal signatures. Instead, I identified unique chemical groups (i.e., putative natal sources) using hierarchical cluster analysis (HCA). To minimize potential temporal variation in the natal source signatures of the juveniles, the dataset was reduced to include only fish that hatched at similar times across all 6 collection sites. In 2006, juveniles that hatched within a 60 d period (9 Feb – 9 Apr) were analyzed; retaining 82 % of the original dataset. In 2008, fish that hatched within an 80 d period (1 Jan – 20 Mar) were analyzed, retaining 80 %

of the original dataset. Using the elemental signature from the core of the juvenile otoliths, representing the first 10 d of life, hierarchical cluster analysis (PC-ORD vol. 6.158; McCune et al. 2002) was used to identify chemically distinct groups. The HCA was run using a Euclidean (Pythagorean) distance measure and the Ward's linkage method. The resulting dendrogram was scaled by Wishart's objective function converted to a percentage of information remaining. The number of chemically distinct groups was quantified by pruning the dendrogram at 50% information remaining. The spatial distribution of each group and their relative contributions to the juvenile recruits was then quantified.

Results

Comparison of larval Pacific cod from the Bering Sea and Gulf of Alaska

In both years, hatch dates of larvae did not differ between basins (Mann-Whitney U Test, $P > 0.30$), indicating that differences observed in otolith chemistry between the Bering Sea and Gulf of Alaska were not confounded by temporal variation (Appendix Table 1). Global mean (±SE) hatch date for larvae was 26 April ± 0.78 d in 2006 and 5 May ± 0.7 d in 2008. Larvae collected in the Bering Sea and Gulf of Alaska in 2006 differed only in size at capture (Mann-Whitney U Test, $P = 0.02$). Larvae collected in the Bering Sea were shorter (SL, mean ± SE, 9.25 ± 0.28 mm) than those collected in the Gulf of Alaska (10.2 ± 0.24 mm). Overall, mean (± SE) age

and average somatic growth rate were 24 ± 0.77 d and 0.22 ± 0.01 mm·d^{-1}, respectively. In 2008, larvae collected in the Bering Sea and Gulf of Alaska differed only in size and age at capture (Mann-Whitney U Test, $P < 0.001$). Larvae collected in the Bering Sea were shorter (SL, mean ± SE, 6.44 ± 0.12 mm) and younger at capture (9 ± 0.43 d) than those collected in the Gulf of Alaska (SL, 8.10 ± 0.22 mm; age, 21 ± 1.2 d). Average somatic growth rate (0.19 ± 0.01 mm·d^{-1}) did not differ between basins (Mann-Whitney U Test, $P > 0.92$).

In 2006, Li and Cu were below background levels and dropped from analysis. Otolith elemental ratios differed between basins (MANOVA; $F_{5, 69} = 8.43$, $P < 0.001$; Table 3.1). Larvae collected in the Gulf of Alaska were distinguished from larvae collected in the Bering Sea by lower Zn:Ca and Sr:Ca (Tukey HSD, $P < 0.04$), and slightly lower Mn:Ca ($P = 0.08$). Mean Mg:Ca and Ba:Ca were similar between basins ($P > 0.92$). Using otolith Mn:Ca, Zn:Ca, Sr:Ca and Ba:Ca, 80% of the larvae in 2006 were successfully assigned to their basin of origin (Table 3.2). Although Mn:Ca and Ba:Ca were not significantly different between basins, their inclusion improved classification success (DFA, Wilks' Lambda: 0.62; $F_{4,70} = 10.6$; $P < 0.001$).

In 2008, Li, Cu and Zn were below background levels and dropped from analysis. Elemental ratios differed between basins (MANOVA; $F_{4,111} = 11.6$, $P < 0.0001$; Table 3.1). As observed in 2006, larvae collected in the Gulf of Alaska were distinguished from those collected in the Bering Sea by lower Mn:Ca (Tukey HSD, $P < 0.03$) and Sr:Ca ($P = 0.03$). Additionally, larvae collected in the Gulf of Alaska in

2008 were also distinguished by lower Mg:Ca ($P < 0.001$). Using Mg:Ca, Mn:Ca and Sr:Ca, 70 % of the larvae in 2008 were correctly assigned to their collection basin (DFA, Wilks Lambda = 0.72; $F_{3,112} = 14.8$; $P < 0.001$; Table 3.2).

Spatial variation in juvenile Pacific cod

In both 2006 and 2008, differences were observed in biological characteristics of juveniles collected throughout the southeastern Bering Sea (Table 3.3). Size at capture, age, and hatch date of juveniles differed among collection sites in both years (Kruskal-Wallis ANOVA by Ranks, $P < 0.001$). Additionally, in 2008 average somatic growth rate of juveniles also differed among collection sites ($P = 0.001$). There was no discernable regional pattern in these metrics within or between years.

There was spatial variation in the elemental ratios at the otolith edge of juvenile cod collected throughout the southeastern Bering Sea in both 2006 and 2008. Li and Cu were below background levels and excluded from analysis in both years. Elemental signatures at the otolith edge of juveniles collected from PE, MS, BB, PM and UI varied both within and between years (Figure 3.5). In both years, Mn:Ca was consistently lower at western collection sites (PE, MS and UI), and Ba:Ca was consistently lower for offshore sites (PE and MS). In both years, Mg:Ca was the most variable across collection locations, with 3 of 5 sites showing opposite trends in

relative concentrations. Mn:Ca and Ba:Ca had the highest variation across sites (as indicated by higher residual values) and Sr:Ca varied the least across sites.

In 2006, Mg:Ca, Mn:Ca, Zn:Ca, Sr:Ca and Ba:Ca at the edge of the juvenile otoliths varied among sites (Appendix Table 2; ANOVA, $P < 0.001$). The three northern collection sites (PW, PE and MS) were significantly lower in Mn:Ca than the three southern collection sites (BB, PM and UI; ANOVA, $F_{1, 160} = 113.4$ $P < 0.001$). Juveniles collected from UI and PM had the highest Ba:Ca (ANOVA, $F_{1, 160} = 110.7$, $P < 0.001$). Sr:Ca displayed a decreasing trend following sites clockwise from CN to UI. Otolith Mg and Zn varied, but not in a systematic fashion.

Juveniles were assigned to their collection site based on otolith edge signatures in 2006 with a relatively high accuracy (split data set; mean = 79% using Mg:Ca, Mn:Ca, Zn:Ca, Sr:Ca and Ba:Ca; Wilks Lambda = 0.04; $F_{25, 265} = 14.4$; $P < 0.001$). Classifications based on the jackknife procedure resulted in similar classification success (mean = 79 %; Wilks Lambda = 0.04; $F_{25, 566} = 32.4$; $P < 0.001$). Juveniles collected at MS had the highest classification success (100%) (Figure 3.4). This site was characterized by high otolith Zn:Ca and Sr:Ca and low Mn:Ca and Ba:Ca compared to the other collection sites.

In 2008, Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca at the edge of the juvenile otoliths varied across collection sites (ANOVA, $P < 0.001$; Appendix Table 2). As in 2006, juveniles collected from the northern sites (PE, MS and CN) had lower Mn:Ca (ANOVA, $F_{1,163} = 267.4$, $P < 0.001$) than the southern sites (BB, PM, and UI).

Additionally, juveniles collected from the northern sites also had lower otolith Ba ($F_{1,163}$ = 205.2, P < 0.001). As in 2006, Sr:Ca decreased across collection sites moving clockwise from CN to UI. While no systematic pattern was observed for otolith Mg 2006, Mg decreased across collection sites from PE to PM in 2008.

Seventy-one percent of juveniles were correctly assigned to their collection site in 2008 based on Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca collected from the otolith edge (Table 3.4; Wilks Lambda = 0.05; $F_{20, 243}$ = 17.6; P < 0.001). Classification success was similar for jackknifed classifications (mean = 63%; Wilks Lambda = 0.05; $F_{20, 518}$ = 37.0; P < 0.001). As in 2006, juveniles collected at MS had the highest classification success (93%) and were similarly characterized by higher otolith Sr:Ca and lower Mn:Ca and Ba:Ca.

Natal sources of juvenile Pacific cod recruits

The number and distribution of natal sources contributing to juvenile recruits throughout the southeastern Bering Sea were estimated based on otolith core chemistry. In both years, a direct comparison of natal sources between larvae collected in May with the juvenile recruits collected in August and September was not possible because the larvae hatched later than the juveniles (Figure 3.6). Mean hatch date of larvae in 2006 and 2008 was April 28 and May 6, respectively, whereas mean hatch date of juveniles in 2006 and 2008 was March 20 and February 12, respectively.

Therefore, temporal patterns in otolith chemistry could not be accounted for when comparing natal source signatures of the two groups. Thus, the larval collections were not used as a known geographic source for the juvenile recruits.

Variation in otolith composition (Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca) was used to identify unique groups (i.e., presumptive natal sources) using hierarchal cluster analysis. Four chemically distinct natal sources contributed to the juvenile recruits in 2006 and three sources contributed in 2008 (Figure 3.7; Table 3.5). In 2006 two sources were dominant and accounted for 56% and 30% of the juveniles collected throughout the southeastern Bering Sea. The remaining two sources accounted for 11% and 3% of the juveniles. The four natal sources were dispersed across most sites; however, the Port Moller (PM) site was comprised of juveniles of a single source. In 2008, two sources were dominant and accounted for 53% and 40% of the juveniles and the remaining source accounted for 8%. Unlike 2006, the three sources were not as evenly distributed across juvenile collection locations. Juveniles collected from two sites, BB and PM, were comprised of one contributing source. The remaining four sites were comprised of the other two sources.

In 2006, size at capture, age, and average somatic growth rate did not differ among the presumptive natal sources (Kruskal-Wallis ANOVA by Ranks, $P > 0.13$; Appendix Table 3). Overall mean (± SE) values for size at capture, age at capture and average somatic growth rate across the four sources were: 65 ± 0.89 mm, 165 ± 2 d, and 0.37 ± 0.01 $mm \cdot d^{-1}$, respectively. Mean hatch dates differed due to one source

(smallest source group accounting for only 4 juveniles) which only differed from 1 of the other 3 sources (Kruskal-Wallis ANOVA by Ranks, $P = 0.03$). In 2008, none of the biological characteristics differed among the three putative natal sources (Kruskal-Wallis ANOVA by Ranks, $P > 0.17$). Overall mean (± SE) values for size at capture, age at capture, hatch date and average somatic growth rate across the three sources were: 70.7 ± 0.66 mm, 225 ± 2 d, 6 February ± 2 d, and 0.29 ± 0.002 $mm \cdot d^{-1}$, respectively.

Discussion

The Unimak Pass-Alaska Peninsula (UP-AP) region is assumed to support the majority of spawning activity for southeastern Bering Sea Pacific cod. Commercial fishing of Pacific cod in the Bering Sea is concentrated within the UP-AP region where some of the largest known spawning aggregations congregate in the winter months. Ormseth et al. (2008) observed the largest aggregations of female Pacific cod in spawning condition within the UP-AP region, although significant aggregations were also located throughout the Aleutian Islands and along the shelf break near the Pribilof Islands. These findings support earlier observations on seasonal migrations of tagged Pacific cod (Shimada & Kimura 1994). Adult cod displayed directed winter (Jan-Mar) migration to the UP-AP spawning region from all directions throughout the Bering Sea and eastern Aleutian Islands. While cod also migrated to other known

spawning regions (Pribilof Islands, shelf break, eastern Aleutian Islands), the highest densities migrated to the UP-AP region with some individuals travelling >1000 km from their original point of capture. Shimada and Kimura (1994) also observed emigration of Pacific cod to the western Gulf of Alaska, suggesting exchange may occur between the two management units. Additionally, Unimak Pass represents the major conduit from the Gulf of Alaska into the Bering Sea, potentially transporting larvae from the Gulf of Alaska into the Bering Sea. Therefore, cod hatched within the western Gulf of Alaska along the Alaska Peninsula may contribute recruits to the Bering Sea population.

The original goal of this study was to quantify the contribution of UP-AP larval sources to eastern Bering Sea recruits using otolith chemistry and structure. Secondarily, our goal was to assess the feasibility to quantify the contribution of Gulf of Alaska sources to the Bering Sea recruits using larvae collected from the western Gulf of Alaska. Samples for this study were collected opportunistically from established sampling cruises by the Eco-FOCI and BASIS surveys. Hatch-date analysis revealed interesting life history information about Bering Sea Pacific cod even though otolith natal signatures of the larval sources and juvenile recruits could not be compared because of a temporal mismatch in hatch dates. Although Pacific cod have a protracted spawning season (December-May) it appears that the majority of juvenile recruits hatched within a much shorter time period: mean hatch dates were 20 March and 12 February in 2006 and 2008, respectively. Additionally, an interesting

observation was the month difference in mean hatch dates of recruits between years, which may be due to annual differences in the timing of peak spawn throughout the reproductive season. Loggerwell and Neidetcher (2009) observed in increase in the occurrence of spawning females toward the end of February in 2006 and 2008 which is consistent with hatch-date estimates of juvenile recruits in this study. However, more information is needed on interannual variation in spawn timing of Bering Sea Pacific cod to more fully evaluate spawn timing as the mechanism causing the difference in hatch dates of recruits.

I assumed that the juvenile samples comprehensively represented the population of Pacific cod recruiting to the southeastern Bering Sea shelf. Evidence in support of this assumption includes: the area sampled across the shelf, and the sizes and hatch-dates of the fish collected. Samples used in this study covered the observed distribution of juvenile cod collected in the Bering Sea (Hurst, unpublished data). The fish sizes were consistent with those observed in other Bering Sea sampling programs using different types of collection gear (e.g., Hurst et al. in review; unpublished data). Further, this study and other sampling programs (e.g., Duffy-Anderson et al. 2006) have collected fish in the summer and fall which hatched throughout the spawning season (January through May; based on age-size relationships from this study and Narimatsu et al. 2007). It is possible, however, that my samples could be biased if late-hatching (May) fish recruiting to the shelf by the August BASIS survey were smaller than the minimum 30 mm (SL) fish size selected by the gear. However, based on my

data, late-hatching fish would be at least 40 mm to 46 mm (SL) in size by the time of the August collections. My study samples were above this minimum size (50 to 111 mm SL) and also included fish which hatched late in the spawning season.

I was able to discriminate between larval Pacific cod collected within the Bering Sea and Gulf of Alaska suggesting that fish hatched within the Gulf of Alaska could potentially be identified among Bering Sea recruits, enabling the relative contribution of Gulf of Alaska natal sources to the Bering Sea population to be quantified in future studies. I also found evidence of variation in otolith chemistry among larvae within each ocean basin (analysis not presented; Appendix Figure 1 and Figure 2). In general, Gulf of Alaska larvae were chemically distinct from Bering Sea larvae in both years. The analysis also suggested the presence of two chemically distinct sources contributing to the Bering Sea larvae collected in 2006 and 2008. Therefore, larvae collected in the Bering Sea may have dispersed from multiple spawning locations, potentially up to 240 km away by the time they reach 14 d of age (based on a mean current flow in the Alaska Coastal Current ~ 2 $cm \cdot s^{-1}$; Stabeno et al. 2002). As a result, it may be challenging to accurately characterize larvae that originated within the Unimak Pass-Aleutian Island region of the Bering Sea. However, the variation observed within the Unimak Pass-Aleutian Island region in the Bering Sea should not be a problem when characterizing sources as long as the larval otolith chemistry is distinct for each spawning region (Aleutian Islands, Pribilof Islands, Unimak Pass-Alaska Peninsula region in the Bering Sea and Gulf of Alaska). It is

likely that larval otolith chemistry will be distinct among spawning regions given the differences observed in the UP-AP region between the Gulf of Alaska and Bering Sea, and the scales spatial variation observed in the juvenile otolith edge chemistry throughout the southeastern Bering Sea.

It is important to understand the spatial scales at which variation in otolith chemistry occurs to prevent inappropriate interpretations of field data. Other studies characterizing natal sources of marine fishes have observed variation in otolith signatures among individual egg batches collected from a common site, likely due to maternal effects and/or small-scale environmental variation (e.g., Ruttenberg & Warner 2006, Ruttenberg et al. 2008, Standish et al. 2008). Variation among clutches within sites could confound interpretations of individual assignments of fish to sources, particularly if clutches from two different sites are chemically similar but geographically distant. For example, Neubauer at al. (2010) observed significant chemical variation among natal sources at small spatial scales (e.g., among clutches within sites, and between sites within regions) but not among regions (i.e., marine reserves) indicating that classifying fish to natal sources might be difficult given the overlap in otolith signatures across regions. For this study, it is important to note that the within basin variation among larval cod may be the result of multiple spawning sources mixing prior to capture. Therefore, to more accurately characterize natal sources within the Unimak Pass-Alaska Peninsula region, larval cod need to be

collected closer to where they hatched. However, this may be challenging given that cod deposit semi-demersal eggs down to 265 m depths (Palsson 1990).

I demonstrated that there was substantial geospatial variation in the otolith edge chemistry of juvenile cod collected throughout the southeastern Bering Sea. Many studies utilizing otolith microchemistry focus on estuarine or freshwater environments because detecting differences in otolith signatures can be challenging in open ocean environments where water masses are potentially more homogeneous. Freshwater and estuarine habitats generally exhibit larger gradients in temperature and the potential for greater local variation in water chemistry due to watershed geology (e.g., bedrock, sediment load, and groundwater transport and retention) (e.g., Elsdon et al. 2008). Even so, several studies investigating natal sources (e.g., Neubauer at al. 2010) and movement (e.g., Thorrold et al. 2001) and stock structure (e.g., Campana et al. 2000, Jónsdóttir et al. 2006) of fishes in the marine environment have observed useful variation in otolith microchemistry across various spatial scales. Gao and Beamish (2003) observed spatial variation in stable isotope composition of Pacific halibut, *Hippoglossus stenolepis*, otoliths related to movement patterns of tagged fish off the coast of British Columbia. Gao et al. (2004) posited the presence of at least three different spawning stocks or subpopulations among juvenile sablefish, *Anaplopoma fimbria*, off the coasts of Oregon and Washington based on variation observed in otolith stable isotope compositions. Gao et al. (2005) found differences between two groups of Pacific cod, possibly differentiating two recognized spawning

stocks within North Puget Sound, using a combination of otolith trace element and stable isotope analyses. Fitzgerald et al. (2004) observed similar otolith elemental profiles unique to sites among juvenile walleye pollock, *Theragra chalcogramma*, collected from locations throughout the northern Gulf of Alaska, Alaska Peninsula and eastern Bering Sea, suggesting fish within a site moved together prior to capture. We observed high classification accuracy (>71%) of juvenile cod across two years and six sites (~100 – 800 km apart) within the eastern Bering Sea. The scale of variation we observed for Pacific cod indicates that migration patterns of fish can be investigated in the Bering Sea region.

Otolith incorporation of certain elements is related to their availability in the water; however, incorporation of elements into the otolith can also be influenced by other physical (e.g., temperature) and biological (e.g., growth) factors (for reviews see Campana 1999 and Elsdon et al. 2008). DiMaria et al. (2010) demonstrated that otolith Sr and Ba were inversely related to temperature from 2-8°C in larval Pacific cod, and Miller et al. (2009) demonstrated that otolith Mn was inversely related to temperature for black rockfish (*Sebastes melanops*), a fully marine species. In this study, otolith Sr:Ca and Mn:Ca were higher in larval cod collected within the Bering Sea compared to fish from the Gulf of Alaska, which may be due to consistently cooler water temperatures within the Bering Sea. However, otolith edge Sr:Ca and Ba:Ca of juvenile cod were not related to SST across collection sites ($r^2 < 0.06$); although, this was not surprising as SST only varied up to 1°C across collection sites in both years.

Juveniles collected from sites along the Alaska Peninsula (<50 km from shore) generally had higher otolith Ba and lower Sr than sites over the open shelf in both years, which may in part be due to freshwater influences being entrained along the Alaska Peninsula by the Bering Coastal Current; Ba is generally higher and Sr lower in freshwater than in saltwater (for review see Gillanders 2005).

Another important consideration for the interpretation of field data is whether or not otolith composition is influenced by somatic growth or otolith precipitation rate. It is unlikely that variation in individual growth rates is a significant factor contributing to the otolith chemical variation observed in this study given that average somatic growth rates of juveniles were generally homogeneous across collection sites. Additionally, DiMaria et al. (2010) observed no effect of somatic or otolith growth on elemental composition. Therefore the observed variation in otolith edge signatures across juvenile collection sites may be due more to differences in elemental concentrations of the water and other unmeasured factors such as diet (e.g., Buckel et al. 2004) or an interactive effect among influencing factors (e.g., Elsdon & Gillanders 2004). Although we quantified temperature and growth effects on otolith elemental incorporation for larval cod, otolith incorporation may differ in the juvenile life stage. Therefore, further research is needed to evaluate potential otogentic differences in otolith elemental incorporation between larval and juvenile life stages (currently ongoing) which could influence interpretations of field data.

I quantified multiple unique chemical groups (i.e., putative natal sources) contributing to the eastern Bering Sea recruits in both years that were generally mixed across collection sites. Juveniles collected at Port Moller consistently had one contributing natal source each year suggesting that larval and juvenile cod may follow discrete pathways depending upon location and timing of spawn, although the mechanisms driving this pattern are unclear. However, given the mixed contribution of natal sources across the other sites in both years, it is likely that the majority of larval and early juvenile cod generally mix throughout development. Some caution should be taken when interpreting contributions of the smallest putative source in 2006 (n=4). Although fish in this source hatched later in the spawning season, mean hatch dates differed significantly from only one of the remaining three sources. Regardless, the mixed distributions of the remaining three natal sources in 2006 still holds given that the hatch-dates among these sources did not differ. Additionally, no one source dominated the juvenile recruits in either year, suggesting that other known spawning regions, such as the eastern Aleutian Islands, Pribilof Islands, and western Alaska Peninsula within the Gulf of Alaska, in addition to the UP-AP region, may contribute significantly to the eastern Bering Sea Pacific cod population. However, to quantify source contributions from other spawning regions, larvae need to be collected throughout all regions and throughout the spawning season to fully characterize and map potential natal sources of Bering Sea recruits.

The general distribution pattern of larval source contributions of the juvenile population in the Bering Sea may be due to patterns of flow across the shelf in warm and cold years. The annual advance and retreat of sea ice is one of the defining characteristics of the eastern Bering Sea shelf (Stabeno et al. 1999). Year 2006 was a transition from a warm (2001 to 2005) to cold (2007 to 2010) regime; the extent of sea ice in the spring was less and temperatures were warmer than in 2008 (Overland et al. 2009). Stabeno et al. (in review) observed that general flow of surface currents across the Bering Sea shelf in warm years was weaker and more variable in direction than during cold years. The mixed distribution of larval sources in 2006 may be due to fish having more control over their swimming direction from spawning regions due to weaker currents. Conversely, strong, directed currents in 2008 may have resulted in fish dispersing along discrete pathways following major flow patterns.

This is the first study to investigate the contribution of natal sources of Pacific cod in the southeastern Bering Sea through the application of otolith geochemistry and microstructure. Given the potential for larvae from multiple hatch locations to mix prior to capture, it may be challenging to characterize larval sources of cod and assign recruits back to known locations within the UP-AP region. However, there is potential to evaluate the relative proportions that Gulf of Alaska larvae contribute to the Bering Sea Pacific cod population using otolith natal signatures. Further, we were able to assign juveniles to their collection site with relatively high accuracy, indicating that otolith analysis can be used to investigate movement of Pacific cod across their

lifespan throughout the southeastern Bering Sea. Multiple sources contributed to the juvenile recruits in both years, and their distribution and relative contributions suggest that other known spawning sources besides the Unimak Pass-Alaska Peninsula region may contribute significantly to the eastern Bering Sea Pacific cod population.

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FIGURE LEGEND

Figure 3.1: General map of study region. Larval Pacific cod were collected by the Eco-FOCI program in the Bering Sea and Gulf of Alaska (dotted lines). Juvenile Pacific cod were collected throughout the southeastern Bering Sea by the BASIS survey (dashed lines).

Figure 3.2: Schematic of a polished transverse section of a juvenile Pacific cod otolith. Solid arrow indicates the path across the otolith from which the chemistry data were collected and fish aged. The path for all juveniles fell within the quadrant highlighted by the dashed lines, from core to edge. Chemistry data representing the first and last 10 d of life for each juvenile was analyzed (grey shaded boxes at core (a) and edge (b), respectively).

Figure 3.3: Collection sites in 2006 (a) and 2008 (b) for larval Pacific cod. Each point represents a single tow. For both years, multiple tows were grouped into two sites: Bering Sea (BS) and Gulf of Alaska (GOA).

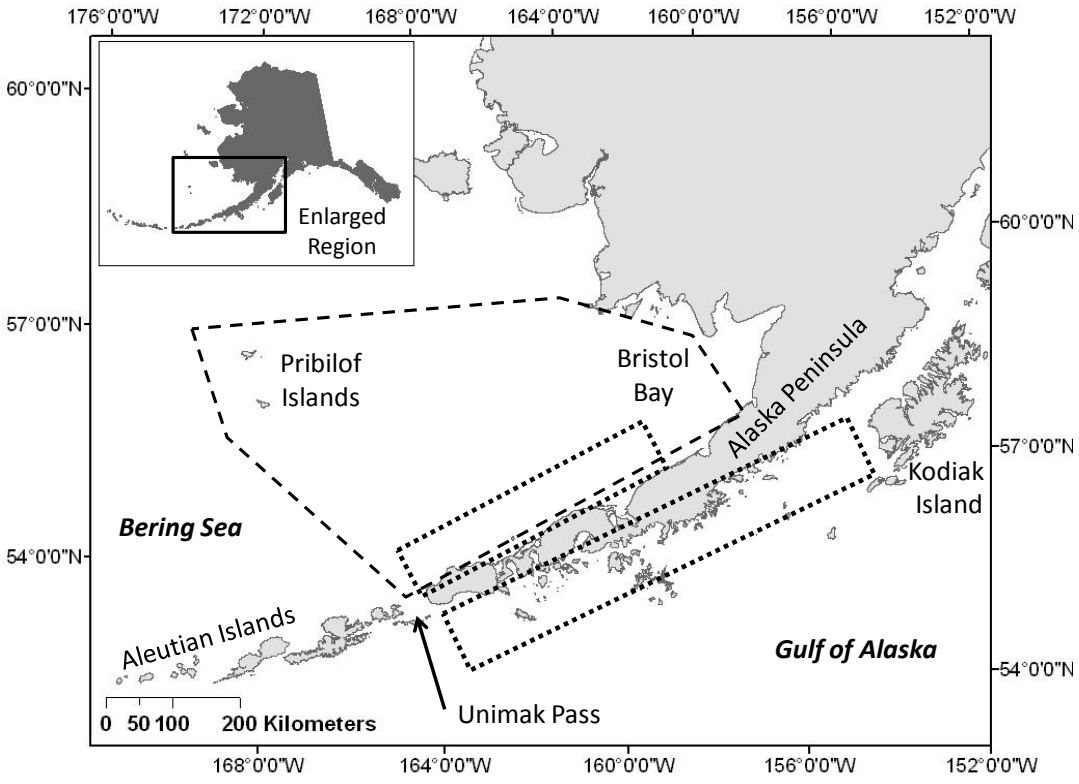

176°0'0"W
172°0'0"W
168°0'0"W
164°0'0"W
160°0'0"W
156°0'0"W
152°0'0"W
60°0'0"N
57°0'0"N
54°0'0"N
Enlarged Region
Pribilof Islands
Bristol Bay
Alaska Peninsula
Kodiak Island
Bering Sea
Gulf of Alaska
Aleutian Islands
Unimak Pass
0 50 100 200 Kilometers

Figure 3.1

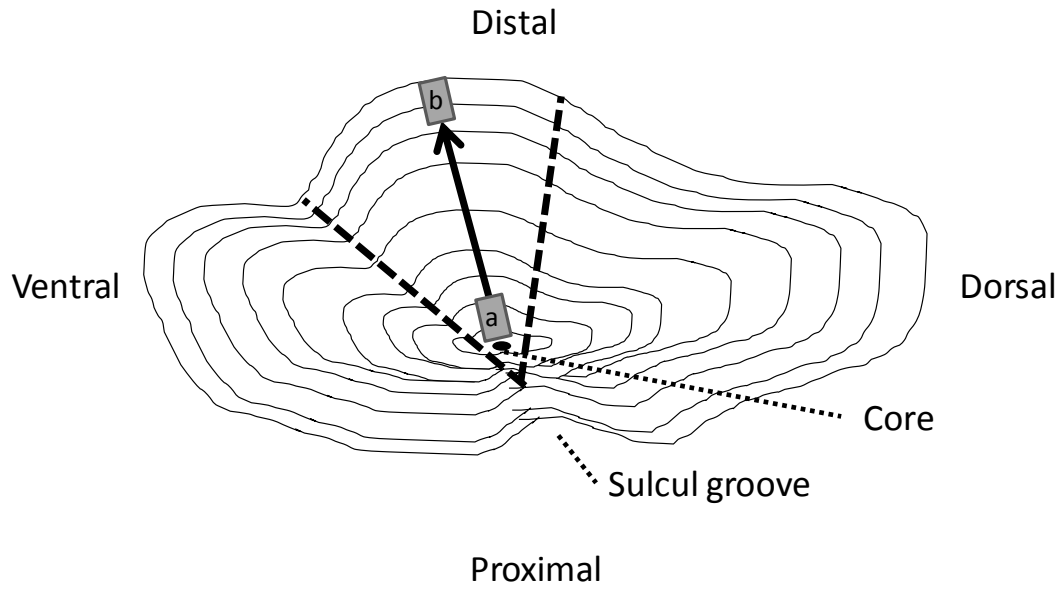


Figure 3.2

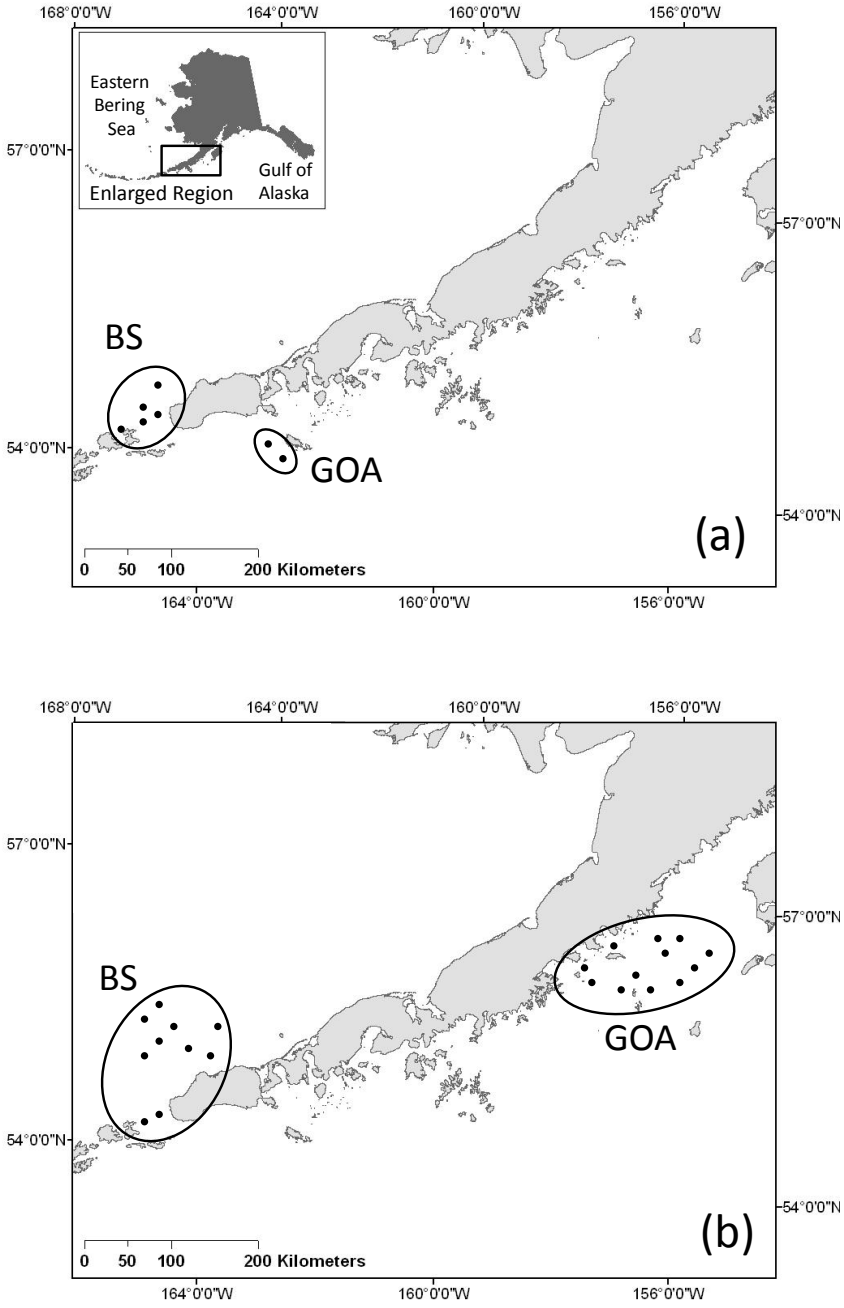
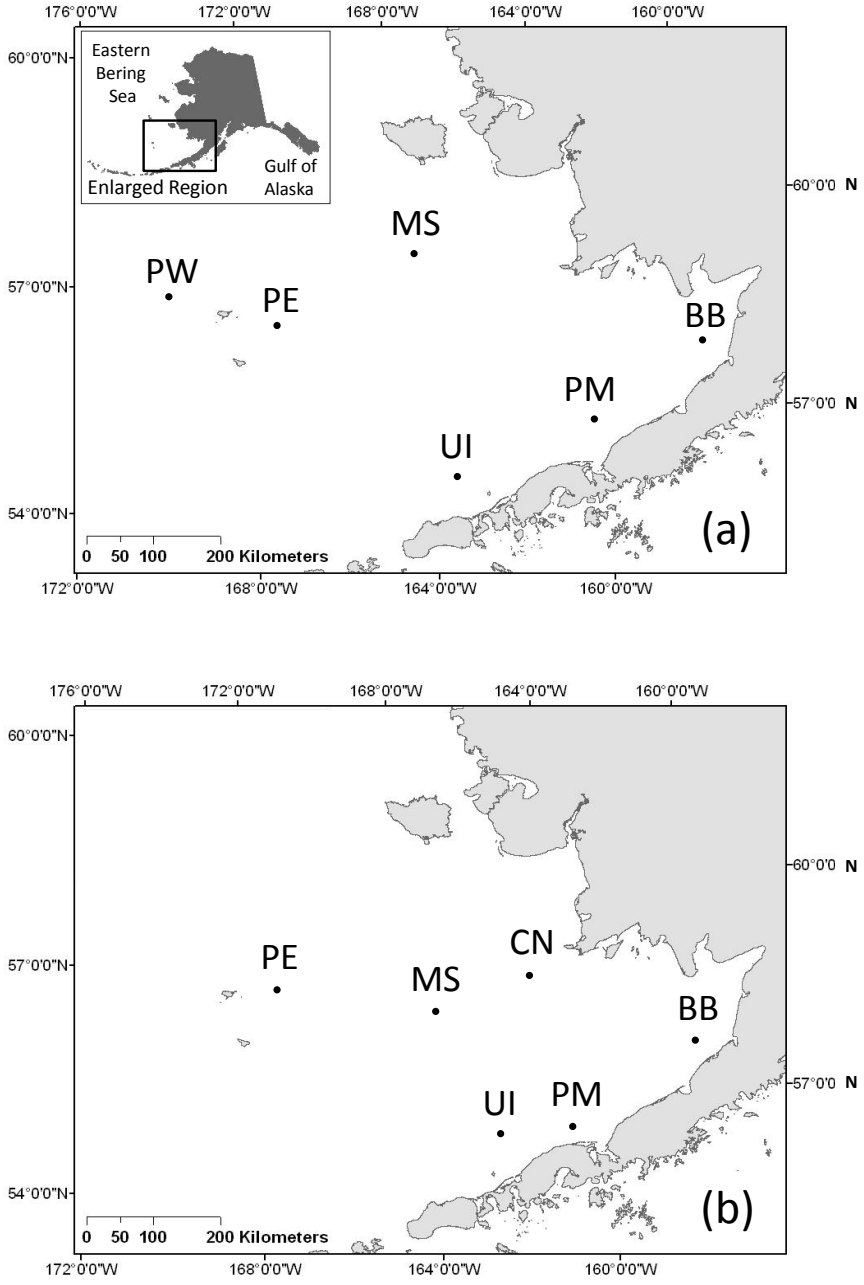

168°0'0"W
164°0'0"W
160°0'0"W
156°0'0"W
57°0'0"N
54°0'0"N
Eastern
Bering
Sea
Gulf of
Alaska
Enlarged Region
BS
GOA
(a)
0 50 100 200 Kilometers
(b)

Figure 3.3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160°0'0"W
60°0'0"N
57°0'0"N
54°0'0"N
Eastern Bering Sea
Gulf of Alaska
Enlarged Region
MS
PW
PE
BB
PM
UI
0 50 100 200 Kilometers
(a)
60°0'0 N
57°0'0 N
CN
(b)

Figure 3.4

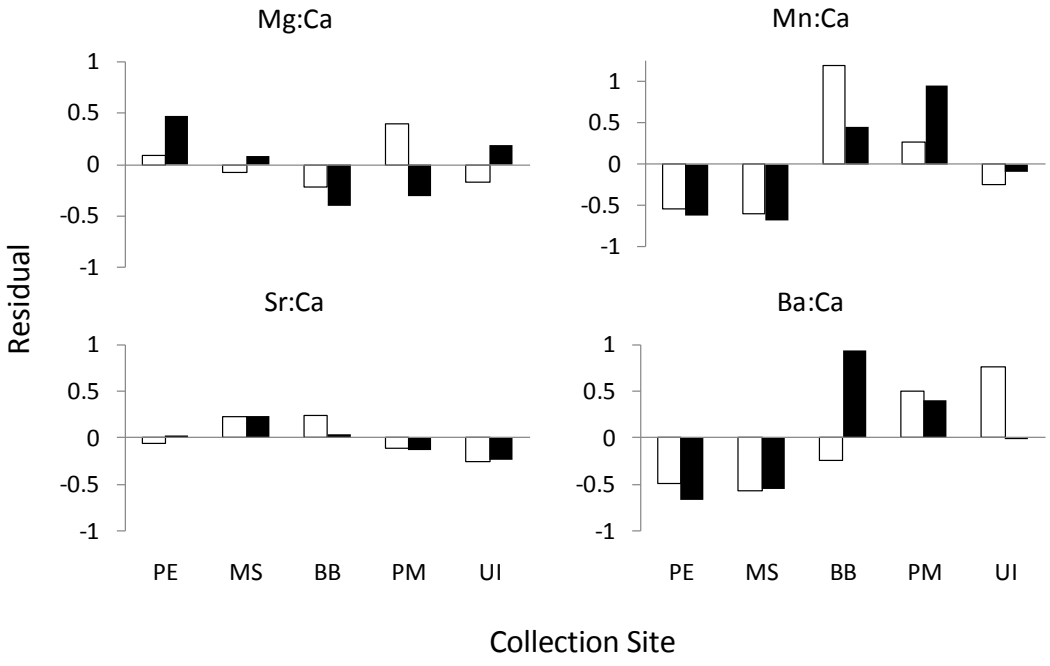

Mg:Ca
Mn:Ca
Sr:Ca
Ba:Ca
Residual
1
0.5
0
-0.5
-1
PE
MS
BB
PM
UI
Collection Site

Figure 3.5

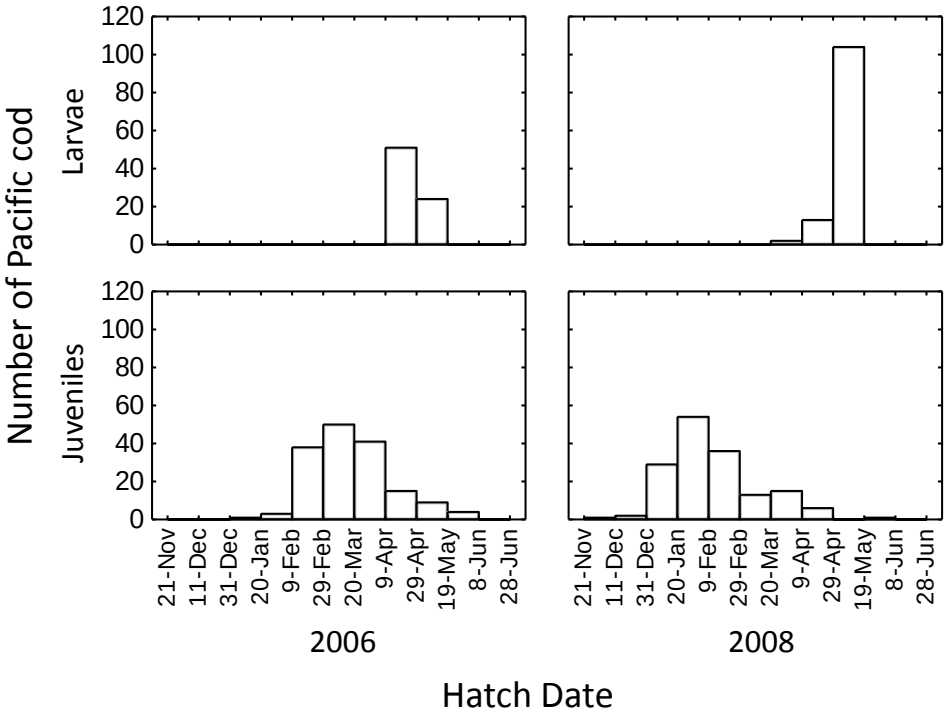
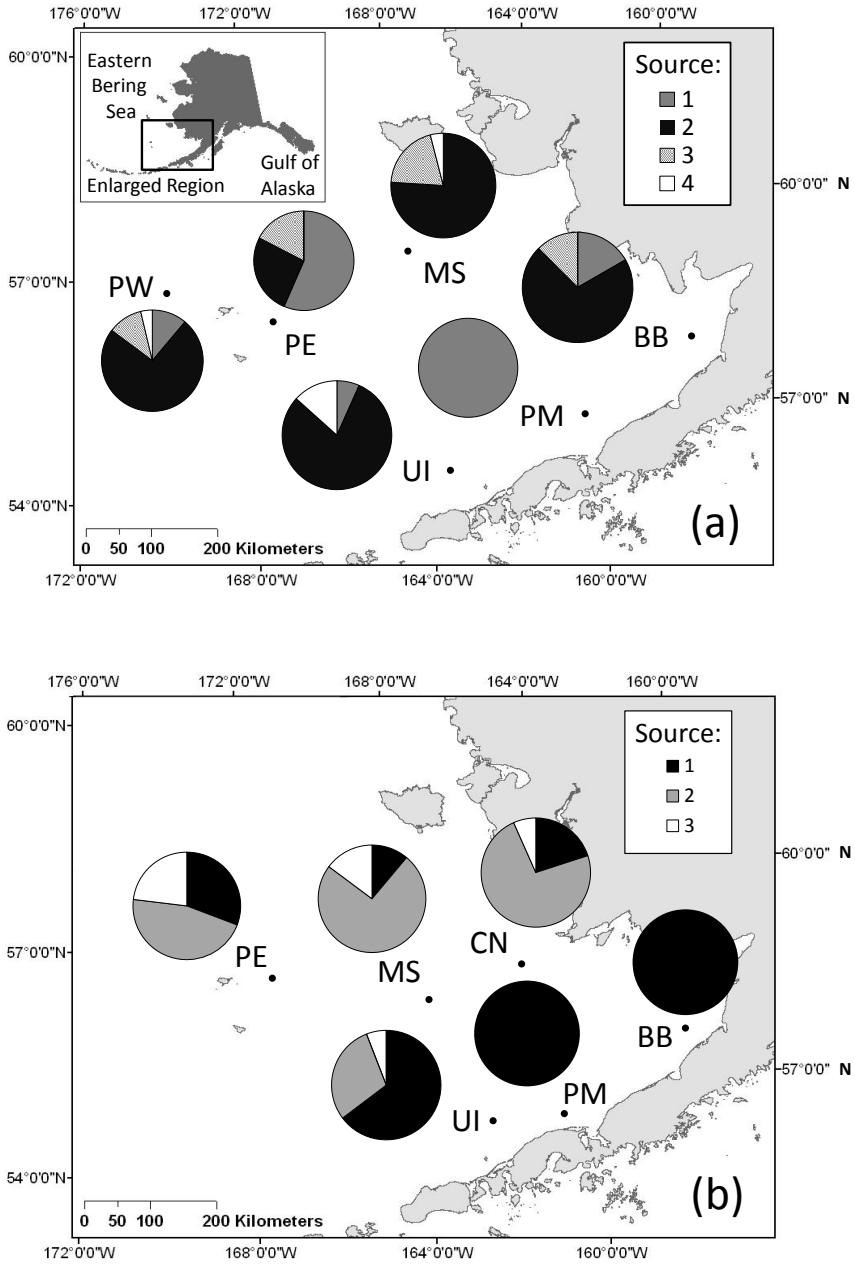

Number of Pacific cod
Larvae
Juveniles
120
100
80
60
40
20
0
21-Nov
11-Dec
31-Dec
20-Jan
9-Feb
29-Feb
20-Mar
9-Apr
29-Apr
19-May
8-Jun
28-Jun
2006
2008
Hatch Date

Figure 3.6


176°0'0"W
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160°0'0"W
60°0'0"N
57°0'0"N
54°0'0"N
60°0'0" N
57°0'0" N
Eastern Bering Sea
Gulf of Alaska
Enlarged Region
Source:
1
2
3
4
PW
PE
MS
BB
PM
UI
0 50 100 200 Kilometers
(a)
Source:
1
2
3
CN
(b)

Figure 3.7

TABLE LEGEND

Table 3.1: Otolith chemistry of larval Pacific cod collected within the Bering Sea (BS) and Gulf of Alaska (GOA) in 2006 and 2008. Mean values (± SE) for untransformed elemental ratios: Mg:Ca (mmol·mol^{-1}), Mn:Ca (µmol·mol^{-1}), Zn:Ca (µmol·mol^{-1}), Sr:Ca (mmol·mol^{-1}) and Ba:Ca (µmol·mol^{-1}). Homogeneous groups were determined using Tukey HSD post-hoc analysis (ANOVA).

Table 3.2: Classification (DFA; cross-validated) of larval Pacific cod in 2006 and 2008. Individuals were classified to the Bering Sea (BS) or Gulf of Alaska (GOA). Basins were characterized by Mn:Ca Zn:Ca, Sr:Ca and Ba:Ca in 2006, and by Mg:Ca, Mn:Ca and Sr:Ca in 2008.

Table 3.3: Biological characteristics of juvenile recruits collected across the southeastern Bering Sea in 2006 and 2008. Mean (range), untransformed values for size at capture (SL, mm), age (d), hatch date, and average somatic growth rate (mm·day^{-1}). Juveniles were collected at 6 sites each year: Pribilof Islands West (PW); Pribilof Islands East (PE); mid-shelf (MS); Cape Newenham (CN); Bristol Bay (BB); Port Moller (PM); and Unimak Island (UI). Homogeneous groups were determined using multiple comparisons (Kruskal-Wallis ANOVA by Ranks).

Table 3.4: Classification (DFA; split dataset) of juvenile recruits to collection site in 2006 and 2008. Juveniles were collected throughout the southeastern Bering Sea: Pribilof Islands West (PW); Pribilof Islands East (PE); mid-shelf (MS); Cape Newenham (CN); Bristol Bay (BB); Port Moller (PM); and Unimak Island (UI). Collection sites were characterized by Mg:Ca, Mn:Ca, Zn:Ca, Sr:Ca and Ba:Ca in 2006 and by Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca in 2008. Data were $Log_{10}(x+1)$-transformed prior to analysis.

Table 3.5: Otolith chemistry of juvenile recruits classified to natal sources contributing to the southeastern Bering Sea in 2006 and 2008. Mean (± SE) ratios of Li:Ca (µmol·mol^{-1}), Mg:Ca (mmol·mol^{-1}), Mn:Ca (µmol·mol^{-1}), Sr:Ca (mmol·mol^{-1}), and Ba:Ca (µmol·mol^{-1}). All otolith elemental ratios are the untransformed values. Homogeneous groups were determined using Tukey HSD post-hoc analysis ANOVA).

Table 3.1

Year	Site	N	Mg:Ca	Mn:Ca	Zn:Ca	Sr:Ca	Ba:Ca
2006	BS	25	1.65 (0.44) a	0.77 (0.20) a	4.18 (0.53) a	3.45 (0.07) a	1.77 (0.14) a
	GOA	50	1.07 (0.20) a	0.46 (0.06) a	1.81 (0.39) b	3.15 (0.11) b	1.86 (0.14) a
2008	BS	58	2.28 (0.42) a	1.11 (0.16) a	0.43 (0.07) a	3.39 (0.05) a	1.35 (0.06) a
	GOA	58	0.69 (0.06) b	0.46 (0.07) b	0.52 (0.11) a	3.18 (0.07) b	1.33 (0.08) a

Table 3.2

Year	Site	BS (n)	GOA (n)	N	% Correct	% Overall
2006	Bering Sea (BS)	15	10	25	60	80
	Gulf of Alaska (GOA)	5	45	50	90	
2008	Bering Sea (BS)	38	20	58	66	69
	Gulf of Alaska (GOA)	16	42	58	72	

Table 3.3

Year	Site	N	SL	Age	Hatch Date	Growth Rate
2006	PW	27	67.1 (58.0-83.0) [b]	165 (141-198) [b]	Mar 17 (Feb 12 – Apr 10) [a]	0.36 (0.32-0.46) [a]
	PE	28	67.7 (55.0-83.0) [b]	172 (132-213) [b]	Feb 29 (Jan 19 – Apr 9) [a]	0.37 (0.27-0.49) [a]
	MS	26	64.4 (51.0-83.0) [b]	167 (138-206) [b]	Mar 8 (Jan 29 – May 11) [a]	0.36 (0.28-0.48) [a]
	BB	25	76.0 (57.0-111) [c]	178 (145-202) [b]	Mar 17 (Feb 22 – Apr 19) [a, b]	0.39 (0.34-0.59) [a]
	PM	26	57.3 (50.0-66.0) [a]	143 (95-190) [a]	Apr 7 (Feb 20 – May 25) [b, c]	0.38 (0.28-0.50) [a]
	UI	29	56.4 (50.0-65.0) [a]	137 (90-175) [a]	Apr 14 (Mar 7 – May 31) [c]	0.38 (0.34-0.55) [a]
2008	PE	29	58.7 (50.0-72.0) [c]	186 (112-238) [d]	Mar 23 (Jan 31 – June 6) [c]	0.29 (0.23-0.47) [a, c]
	MS	30	71.1 (64.0-79.0) [a]	235 (200-283) [a]	Jan 28 (Dec 12 – Mar 5) [a]	0.28 (0.23-0.30) [a, b]
	CN	30	71.5 (65.0-80.0) [a]	228 (206-254) [a, c]	Feb 3 (Jan 8 – Feb 25) [a, b]	0.29 (0.26-0.33) [a, b]
	BB	25	65.7 (55.0-86.5) [b]	207 (139-239) [b]	Feb 20 (Jan 19 – Apr 29) [b]	0.30 (0.29-0.43) [b]
	PM	23	79.2 (58.5-101) [d]	236 (186-272) [a]	Jan 23 (Dec 19 – Mar 14) [a]	0.32 (0.29-0.48) [c]
	UI	20	67.3 (55.0-80.0) [a, b]	212 (165-257) [b, c]	Feb 12 (Dec 30 – Mar 31) [a, b]	0.30 (0.29-0.36) [a, c]

Table 3.4

Year	Site	PW	PE	MS	BB	PM	UI	N	% Correct	Overall
2006	**PW**	**7**	3	1	0	0	2	13	54	79
	PE	0	**13**	0	0	0	1	14	93	
	MS	0	0	**13**	0	0	0	13	100	
	BB	2	0	1	**10**	0	0	13	77	
	PM	2	1	0	1	**9**	0	13	69	
	UI	1	1	1	0	0	**12**	15	80	

		PE	MS	CN	BB	PM	UI			
2008	**PE**	**11**	2	0	0	0	1	14	79	71
	MS	2	**11**	2	0	0	0	15	93	
	CN	1	4	**9**	0	0	1	15	60	
	BB	0	0	0	**9**	4	0	13	69	
	PM	0	0	0	3	**12**	0	15	80	
	UI	0	1	2	0	1	**6**	10	60	

Table 3.5

Year	Source	N	Li:Ca	Mg:Ca	Mn:Ca	Sr:Ca	Ba:Ca
2006	1	40	0.85 (0.05) [a]	0.54 (0.03) [a]	0.28 (0.01) [a]	2.06 (0.04) [a]	0.43 (0.03) [b]
	2	74	0.56 (0.01) [b]	0.25 (0.01) [b]	0.26 (0.01) [a]	2.13 (0.03) [a, b]	0.60 (0.02) [c]
	3	15	0.87 (0.03) [a]	0.32 (0.03) [b]	0.33 (0.04) [a]	2.26 (0.07) [b]	1.12 (0.04) [a]
	4	4	0.78 (0.05) [a, b]	0.27 (0.04) [b]	1.85 (0.25) [b]	2.80 (0.14) [c]	1.01 (0.17) [a]
2008	1	70	0.86 (0.04) [a]	0.29 (0.02) [b]	0.26 (0.01) [b]	2.25 (0.04) [a]	0.76 (0.04) [a]
	2	53	2.53 (0.09) [b]	0.56 (0.02) [a]	0.22 (0.01) [a]	2.15 (0.04) [a, b]	0.55 (0.04) [b]
	3	10	5.18 (0.22) [c]	0.60 (0.04) [a]	0.18 (0.01) [a]	2.08 (0.05) [b]	0.28 (0.02) [c]

CHAPTER 4 – GENERAL CONCLUSION

This is the first study to investigate the contribution of natal sources of Pacific cod in the southeastern Bering Sea through the application of otolith chemistry and microstructure. The ability to determine the relative contribution of spawning regions to the sustained productivity of fished Pacific cod populations within the Bering Sea may lie in building upon these efforts.

A better understanding of the mechanism controlling otolith elemental incorporation is important for interpreting patterns observed in field-collected fish. Therefore, I conducted a controlled laboratory experiment to determine the effects of temperate and growth rate on elemental incorporation into the otoliths of larval cod. I observed inverse relationships between water temperature and otolith Sr and Ba but not Mg. Although abundant in the marine environment, Mg is also important for maintaining physiological functions such as bone development. Therefore, physiological regulation of internal concentrations may be stricter for Mg than Sr and Ba in larval Pacific cod. However, similar laboratory validation experiments are needed for juvenile cod to rule out any ontogenic effects on otolith elemental incorporation.

In the field samples, I observed higher otolith Sr and Mn in Bering Sea larvae than Gulf of Alaska larvae in both 2006 and 2008. Although otolith Mn concentrations were below detection limits in my laboratory study, Miller (2009) observed an inverse

relationship between temperature and otolith Mn in a laboratory study on black rockfish (*Sebastes melanops*), a marine species. The cooler water temperatures in the Bering Sea compared to the Gulf of Alaska at the time of collection might explain the pattern of consistently higher otolith Sr and Mn observed in the Bering Sea. However, concentrations of otolith edge Sr and Ba of juvenile cod were not correlated with water temperature at capture, which was not surprising given that temperatures only varied up to 1°C across collection sites in both years. Therefore, observed variation in otolith edge signatures of juveniles may be due to other factors such as differences in elemental water concentrations across collection sites or interactive effects of influencing factors. There was no evidence for a significant growth effect on otolith incorporation in the laboratory experiment, and average somatic growth rates were generally homogenous among larval and juvenile field collections. Therefore, variation in individual growth rates is an unlikely factor influencing observed patterns in otolith chemistry of larvae and juveniles in the field.

Given the potential for larvae to disperse large distances (up to 240 km) in 10 to 14 d, it may be difficult to characterize larval sources of Pacific cod and assign recruits back to specific locations within the Unimak Pass-Alaska Peninsula region. However, because Gulf of Alaska larvae were chemically distinct from larvae collected in the Bering Sea in both years, there is potential to evaluate the relative proportions that Gulf of Alaska sources along the Alaska Peninsula contribute to the Bering Sea Pacific cod population using otolith natal signatures. We were able to

assign juveniles to their collection site with relatively high accuracy, indicating that otolith analysis can likely be used to investigate movement patterns of Bering Sea Pacific cod. Multiple putative sources contributed to the juvenile recruits in both years, and their distribution and relative contributions suggest that other known spawning regions, aside from the Unimak Pass-Alaska Peninsula region, may contribute significantly to the eastern Bering Sea Pacific cod population.

To continue research evaluating source contributions of Pacific cod to the Bering Sea, the most comprehensive approach would be to collect larvae throughout all known spawning regions (Aleutian Islands, Pribilof Islands, and Unimak Pass-Alaska Peninsula) and throughout the spawning season (January-May) to fully characterize sources and assign recruits back to spawning regions. Because larval collections within the Bering Sea Unimak Pass-Alaska Peninsula region are potentially composed of mixed sources after 10 to 14 d post-hatch, larvae also need to be collected closer to their true hatch location to accurately characterize sources. However, given that there is greater interest in evaluating how different spawning regions contribute to the Bering Sea cod population (not only the UP-AP), mixed source contributions to the UP-AP region may not be an issue as long as the otolith chemistry of larvae collected from each known spawning region are chemically distinct.

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APPENDIX

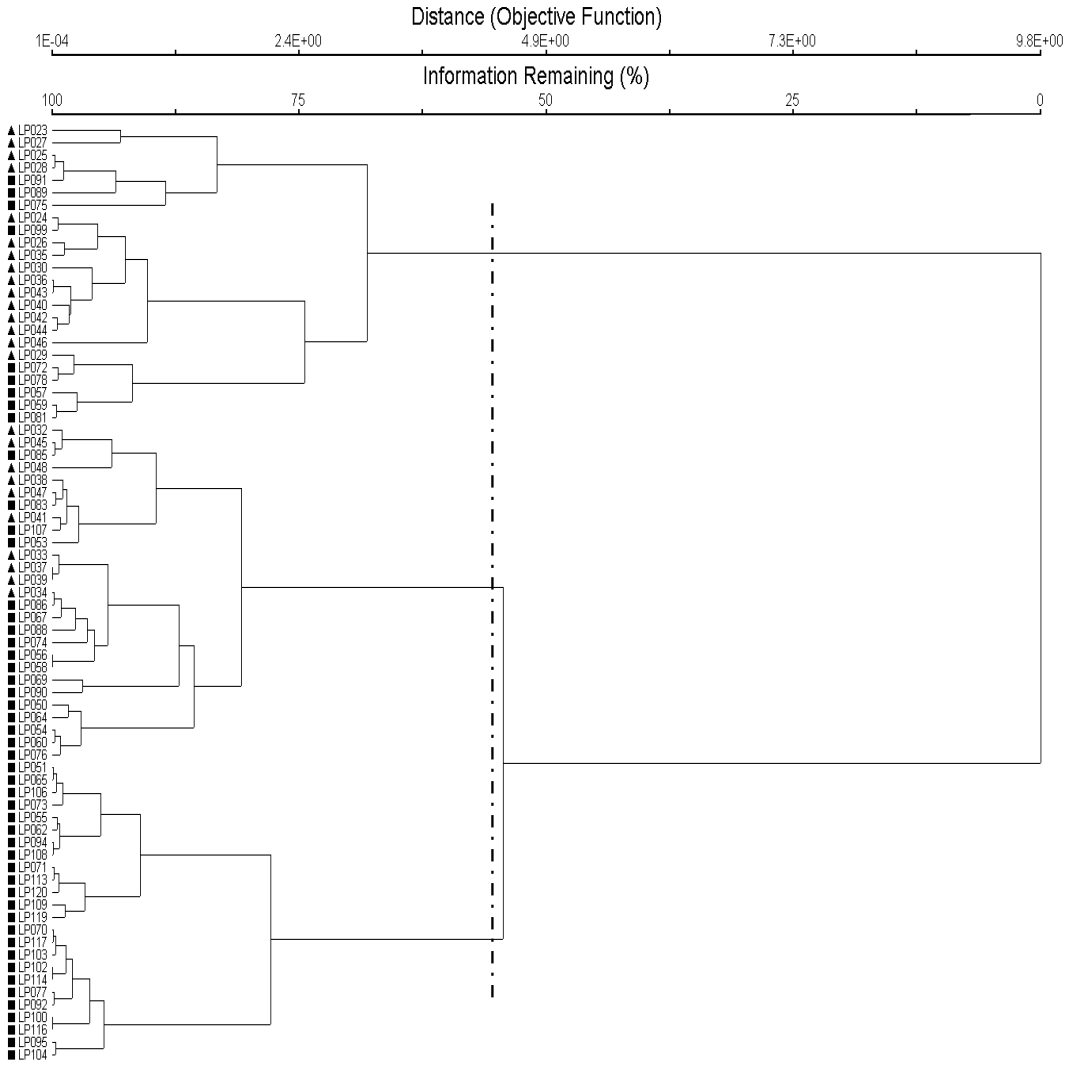

Figure 1: Cluster analysis of all 2006 larvae collected within the Bering Sea (triangle) and Gulf of Alaska (square). Dashed line represents cut-off for group quantification.

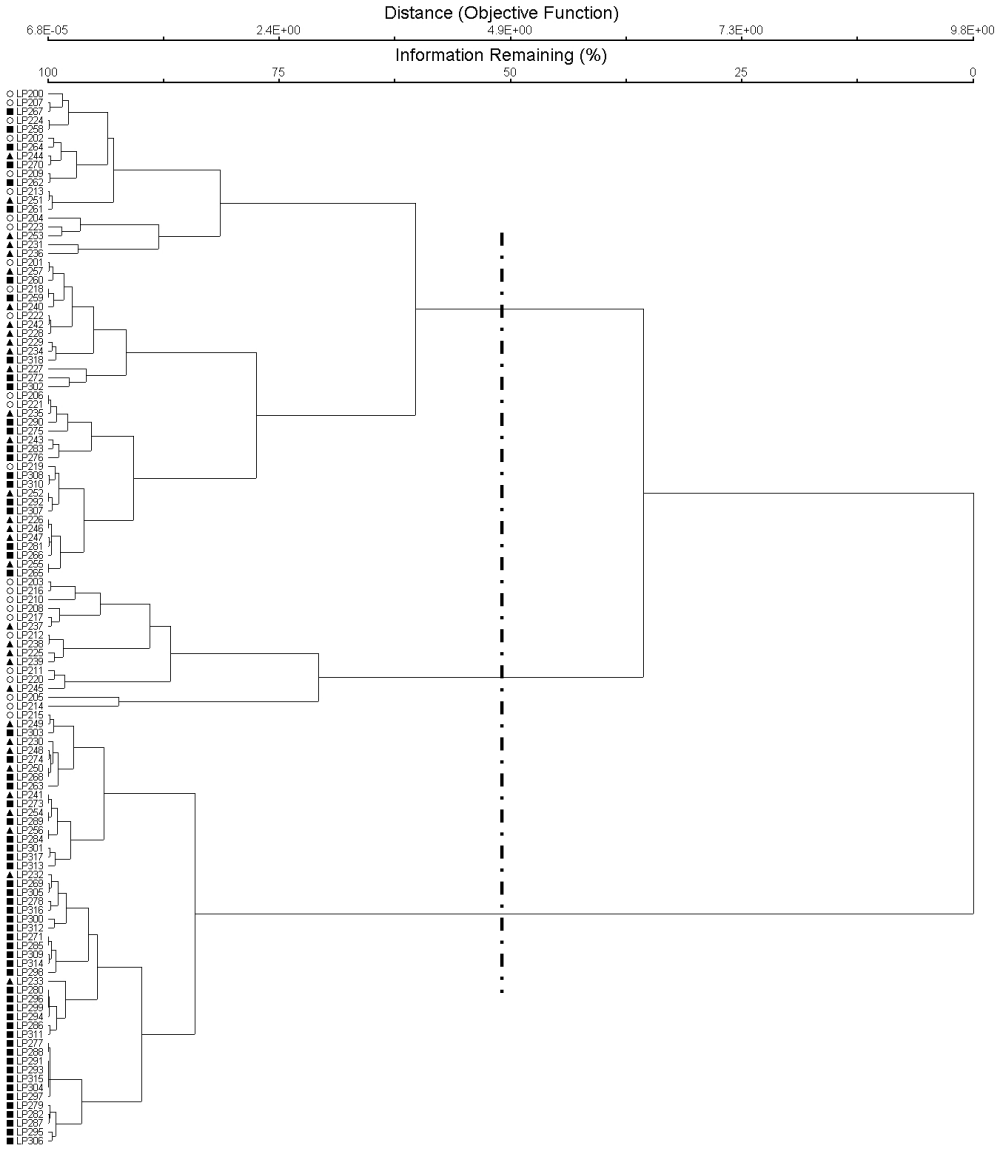

Figure 2: Cluster analysis of all 2008 larvae collected within the Bering Sea (triangle), the two tows within Unimak Pass (circle), and the Gulf of Alaska (square). Dashed line represents cut-off for group quantification.

Table 1: Biological characteristics of larval Pacific cod collected within the Bering Sea (BS) and Gulf of Alaska (GOA) in 2006 and 2008. Mean values (range) for size at capture (SL, mm), age at capture (d), hatch date, and average somatic growth rate ($mm \cdot d^{-1}$). Homogeneous groups were determined using multiple comparisons (Mann-Whitney U Test).

Year	Site	N	SL	Age	Hatch Date	Growth Rate
2006	BS	25	9.25 (5.64 - 11.5) [a]	23 (13 - 34) [a]	Apr 26 (Apr 14 – May 4) [a]	0.22 (0.07 - 0.51) [a]
	GOA	50	10.2 (7.18 – 13.5) [b]	25 (10 - 39) [a]	Apr 27 (Apr 14 – May 12) [a]	0.22 (0.15 - 0.34) [a]
2008	BS	58	6.44 (4.73 – 8.80) [a]	9 (4 - 18) [a]	May 7 (Apr 30 – May 13) [a]	0.20 (0.01 - 0.43) [a]
	GOA	58	8.10 (4.62 – 13.9) [b]	21 (8 - 50) [b]	May 4 (Apr 5 – May 16) [a]	0.17 (0.01 - 0.31) [a]

Table 2: Otolith chemistry of juvenile recruits representing the last 10 d of life. Mean (± SE), untransformed ratios of Mg:Ca (mmol·mol^{-1}), Mn:Ca (µmol·mol^{-1}), Sr:Ca (mmol·mol^{-1}), and Ba:Ca (µmol·mol^{-1}). Recruits were collected at sites throughout the southeastern Bering Sea: Pribilof Islands West (PW);Pribilof Islands East (PE); mid-shelf (MS); Cape Newenham (CN); Bristol Bay (BB); Port Moller (PM); and Unimak Island (UI). Sites are listed in order from north to south in a clockwise direction. Homogeneous groups were determined using Tukey HSD post-hoc analysis (ANOVA).

Year	Site	N	Mg:Ca	Mn:Ca	Zn:Ca	Sr:Ca	Ba:Ca
2006	PW	27	0.31 (0.02) $^{a, b}$	1.01 (0.06) ^{a}	0.63 (0.07) $^{a, b}$	1.99 (0.04) $^{a, b}$	0.51 (0.09) ^{a}
	PE	28	0.38 (0.02) ^{b}	0.63 (0.04) ^{a}	0.42 (0.04) $^{a, b}$	2.07 (0.08) ^{b}	0.29 (0.02) ^{a}
	MS	26	0.32 (0.01) $^{a, b}$	0.56 (0.02) ^{a}	1.11 (0.15) $^{c, d}$	2.69 (0.08) ^{c}	0.25 (0.01) ^{a}
	BB	25	0.27 (0.01) ^{a}	2.38 (3.68) ^{c}	0.24 (0.03) ^{a}	2.74 (0.09) ^{c}	0.44 (0.04) ^{a}
	PM	26	0.48 (0.03) ^{c}	1.75 (0.09) ^{b}	0.70 (0.08) $^{b, c}$	1.96 (0.10) $^{a, b}$	0.87 (0.08) ^{b}
	UI	29	0.29 (0.01) ^{a}	1.03 (0.05) ^{a}	1.24 (0.16) ^{d}	1.64 (0.13) ^{a}	1.02 (0.10) ^{b}
2008	PE	29	0.56 (0.02) ^{c}	0.36 (0.02) ^{a}	0.56 (0.09) ^{b}	2.77 (0.10) ^{a}	0.16 (0.01) ^{a}
	MS	30	0.41 (0.01) ^{a}	0.31 (0.02) ^{a}	0.26 (0.03) ^{a}	3.32 (0.10) ^{c}	0.21 (0.01) $^{a, b}$
	CN	30	0.41 (0.01) ^{a}	0.39 (0.02) ^{a}	0.23 (0.03) ^{a}	3.05 (0.10) $^{a, c}$	0.30 (0.02) ^{b}
	BB	25	0.22 (0.01) ^{b}	1.42 (0.10) ^{c}	0.24 (0.04) ^{a}	2.78 (0.06) ^{a}	0.92 (0.07) ^{e}
	PM	23	0.26 (0.01) ^{b}	1.90 (0.25) ^{d}	0.20 (0.03) ^{a}	2.29 (0.05) ^{b}	0.64 (0.05) ^{d}
	UI	20	0.45 (0.02) ^{a}	0.88 (0.10) ^{b}	0.38 (0.12) $^{a, b}$	2.07 (0.11) ^{b}	0.47 (0.06) ^{c}

Table 3: Biological characteristics of juvenile recruits classified to natal sources contributing to the southeastern Bering Sea in 2006 and 2008. Mean (range) values for size at capture (SL, mm), age (d), hatch date, and average somatic growth rate ($mm \cdot day^{-1}$). All characteristics are the untransformed values. In both years all biological characteristics, except hatch dates, were similar among sources (Kruskal-Wallis ANOVA by Ranks, $P > 0.13$).

Year	Source	N	SL	Age	Hatch Date	Growth Rate
2006	1	40	62.8 (51.0-100)	160 (103-197)	Mar 19 (Feb 15 – May 17) [a, b]	0.36 (0.28-0.49)
	2	74	66.9 (51.0-111)	168 (103-202)	Mar 14 (Feb 15 – May 18) [a, b]	0.37 (0.34-0.59)
	3	15	68.9 (60.0-97)	176 (160-202)	Mar 3 (Feb 16 – Mar 15) [a]	0.36 (0.34-0.47)
	4	4	59.8 (51.0-65)	143 (103-172)	Apr 6 (Mar 10 – May 11) [b]	0.36 (0.34-0.41)
2008	1	70	71.6 (57.0-101)	224 (179-256)	Feb 4 (Jan 3 – Mar 16)	0.30 (0.28-0.48)
	2	53	69.7 (59.0-80.0)	226 (188-260)	Feb 5 (Jan 3 – Mar 20)	0.29 (0.23-0.36)
	3	10	69.3 (60.0-79.0)	226 (192-254)	Feb 7 (Jan 8 – Mar 16)	0.29 (0.28-0.30)