

1 **Spatial and temporal variation in otolith elemental signatures of age-0 Pacific cod (*Gadus***  
2 ***macrocephalus*) in the Gulf of Alaska**

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17 **Abstract**

18 Shallow coastal waters of the Gulf of Alaska (GOA) serve as nursery habitats for young-of-year  
19 Pacific cod (*Gadus macrocephalus*). However, little is known regarding the relative contribution  
20 of these areas to the adult offshore stock. Trace elements incorporated into the otolith matrix can  
21 reflect the environmental conditions to which a fish has been exposed during its lifetime. When  
22 analyzed together, a suite of elements can serve as a natural marker, characteristic of a particular  
23 environment. We evaluated the potential of otolith elemental signatures to identify nursery  
24 habitats of age-0 Pacific cod, focusing on spatial patterns within and across the eastern and  
25 western GOA. Fish were collected from shallow nearshore areas within five large embayments in  
26 summer and fall, and element:calcium ratios were measured from two different time stanzas  
27 within the otolith. Elemental ratios were found to change over short (2 month) time periods and  
28 were related to seasonal changes in temperature and salinity. Fish were classified to nursery  
29 habitats using quadratic discriminant analysis based on their otolith elemental signatures;  
30 classification accuracy to individual bays ranged from 30 to 95%, with an overall success rate of  
31 59%. Classification accuracy improved to 78% at greater spatial scales (eastern versus western  
32 GOA). Our results point out the limitations of this application to Gulf of Alaska Pacific cod and  
33 other widely distributed species residing in coastal embayments. While some nursery habitats  
34 impart unique chemical signatures to otoliths, it may not be possible to distinguish among  
35 specific areas without considering additional factors. However, our work demonstrates that  
36 otolith microchemistry may be a useful tool for understanding source contributions to the Pacific  
37 cod population at larger regional scales within the GOA.

38

39 **Keywords:** otoliths, life history, Gulf of Alaska, trace elements, fishery ecology, Pacific cod,  
40 nursery grounds, otolith microchemistry

41

## 42 **1. Introduction**

43  
44 The Gulf of Alaska (GOA) is a complex large marine ecosystem that is seasonally and  
45 spatially variable in oceanography and productivity (Mundy, 2005; Spies, 2007). The GOA  
46 supports a number of important commercial fisheries, including several species of groundfish  
47 and salmon. The coastline of the GOA is highly rugose with small and large embayments,  
48 including inland seas such as Prince William Sound. The GOA is almost completely ringed by  
49 coastal mountain ranges that heavily influence its climate and oceanography, notably by  
50 producing large amounts of freshwater runoff from precipitation and, to a lesser extent, glacial  
51 melt (Weingartner, 2007). Runoff is a significant contributor of fresh water in the GOA and  
52 helps to drive the Alaska Coastal Current, which moves from east to west and dominates the  
53 inner shelf (Stabeno et al., 2004; Weingartner, 2007). Hydrography off the shelf is influenced by  
54 the stronger Alaska Current and Alaskan Stream. The GOA can be divided into two main regions  
55 (western and eastern; Fig. 1) that differ in their physical and biological characteristics. The  
56 eastern GOA has a much narrower shelf than the western GOA, and the influence of freshwater  
57 runoff increases from east to west as inputs accumulate. The eastern and western regions also  
58 display different patterns of sea surface temperature and chlorophyll variability (Bograd et al.,  
59 2005; Waite and Mueter, 2013). These differences are reflected in fish populations; the western  
60 region, particularly around Kodiak Island, supports higher fish biomass but has lower species  
61 richness and diversity than the eastern GOA (Mueter and Norcross, 2002). However, more work  
62 is needed to understand the mechanisms behind the relative differences in fish habitat use  
63 between the regions and how these areas are linked to offshore productivity. Knowledge of the  
64 connectivity between life stages of marine species, as well as habitats used at each stage, is  
65 critical to sustainable fisheries management.

66 Otolith microchemistry has been used in a variety of ecological applications, including  
67 studies of life history patterns and migration (Elsdon et al., 2008). Though otoliths consist  
68 primarily of calcium carbonate (in the form of aragonite) precipitated over a protein matrix, trace  
69 elements are also incorporated and permanently retained as the otolith grows (Campana, 1999;  
70 Campana and Thorrold, 2001). To some degree, the specific combination and concentration of  
71 certain trace elements in otoliths (referred to as “elemental signatures”) often reflect the  
72 particular environment to which a fish has been exposed (Gillanders and Kingsford, 2000) and

73 may serve as natural tags of individual groups or stocks (Campana, 2004; Campana et al., 2000),  
74 allowing discrimination among fish from different natal sources (Barbee and Swearer, 2007).  
75 Furthermore, elemental signatures of early life stages may be compared in adults of the same  
76 cohort to determine connectivity between juvenile and adult fish populations (Gillanders, 2002).

77 Pacific cod (*Gadus macrocephalus*) is widely distributed across Alaska waters and is the  
78 target of a large multi-gear commercial fishery; however, the complex life history of this species  
79 is only partially understood. In the GOA, adult Pacific cod spawn semi-demersal eggs in late  
80 winter through spring near the bottom of continental shelf waters. Evidence of spawning activity  
81 has been identified in the vicinity of Kodiak Island, the Shumagin Islands, and Sanak Island in  
82 the western GOA (Doyle et al., 2009; Rugen and Matarese, 1988), though specific spawning  
83 areas throughout the rest of the GOA have not yet been identified. Soon after hatching, larvae  
84 rise to the surface and are thought to be transported shoreward by the Alaska Coastal Current  
85 (Hurst et al., 2009; Rugen and Matarese, 1988). Age-0 juveniles settle into shallow coastal  
86 nursery areas where they exhibit associations with seagrasses, kelps, and sea cucumber mounds  
87 (Abookire et al., 2007; Laurel et al., 2007; Stoner et al., 2008). Age-1 Pacific cod generally occur  
88 at deeper depths in these nearshore areas, suggesting the continued importance of coastal nursery  
89 habitats beyond the first year of life (Laurel et al., 2009). Protected inner waters support higher  
90 primary productivity and consequently higher availability of zooplankton for juvenile fish  
91 (Cooney, 2007) while simultaneously acting as refugia from predators (Linehan, 2001).  
92 However, the relative contribution of juveniles from different inshore nursery habitats to the  
93 broadly distributed adult offshore population of Pacific cod within the GOA is currently  
94 unknown.

95 Analysis of otolith microchemistry has been used to describe patterns of larval dispersal  
96 and mixing in juvenile Pacific cod from the eastern Bering Sea (EBS). Otolith elemental  
97 signatures of Pacific cod larvae collected in the GOA and EBS were significantly different  
98 between the two regions, indicating the ability to discriminate among larval sources at broad  
99 spatial scales (DiMaria, 2011). Within the EBS, otolith elemental signatures were used to  
100 identify several distinct larval sources and to classify juvenile Pacific cod to site of collection,  
101 suggesting the utility of this approach at smaller spatial scales as well (DiMaria, 2011; Miller et  
102 al., 2016). However, the otolith microchemistry of Pacific cod collected from coastal regions of  
103 the GOA has not yet been investigated. Habitat use of juvenile Pacific cod differs markedly

104 between the EBS and GOA. The EBS has a broad, low-relief continental shelf with few  
105 protected bays and inlets, with juvenile Pacific cod inhabiting shallow coastal waters as well as  
106 pelagic waters over the continental shelf (Hurst et al., 2014). Conversely, in the GOA, the  
107 primary nursery areas of Pacific cod are the shallow waters of numerous discrete embayments  
108 and inlets (Johnson et al., 2012; Laurel et al., 2007). The relative spatial complexity of Pacific  
109 cod nursery habitats in the GOA may therefore result in finer scales of variation in otolith  
110 microchemistry and allow for potentially greater power of discrimination among natal signatures.

111 In this paper, we evaluate the utility of otolith elemental signatures to identify nursery  
112 habitat associations in juvenile (age-0) Pacific cod in the GOA. We examine patterns of variation  
113 in otolith elemental signatures at three spatial scales: 1) between the large, oceanographically and  
114 ecologically different eastern and western regions of the GOA, 2) across nearshore embayments  
115 within each region, and 3) among sampling sites within embayments. The temporal stability of  
116 otolith signatures is assessed by comparing signatures of otoliths collected during summer and  
117 fall of the same year. We next address possible explanatory mechanisms by exploring the  
118 relationships between elemental values and environmental variables. Finally, we test the  
119 potential for otolith elemental signatures to assign juvenile Pacific cod to nursery bay and region.

120

## 121 **2. Materials and methods**

122

### 123 *2.1 Otolith collection and preparation*

124

125 Pacific cod were collected by purse seine during nearshore fish surveys conducted in  
126 2011 as part of the Gulf of Alaska Integrated Ecosystem Research Program (GOAIERP)  
127 (<http://www.nprb.org/gulf-of-alaska-project>). The GOAIERP focused on two large study areas  
128 within the eastern and western regions of the GOA (Fig. 1). The nearshore surveys were  
129 conducted in bays that were representative of the nearshore habitats available in each study area  
130 and varied in size, mean depth, watershed area, and other characteristics (Ormseth et al., 2017).  
131 Habitats (sites) within bays also varied in the degree of exposure, freshwater influence, and  
132 submerged vegetation.

133 We considered three nested spatial levels: region ( $n = 2$ ), bay ( $n = 5$ ), and site ( $n = 12$ ),  
134 and two temporal periods: summer (July-August) and fall (September-October) (Table 1). Pacific

135 cod were collected from two bays in the eastern GOA (Islas Bay and Salisbury Sound) and three  
136 bays in the western GOA (Kiliuda Bay, Izhut Bay, and Port Dick) (Fig. 1). Within each bay,  
137 multiple sites were sampled (Fig. 1). Similar to the selection of bays, seining sites were designed  
138 to be representative of the habitats available within each bay and to cover the spatial extent of  
139 each bay. Pacific cod were not captured at all sampling sites in each bay, but this analysis  
140 included all sites (up to three per bay) where cod were found. All bays were sampled in both the  
141 summer and fall sampling periods. However, in only two of the bays (Kiliuda Bay and Port  
142 Dick) were sufficient numbers of age-0 Pacific cod captured in both seasons to use in these  
143 analyses ( $n > 10$ ). Sufficient sample sizes were available from the remaining bays in only one of  
144 the sampling periods (Table 1).

145 Fish were collected using a small purse seine (14.6 m diameter when deployed, 6 m  
146 depth, 3 mm knotless nylon mesh) during daylight hours. Individuals  $< 120$  mm fork length (FL)  
147 were assumed to be age 0 (Ormseth et al., 2017) and were selected randomly from the catch for  
148 later analysis. A maximum of 12 age-0 cod were collected from each seine haul; this sample size  
149 had been determined to be sufficient for unrelated analysis of fatty acid signatures and was  
150 deemed acceptable for the otolith analysis as well, based on prior studies that have found that  
151 variance usually stabilizes between 10 and 20 samples (J.A. Miller, unpublished data). All fish  
152 were stored on ice until the evening when they were wrapped in two layers of plastic film, placed  
153 in plastic bags, and frozen at  $-40^{\circ}\text{C}$  until subsequent laboratory processing was possible. In each  
154 bay, temperature, salinity, and dissolved oxygen were measured from vertical casts at multiple  
155 stations using an SBE 19plus SeaCAT conductivity-temperature-depth (CTD) recorder (Sea-Bird  
156 Electronics, Bellevue, WA). The CTD sampling occurred within 1-2 days of fish collection, and  
157 CTD stations were located within 1-4 km of the seining sites (Fig. 1). Data were collected by the  
158 CTD continuously throughout the water column and were post-processed to provide average  
159 values at each 1 m depth increment. Because the seining targeted areas with a bottom depth of 6  
160 m or less, only data from the 5 m depth increment were used in this analysis.

161 In the laboratory, fish were weighed to the nearest 0.01 g and measured (FL) to the  
162 nearest millimeter. Otoliths (sagittae) were removed from partially thawed fish using a scalpel  
163 and forceps, wiped on a sponge to remove organic debris, and rinsed with distilled deionized  
164 water. All otoliths were stored dry. Whole otoliths were photographed, aged to confirm that they  
165 were age-0, and any damage to the otolith was noted. Otoliths were ultrasonically cleaned in

166 distilled deionized water for 10 min and placed in acid-washed glass vials using acid-washed  
167 ceramic forceps. Each otolith was then weighed to the nearest 0.0001 g; no significant difference  
168 in weight was found between intact left and right otoliths (paired  $t$ -test:  $t = 1.40$ ,  $df = 290$ ,  $P =$   
169  $0.16$ ). Either the left or right otolith was randomly selected for elemental analysis. Otoliths were  
170 sectioned through the core to produce transverse thin sections and then ground with 3M™  
171 Wetordry™ Tri-M-ite™ paper (240 to 1200 grit) and polished with Buehler AlO<sub>2</sub> powder (12.5  
172  $\mu\text{m}$  and 3.0  $\mu\text{m}$ ). Otolith sections were ultrasonically cleaned in distilled deionized water for 15  
173 min and mounted in random order on acid-washed glass slides.

174

## 175 *2.2 Otolith elemental analysis*

176

177 Laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS) was  
178 conducted at Oregon State University's W. M. Keck Collaboratory for Plasma Spectrometry  
179 (Corvallis, OR, USA) to quantify the analytes <sup>7</sup>Li, <sup>11</sup>B, <sup>24</sup>Mg, <sup>25</sup>Mg, <sup>43</sup>Ca, <sup>52</sup>Cr, <sup>55</sup>Mn, <sup>59</sup>Co, <sup>65</sup>Cu,  
180 <sup>66</sup>Zn, <sup>85</sup>Rb, <sup>86</sup>Sr, <sup>111</sup>Cd, <sup>138</sup>Ba, <sup>139</sup>La, and <sup>208</sup>Pb. These isotopes were selected for analysis based on  
181 previous Pacific cod otolith microchemistry studies (DiMaria, 2011; Miller et al., 2016). A  
182 Thermo Scientific XSERIES 2 ICP-MS coupled with a Photon Machines Analyte G2 193 nm  
183 laser was used for analyses. Otoliths were introduced in batches to the LA-ICPMS haphazardly  
184 to prevent systematic bias. Laser spot diameter was 40  $\mu\text{m}$ , scanning speed was 5  $\mu\text{m}/\text{s}$ , and laser  
185 pulse rate was 8 Hz. Elemental data were collected from two laser tracks in each otolith. The first  
186 track passed from the sulcus through the core region to the distal surface; the portion of this track  
187 from the core to the distal edge is hereafter referred to as the “life history transect”, representing  
188 the environmental conditions experienced over the entire lifetime of the fish. A second track  
189 approximately 300  $\mu\text{m}$  long tracing the outermost 40  $\mu\text{m}$  of the otolith along the distal surface is  
190 hereafter referred to as the “edge trace”, representing the conditions experienced during the last  
191 7-10 days of life prior to collection (Fig. 2). Background levels of analytes were measured for 30  
192 s prior to each scan. A glass reference standard, National Institute of Standards and Technology  
193 (NIST) 612, bracketed otolith scans and was used to assess precision of measurements and to  
194 convert elemental count rates to ratios. An aragonite standard, United States Geological Survey  
195 Microanalytical Carbonate Standard MACS-3, was used to assess accuracy. All otoliths were

196 photographed post-ablation and laser track distances were measured using Image-Pro Plus  
197 software (Media Cybernetics).

198 Post-processing of raw data was accomplished using an automated algorithm. To convert  
199 count rates to ratios, first the average background level of each analyte measured prior to  
200 ablation was subtracted from the mean count measured during otolith ablation. Analytes  
201 measured in otoliths were only retained for further analysis if they were on average equal or  
202 greater than two times background levels. Each analyte was normalized to  $^{43}\text{Ca}$ , an internal  
203 standard, to account for variations in instrument sensitivity and ablation rate. NIST 612 values  
204 were then used to convert elemental count rates to molar ratios using the following equation  
205 (Kent and Ungerer, 2006):

$$206 \quad (C_i^O / C_{Ca}^O) = (C_i^{NIST} / C_{Ca}^{NIST}) / (I_{ij}^{NIST} / I_{Ca,k}^{NIST}) \times (I_{ij}^O / I_{Ca,k}^O) \quad (1)$$

207 where  $C_i^O$  is the concentration of element  $i$  in otolith  $O$ ,  $C_{Ca}^O$  is the concentration of the internal  
208 standard Ca in otolith  $O$ ,  $C_i^{NIST} / C_{Ca}^{NIST}$  is the ratio of known concentrations of element  $i$  to Ca in  
209 reference standard NIST 612,  $I_{ij}^{NIST} / I_{Ca,k}^{NIST}$  is the normalized ion yield for isotope  $j$  of element  $i$   
210 and isotope  $k$  of Ca in NIST 612, and  $I_{ij}^O / I_{Ca,k}^O$  is the normalized ion yield for isotope  $j$  of  
211 element  $i$  and isotope  $k$  of Ca in otolith  $O$ . Known concentrations of elements in NIST 612 are  
212 reported in Pearce et al. (1997) and Jochum et al. (2011).

213

### 214 *2.3 Time stanzas of elemental data*

215

216 Two different time stanzas of elemental data were subjected to statistical analyses:

217

218 1) Edge traces were analyzed to determine otolith elemental composition at time of capture to  
219 evaluate temporal stability in twice-sampled bays, as well as to evaluate potential effects of fish  
220 size and environmental variables on elemental ratios. For otoliths where the edge trace passed  
221 through cracks or other areas of poor integrity, the final 40  $\mu\text{m}$  of the life history transect were  
222 used to calculate the elemental ratios representative of time at capture.

223

224 2) Because fish were not collected in both seasons from all bays, we also extracted elemental  
225 data from a synchronous portion of the life history transect of all otoliths. The goal of this  
226 analysis was to remove temporal variation such that elemental signatures representing a common



227 time period could be compared among collection locations. The earliest collection in our study  
228 occurred on July 14-15. Therefore, otolith elemental composition from the 100  $\mu\text{m}$  of the life  
229 history transect preceding July 15 was averaged to calculate the “early July signature” for each  
230 fish. Age-0 Pacific cod are thought to settle in nearshore nursery areas by July (Blackburn and  
231 Jackson, 1982; Laurel et al., 2016; Laurel et al., 2007). Therefore, we did not choose a longer  
232 integration for comparison of contemporaneous signatures in order to exclude elemental ratios  
233 incorporated during the pre-settlement period. Consequently, our “start point” used to calculate  
234 the average composition for each fish increases the likelihood that only elements incorporated at  
235 the capture location would be included in the analysis. For the fish collected on July 14-15, the  
236 early July signature was simply the final 100  $\mu\text{m}$  of the life history transect. For specimens  
237 collected August through October, otolith daily rings (Narimatsu et al., 2007) were counted at  
238 400 $\times$  magnification moving from the distal edge (latest deposited material) toward the core  
239 (earliest deposited material) to determine the point in the life history transect corresponding to  
240 July 15.

241 Rings were counted by a single age reader blindly on two separate occasions; if counts  
242 were within 10% of each other, the second count was retained. For specimens in which counts  
243 differed by >10%, or when daily rings were unclear and difficult to accurately quantify, the point  
244 in the life history transect corresponding to July 15 was estimated using the fractional distance of  
245 the July 15 ring relative to the otolith radius. The fractional distance was determined from clear  
246 specimens collected within the same bay and season and applied to unclear specimens. The 100  
247  $\mu\text{m}$  section of the life history transect immediately preceding the estimated position of the July  
248 15 ring was used to represent the early July elemental signature. The number of specimens for  
249 which this distance was estimated varied by season of collection, as otoliths displayed a general  
250 slow-down (and subsequent compression) of daily ring growth starting in the early fall, which  
251 made rings difficult to count. The distance was estimated for only one specimen collected in the  
252 summer, whereas it was estimated for 48% of specimens collected in the fall.

253

#### 254 *2.4 Statistical analyses*

255

256 Variation in otolith elemental ratios was quantified with univariate and multivariate  
257 analyses. Data were assessed for univariate normality using probability-probability (P-P) plots

258 and the Shapiro-Wilk test, and for homoscedasticity using Levene's test. Based on these results,  
 259 all elemental ratios were ln-transformed following the generalized procedure recommended by  
 260 McCune and Grace (2002) to meet assumptions of parametric statistical analyses. Strong outliers  
 261 (>3 SD from the mean) within the transformed data were excluded from further analysis  
 262 (McCune and Grace, 2002).

263 Differences in fish body length among bays were assessed with non-parametric Kruskal-  
 264 Wallis tests, and post-hoc pairwise comparisons were made using Dunn's procedure (Zar, 1999).  
 265 Potential effects of fish size on otolith edge elemental signatures were evaluated by fitting linear  
 266 mixed effects models to the relationship between each elemental ratio and ln-transformed fish  
 267 length, with collection site considered as a random effect.

268 To determine whether collection location (region, bay) had an effect on otolith elemental  
 269 composition, a hierarchical multivariate analysis of variance (MANOVA) was performed on the  
 270 early July signatures. Bay was nested within the region term, with random errors for site and for  
 271 individuals within site (residuals). Pillai's trace statistic (Zar, 1999) was used to determine  
 272 whether region or bay had significant effects on elemental signatures. Univariate ANOVAs were  
 273 used to identify individual early July elemental ratios contributing to significant differences  
 274 observed in the MANOVA.

275 Seasonal differences in individual elemental ratios within edge traces in Kiliuda Bay and  
 276 Port Dick (the only two bays that were sampled in both seasons) were assessed using univariate  
 277 two-sample *t*-tests.

278 Trends associated with potential environmental correlates were assessed using linear  
 279 regressions of elemental ratios against temperature, salinity, and dissolved oxygen content  
 280 measured at 5 m depth from the CTD cast taken nearest to each fish collection location (Fig. 1).  
 281 Linear regressions were tested for significance using ANOVA (Zar, 1999). Pearson correlation  
 282 coefficients were also calculated.

283 Discriminant analysis (DA) was used to classify fish by collection location (region and  
 284 bay) based on early July elemental signatures. Equality of covariance matrices was assessed  
 285 using Box tests to determine whether to use a linear or quadratic classification function.  
 286 Classification accuracy was tested using a leave-one-out (jack-knife) cross-validation procedure.  
 287 Cohen's kappa statistic, formulated as:

$$288 \kappa = (p_o - \sum_{i=1}^G p_i q_i) / (1 - \sum_{i=1}^G p_i q_i) \quad (2)$$

289 where  $G$  is the number of groups,  $p_o$  is the observed proportion of samples correctly classified,  $p_i$   
290 is the proportion of samples in the  $i$ th group, and  $q_i$  is the proportion of samples classified into  
291 the  $i$ th group, was calculated to assess overall classification accuracy relative to random chance;  
292 a value of  $\kappa = 1.0$  indicates perfect assignment and a value of  $\kappa = 0.0$  indicates no improvement  
293 over chance (Cohen, 1960; McGarigal et al., 2000).

294 All analyses were conducted in either the statistical computing language R v. 3.2.5 (R  
295 Core Team, 2016), SAS (SAS Institute), or the Microsoft Excel statistical add-in package  
296 XLSTAT-Pro<sup>®</sup> v. 2013.1.02 (Addinsoft). Values were considered significant at  $\alpha < 0.05$ .

297

### 298 **3. Results**

299

#### 300 *3.1 Laser ablation-inductively coupled plasma mass spectrometry*

301

302 Otoliths were successfully analyzed from 363 age-0 Pacific cod captured in various  
303 locations in the eastern and western GOA (Table 1). Of these, 16 and 14 otoliths were dropped,  
304 respectively, from the analyses of the edge traces and early July segments due to outliers ( $>3$  SD  
305 from the mean) in the elemental data. These outliers were omitted because of their potential  
306 disproportionate influence on results of the MANOVA and discriminant analysis.

307 Of the 16 analytes measured, values of  $^{11}\text{B}$ ,  $^{24}\text{Mg}$ ,  $^{25}\text{Mg}$ ,  $^{43}\text{Ca}$ ,  $^{55}\text{Mn}$ ,  $^{66}\text{Zn}$ ,  $^{86}\text{Sr}$ ,  $^{138}\text{Ba}$ , and  
308  $^{208}\text{Pb}$  were consistently two times or greater than mean background levels and were included in  
309 statistical analyses. Estimates of precision (% CV) based on mean measurements of NIST 612  
310 standard glass were as follows:  $^{11}\text{B} = 17.6\%$ ,  $^{24}\text{Mg} = 7.4\%$ ,  $^{43}\text{Ca} = 2.9\%$ ,  $^{55}\text{Mn} = 6.2\%$ ,  $^{66}\text{Zn} =$   
311  $10.7\%$ ,  $^{86}\text{Sr} = 5.0\%$ ,  $^{138}\text{Ba} = 2.6\%$ , and  $^{208}\text{Pb} = 6.1\%$ . Estimates of accuracy for elemental ratios  
312 (% error based on published MACS-3 calcium carbonate standard values where available) were  
313  $\text{Mg:Ca} = 37.7\%$ ,  $\text{Mn:Ca} = 2.8\%$ ,  $\text{Zn:Ca} = 9.5\%$ ,  $\text{Sr:Ca} = 4.4\%$ ,  $\text{Ba:Ca} = 9.9\%$ , and  $\text{Pb:Ca} =$   
314  $5.6\%$ . Though accuracy was generally low for Mg:Ca, relative precision of MACS-3 runs was  
315 high (3.6% relative standard deviation), indicating that differences between measured ratios and  
316 known ratios of Mg:Ca were consistent.

317

#### 318 *3.2 Effects of fish size on elemental ratios*

319

320 There were significant differences in fish body length among bays in summer (Kruskal-  
321 Wallis:  $K = 38.686$ ,  $df = 2$ ,  $P < 0.001$ ) and fall (Kruskal-Wallis:  $K = 33.568$ ,  $df = 3$ ,  $P < 0.001$ ).  
322 In summer, fish lengths were significantly different among all three bays sampled (Dunn:  $P <$   
323  $0.05$ ). Fish in Islas Bay, sampled earliest, were the smallest (mean FL = 65 mm); fish in Port  
324 Dick were the largest (mean FL = 77 mm) and had the widest range of lengths sampled (Table  
325 1). In fall, although it was sampled earliest, fish in Salisbury Sound were the largest (mean FL =  
326 116 mm), differing significantly from Kiliuda and Izhut Bays. Port Dick was not significantly  
327 different from the other bays in length distribution. Fish length had a significant negative effect  
328 on B:Ca ( $F_{1,11} = 6.05$ ,  $P = 0.015$ ), with no interaction between site and length ( $P = 0.245$ ).  
329 Length also had a significant negative effect on Ba:Ca ( $F_{1,11} = 6.89$ ,  $P = 0.009$ ), but the  
330 relationship differed among bays as indicated by a strong interaction between site and length ( $P$   
331  $< 0.001$ ). Length had a weakly negative effect on Pb:Ca ( $F_{1,11} = 4.84$ ,  $P = 0.028$ ), with a  
332 marginally significant interaction between site and length ( $P = 0.059$ ). Finally, there were  
333 significant interactions between site and length for the elemental ratios Mg:Ca, Mn:Ca, and  
334 Sr:Ca (all  $P < 0.001$ ).

335

### 336 *3.3 Spatial and temporal differences in elemental concentration*

337

338 Significant spatial effects were detected among the multivariate early July signatures at  
339 the levels of region (MANOVA: Pillai's trace = 1.00,  $F_{1,11} = 395.96$ ,  $P = 0.039$ ) and bay (Pillai's  
340 trace = 2.71,  $F_{3,11} = 3.96$ ,  $P = 0.019$ ). There were significant differences between the eastern and  
341 western regions of the GOA for all elemental ratios except Zn:Ca and Pb:Ca. There were  
342 significant differences among bays in all elemental ratios; Islas Bay had higher ratios of B:Ca  
343 and Sr:Ca compared to the other bays, and Ba:Ca and Mg:Ca were both highly variable among  
344 bays (Fig. 3). Individual sites within bays also showed significant variation for all elemental  
345 ratios (Fig. 3).

346 Large temporal differences were noted in several of the elemental ratios analyzed from  
347 edge traces of otoliths collected in both summer and fall from the same bay (Kiliuda Bay and  
348 Port Dick) (Fig. 4). In both bays, average values of B:Ca, Mg:Ca, and Zn:Ca decreased  
349 significantly from summer to fall, while Sr:Ca increased significantly (Fig. 4).  
350 Manganese:calcium decreased significantly in Kiliuda Bay ( $t$ -test:  $t = -8.11$ ,  $df = 68$ ,  $P < 0.001$ )

351 while no significant change was observed in Port Dick ( $t$ -test:  $t = -1.12$ ,  $df = 78$ ,  $P = 0.27$ ) (Fig.  
352 4). Interestingly, Ba:Ca significantly decreased in Kiliuda Bay but increased slightly in Port Dick  
353 from summer to fall (Fig. 4). There was no significant difference between seasons in Pb:Ca for  
354 either bay ( $P > 0.05$ ).

355

### 356 *3.4 Effects of environmental variables on elemental concentration*

357

358 Temperature, salinity, and dissolved oxygen concentrations measured at the time of fish  
359 collections were somewhat variable among bays within each season of collection  
360 (Supplementary Table 1). Temperatures were generally higher in summer than in fall, but  
361 salinity and dissolved oxygen concentrations did not appear to follow any seasonal patterns.

362 Significant relationships were found between environmental variables and ratios of some  
363 elements at the otolith edge (Fig. 5; Supplementary Table 2). All elemental ratios except for  
364 Pb:Ca were significantly related to temperature. Five of the elemental ratios (B:Ca, Mg:Ca,  
365 Mn:Ca, Zn:Ca, and Ba:Ca) were positively related to temperature. Only Sr:Ca was negatively  
366 correlated with temperature ( $r = -0.628$ ). All elemental ratios except for B:Ca and Pb:Ca were  
367 significantly related to salinity. Elemental ratios that were positively related to salinity were  
368 Mg:Ca, Zn:Ca, and Ba:Ca, which had the highest correlation ( $r = 0.497$ ). Both Mn:Ca and Sr:Ca  
369 were negatively related to salinity. Only B:Ca, Sr:Ca, and Mg:Ca were significantly related to  
370 dissolved oxygen concentration; B:Ca and Mg:Ca were positively correlated ( $r = 0.36$  and  $r =$   
371  $0.24$ , respectively) and Sr:Ca was negatively correlated ( $r = -0.34$ ).

372

### 373 *3.5 Classifying elemental signatures*

374

375 Quadratic discriminant analyses (DA) were used to group specimens at the regional and  
376 bay level based on early July elemental signatures (Fig. 6). All seven elements (B, Mg, Mn, Zn,  
377 Sr, Ba, and Pb) were used to create the classification functions for discriminating among bays.  
378 The initial variables that had the largest relative contribution (standardized canonical  
379 coefficients) to the discriminant functions were Ba:Ca, Sr:Ca, and B:Ca. Classification success to  
380 the bay level averaged 59%, and was greater than the value expected by chance ( $\kappa = 0.48$ ). Port  
381 Dick had the largest percentage of specimens assigned correctly in the cross-validation (95%),

382 followed by Islas Bay (75%) (Table 2). Classification success was lower for Kiliuda Bay, Izhut  
383 Bay, and Salisbury Sound (Table 2), indicating some degree of overall similarity among these  
384 bays in the elemental ratios measured.

385 The five elements (B, Mg, Mn, Sr, and Ba) for which significant differences were found  
386 between the western and eastern GOA were used to create the classification functions for  
387 discriminating between the two regions. The initial variables Sr:Ca and Ba:Ca had the greatest  
388 relative contribution to the discriminant functions. Classification accuracy was higher at the  
389 regional level than at the bay level, with an overall success rate of 78% (Table 2), greater than  
390 what could be ascribed to chance alone ( $\kappa = 0.50$ ).

391

#### 392 **4. Discussion**

393

394 Our results suggest the potential for identifying juvenile sources of Pacific cod in the  
395 GOA based on otolith elemental signatures, while recognizing some of the challenges associated  
396 with using this approach to understand connections between nursery areas and the wider GOA  
397 Pacific cod population. Significant spatial and temporal variation in otolith microchemistry was  
398 observed, although the ability to correctly classify juveniles to their sources varied according to  
399 spatial scale. Discriminant analysis based on otolith elemental signatures revealed high  
400 classification accuracy at the regional scale (78%). However, while significant differences in  
401 otolith elemental signatures were found among individual embayments within the GOA, the  
402 overall rate of accurate classification to collection bay was only 59%. Our results are similar to  
403 those of Stanley et al. (2016), who had improved classification success at larger spatial scales for  
404 juvenile Atlantic cod (*G. morhua*) in coastal Newfoundland. Indeed, as suggested by Stanley et  
405 al. (2016), fine-scale variation caused by localized environmental conditions could hinder  
406 assignments to individual nursery embayments.

407 Classification accuracy varied markedly among bays, with the highest rates of success for  
408 Port Dick (95%) and Islas Bay (75%). Conversely, discriminatory power was relatively low for  
409 Izhut Bay (52%), Kiliuda Bay (44%), and especially Salisbury Sound (30%). (Due to the  
410 elimination of outliers from our sample set, true error rates may be slightly higher.) Certain  
411 embayments may have hydrological or chemical features which impart unique elemental  
412 signatures in Pacific cod otoliths. Otoliths from Port Dick had the most distinct signature, which

413 appeared to be driven by extremely low ratios of Ba:Ca. Of the bays analyzed, Port Dick is the  
414 only one with direct glacial influence. The Port Dick watershed includes part of the Grewingk-  
415 Yalik Glacier Complex, a ~400 km<sup>2</sup> icefield formed approximately 23,000 years ago during the  
416 Pleistocene Epoch (Hall et al., 2005; Zimmerman et al., 2016). Melting of this icefield has  
417 accelerated since 1950 (VanLooy et al., 2006), and chemical weathering in glaciers affects the  
418 elemental composition of freshwater runoff (Anderson et al., 1997). Indeed, Port Dick also had  
419 some of the lowest salinity values recorded of the bays analyzed. Typically, Ba:Ca ratios in  
420 nearshore marine carbonates display a negative relationship with salinity and can act as a proxy  
421 for coastal freshwater runoff (Chan et al., 2011). However, we detected a significant positive  
422 relationship between Ba:Ca and salinity, similar to Hetzinger et al. (2013), who concluded that  
423 low Ba:Ca ratios in coralline algae (coinciding with low salinities) in the coastal Northwest  
424 Atlantic were due to increased ice melt-driven stratification, resulting in less advection of  
425 barium-enriched deep water to the surface. There is some evidence that iron and manganese  
426 oxyhydrides may induce barium removal through coprecipitation, though this typically occurs at  
427 the interface between oxic seawater and highly saline anoxic waters in the deep ocean (De Lange  
428 et al., 1990). Elevated manganese levels have been reported in the surface sediments of glacially  
429 fed fjords, especially in their inner regions (Wehrmann et al., 2014), which could explain the  
430 relatively high Mn:Ca otolith ratios observed in the Taylor site in Port Dick, and possibly also  
431 the lower Ba:Ca as higher levels of dissolved Mn<sup>2+</sup> would be available to sequester free Ba<sup>2+</sup>  
432 ions. In contrast, Islas Bay, the other embayment with a distinctive signature, has no glacial  
433 influence. However, Islas Bay is relatively small, shallow, and exposed to the open waters of the  
434 eastern GOA shelf, and appears to have hydrographic characteristics similar to those in the open  
435 ocean (Ormseth et al., 2017). The basis for the comparative lack of differentiation among the  
436 remaining three embayments is not readily apparent but may be related to oceanographic  
437 characteristics. Kiliuda Bay, a large shallow embayment, is physically and biologically  
438 heterogeneous, with a sill separating its inner and outer areas. Izhut Bay is relatively open, with a  
439 deep central section and multiple small coves along its coastline. Salisbury Sound is not a closed  
440 embayment, instead connecting to the waters of the Inside Passage in southeast Alaska (Ormseth  
441 et al., 2017). These three embayments share two features that are in contrast to Port Dick and  
442 Islas Bay: none are influenced by glaciers, and all appear to have reduced exchange with GOA  
443 shelf waters.

444           The distinction between otolith elemental signatures at the regional level likely also  
445 resulted from differences in physical processes. The eastern and western regions of the GOA are  
446 quite different from each other in their oceanography, which could explain why classification  
447 accuracy improved at these larger spatial scales. The rugged mountainous coastline of the GOA  
448 is highly articulated and experiences significant amounts of precipitation and glacial runoff,  
449 which is pronounced in the southeast region. Further, regional differences in temperature,  
450 seasonal upwelling, and riverine inputs could have also impacted the observed signatures. The  
451 higher classification success at the regional level was likely driven in part by the distinct  
452 signatures observed in Port Dick (in the western GOA) and Islas Bay (in the eastern GOA),  
453 highlighting the need for additional future sampling within these large regions to verify their  
454 differences.

455           In addition to the elemental composition of the ambient water, the uptake and  
456 incorporation of trace elements into fish otoliths is also influenced by physiological regulation  
457 and environmental conditions (see reviews by Campana, 1999 and Elsdon et al., 2008).  
458 Temperature and salinity can affect the incorporation of Sr and Ba, although these effects appear  
459 to vary widely among species, and interactions among temperature, salinity, and ambient  
460 elemental concentration may also affect uptake rates (Collingsworth et al., 2010; de Vries et al.,  
461 2005; Elsdon and Gillanders, 2003, 2004, 2005; Izzo et al., 2017; Miller, 2011; Stanley et al.,  
462 2015). Ion uptake may be further mediated by physiological biokinetic processes and growth  
463 rate, particularly in the case of Mg:Ca, Mn:Ca, and Sr:Ca, though it can be difficult to tease apart  
464 these effects from environmental influences on elemental incorporation (Sturrock et al., 2015).  
465 Seasonal variations in blood plasma chemistry, which is under strict physiological control, can  
466 influence otolith elemental composition; physiological effects on ion transport can be moderated  
467 by environmental factors such as temperature and salinity (Sturrock et al., 2015; Sturrock et al.,  
468 2014). The seasonal trends that we observed likely highlight the potential influence of temporally  
469 varying water chemistry characteristics, other environmental influences, or covarying  
470 physiological factors on otolith elemental signatures. Significant differences were detected  
471 between summer and fall for all ratios except Pb:Ca. With the exception of Ba:Ca and Mn:Ca,  
472 seasonal trends in elemental ratios were similar in Port Dick and Kiliuda Bay. All otolith  
473 elemental ratios (except Pb:Ca) had significant relationships with temperature, and all elemental  
474 ratios (except Pb:Ca and B:Ca) had significant relationships with salinity. In a laboratory study



475 of larval Pacific cod, DiMaria et al. (2010) found significant effects of temperature on the  
476 incorporation of both Sr and Ba, although interestingly, they did not observe any temperature  
477 effect on incorporation of Mg. The results of the present study suggest effects of fish size on  
478 certain elemental ratios. However, interactions between site and length (with the exception of  
479 B:Ca) indicate that these effects may be due to spatial variation among sites rather than length  
480 differences. Though the interaction between site and length was not statistically significant for  
481 B:Ca, the relationships between length and this elemental ratio within sites were both positive  
482 and negative in direction. Additionally, DiMaria et al. (2010) did not observe any effects of  
483 somatic growth rate or otolith precipitation rate on incorporation of trace elements into otoliths  
484 of Pacific cod larvae.

485         These results illustrate the limitation of expanding our approach to tracking dispersal and  
486 recruitment in GOA Pacific cod and other nearshore coastal inhabitants. While every effort was  
487 made to include specimens from representative nearshore habitats, because of the enormous  
488 spatial scale of the GOA and the large number of shallow coastal habitats utilized by this species,  
489 it would be logistically impractical to sample all potential nursery areas. As a result, bay-specific  
490 signatures identified in this study may not be unique in the context of the entire GOA. The  
491 temporal differences observed in the bays with samples available from both seasons (Port Dick  
492 and Kiliuda Bay) demonstrated that otolith elemental composition is capable of large changes  
493 over relatively short (~2 month) timescales. Unfortunately, the fact that fish were not captured in  
494 all bays in both seasons limited our ability to more comprehensively compare the relative  
495 importance of temporal variation within bays to the spatial variation among bays. The  
496 unavailability of samples from all bays in both seasons also necessitated supplementing the  
497 analysis of edge signatures with analysis of synchronous periods among the life history transects.  
498 If “checks” associated with life history events (e.g., a settlement check) had been present within  
499 the otolith microstructure, we could have used them as a reference point to identify synchronous  
500 periods in otolith transects. However, in this study and in Miller et al. (2016), no such checks in  
501 Pacific cod otoliths could be identified. Therefore, we used counts of otolith daily rings to  
502 identify and evaluate contemporaneous sections of the life history transect. Our spatial  
503 comparison of elemental signatures was based on a relatively short portion of the life history  
504 transect corresponding to early July for two reasons: using elemental ratios pre-July could  
505 potentially result in the inclusion of pre-settlement signatures (e.g., not representative of nursery

506 habitats), and using elemental ratios post-July would result in a large reduction in sample size,  
507 particularly with respect to the eastern GOA. An exploratory analysis was performed using a  
508 larger integration (early July to the otolith edge) for all fall-collected fish. Compared to the early  
509 July signatures for just the fall-collected fish, this longer integration resulted in higher  
510 discriminatory power at the bay level (from 54% to 62% accuracy), but reduced power at the  
511 larger regional level (from 80% to 75% accuracy), likely due to the much smaller sample size  
512 and representation of only one bay in the eastern GOA (data not shown). While a larger  
513 integration would be desirable, making it easier to isolate the same period in adult fish to track  
514 them back to nursery habitats, this was not advisable given our sampling limitations.

515         The ability to accurately classify young-of-year Pacific cod to their nursery habitats is  
516 necessary in order to use otolith elemental signatures to link juvenile sources to the adult  
517 offshore population. In other studies, fish otolith chemistry has successfully been used to  
518 differentiate among juvenile fish in both nearshore estuarine habitats and the open ocean  
519 (Anstead et al., 2015; Clarke et al., 2009; Miller et al., 2016), as well as to describe connectivity  
520 between life stages (Anstead et al., 2016; Gillanders, 2002; Walther et al., 2008). However, our  
521 ability to classify juvenile Pacific cod to individual embayments varied markedly, and  
522 conceivably, other unsampled nursery habitats in the GOA could impart similar signatures to our  
523 collection locations, making the application of this approach challenging at smaller spatial scales.  
524 Indeed, the extremely heterogeneous and complex nature of nearshore habitats within the GOA  
525 may preclude such applications, as the cost and labor associated with complete sampling of  
526 embayments would be prohibitive, even if unique signatures could be identified for each  
527 embayment. Nonetheless, the otolith microchemistry approach may still have promise in terms of  
528 differentiating between adults originating from the eastern and western regions of the GOA.  
529 Further work could explore the inclusion of factors such as otolith stable oxygen isotopes to  
530 improve discriminatory power at smaller spatial scales.

531         Genetic studies have found that population subdivision exists across the extended range  
532 of Pacific cod due to geographic barriers and isolation by distance (Canino et al., 2010;  
533 Cunningham et al., 2009; Spies, 2012), and consequently, Pacific cod is currently managed as  
534 three separate stocks in Alaskan waters: the GOA, EBS, and Aleutian Islands (AI). Tagging  
535 studies have shown that adult Pacific cod undertake seasonal migrations within the EBS for the  
536 purposes of spawning and feeding, and that some individuals move between all three regions

537 (Rand et al., 2014; Shimada and Kimura, 1994). There is no evidence for multiple stocks within  
538 the GOA, although our knowledge of the degree of population structure within the GOA itself is  
539 limited. However, the present study suggests that it is possible to discriminate among juvenile  
540 Pacific cod at gross scales within the GOA, which could be used to determine the relative  
541 contributions of the eastern and western GOA to the adult stock. Ultimately, understanding the  
542 contribution of juvenile sources to the adult population on a broad regional scale could elucidate  
543 patterns of connectivity of Pacific cod within the GOA, and would be informative to  
544 management of this commercially important species.

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560

561 **References**

- 562 Abookire, A.A., Duffy-Anderson, J.T., Jump, C.M., 2007. Habitat associations and diet of  
563 young-of-the-year Pacific cod (*Gadus macrocephalus*) near Kodiak, Alaska. *Mar. Biol.*  
564 150, 713-726.
- 565 Anderson, S.P., Drever, J.I., Humphrey, N.F., 1997. Chemical weathering in glacial  
566 environments. *Geology* 25, 399-402.
- 567 Anstead, K.A., Schaffler, J.J., Jones, C.M., 2015. Coastwide otolith signatures of juvenile  
568 Atlantic menhaden, 2009-2011. *Trans. Am. Fish. Soc.* 144, 96-106.
- 569 Anstead, K.A., Schaffler, J.J., Jones, C.M., 2016. Coast-wide nursery contribution of new  
570 recruits to the population of Atlantic menhaden. *Trans. Am. Fish. Soc.* 145, 627-636.
- 571 Barbee, N.C., Swearer, S.E., 2007. Characterizing natal source population signatures in the  
572 diadromous fish *Galaxias maculatus*, using embryonic otolith chemistry. *Mar. Ecol.*  
573 *Prog. Ser.* 343, 273-282.
- 574 Blackburn, J.E., Jackson, P.B., 1982. Seasonal composition and abundance of juvenile and adult  
575 marine finfish and crab species in the nearshore zone of Kodiak Island's eastside during  
576 April 1978 through March 1979. Alaska Department of Fish and Game Final Report 03-  
577 5-022-69, Kodiak, AK, pp. 377-570.
- 578 Bograd, S.J., Mendelssohn, R., Schwing, F.B., Miller, A.J., 2005. Spatial heterogeneity of sea  
579 surface temperature trends in the Gulf of Alaska. *Atmosphere-Ocean* 43, 241-247.
- 580 Campana, S.E., 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and  
581 applications. *Mar. Ecol. Prog. Ser.* 188, 263-297.
- 582 Campana, S.E., 2004. Otolith elemental composition as a natural marker of fish stocks, in:  
583 Cadrin, S.X., Friedland, K.D., Waldman, J.R. (Eds.), *Stock Identification Methods:*  
584 *Applications in Fishery Science.* Elsevier Academic Press, Amsterdam, pp. 227-245.
- 585 Campana, S.E., Chouinard, G.A., Hanson, J.M., Frechet, A., Bratley, J., 2000. Otolith elemental  
586 fingerprints as biological tracers of fish stocks. *Fish. Res.* 46, 343-357.
- 587 Campana, S.E., Thorrold, S.R., 2001. Otoliths, increments, and elements: keys to a  
588 comprehensive understanding of fish populations? *Can. J. Fish. Aquat. Sci.* 58, 30-38.
- 589 Canino, M.F., Spies, I.B., Cunningham, K.M., Hauser, L., Grant, W.S., 2010. Multiple ice-age  
590 refugia in Pacific cod, *Gadus macrocephalus*. *Mol Ecol* 19, 4339-4351.
- 591 Chan, P., Halfar, J., Williams, B., Hetzinger, S., Steneck, R., Zack, T., Jacob, D.E., 2011.  
592 Freshening of the Alaska Coastal Current recorded by coralline algal Ba/Ca ratios. *J.*  
593 *Geophys. Res.* 116.
- 594 Clarke, L.M., Walther, B.D., Munch, S.B., Thorrold, S.R., Conover, D.O., 2009. Chemical  
595 signatures in the otoliths of a coastal marine fish, *Menidia menidia*, from the northeastern  
596 United States: spatial and temporal differences. *Mar. Ecol. Prog. Ser.* 384, 261-271.
- 597 Cohen, J., 1960. A coefficient of agreement for nominal scales. *Educ. Psychol. Meas.* 20, 37-46.
- 598 Collingsworth, P.D., Van Tassell, J.J., Olesik, J.W., Marschall, E.A., 2010. Effects of  
599 temperature and elemental concentration on the chemical composition of juvenile yellow  
600 perch (*Perca flavescens*) otoliths. *Can. J. Fish. Aquat. Sci.* 67, 1187-1196.
- 601 Cooney, T., 2007. The marine production cycle, in: Spies, R.B. (Ed.), *Long-term Ecological*  
602 *Change in the Northern Gulf of Alaska.* Elsevier, Amsterdam, pp. 47-60.
- 603 Cunningham, K.M., Canino, M.F., Spies, I.B., Hauser, L., 2009. Genetic isolation by distance  
604 and localized fjord population structure in Pacific cod (*Gadus macrocephalus*): limited  
605 effective dispersal in the northeastern Pacific Ocean. *Can. J. Fish. Aquat. Sci.* 66, 153-  
606 166.

- 607 De Lange, G.J., Catalano, G., Klinkhammer, G.P., Luther, G.W., 1990. The interface between  
608 oxic seawater and the anoxic Bannock brine; its sharpness and the consequences for the  
609 redox-related cycling of Mn and Ba. *Mar. Chem.* 31, 205-217.
- 610 de Vries, M.C., Gillanders, B.M., Elsdon, T.S., 2005. Facilitation of barium uptake into fish  
611 otoliths: influence of strontium concentration and salinity. *Geochim. Cosmochim. Acta*  
612 69, 4061-4072.
- 613 DiMaria, R.A., 2011. Natal source contributions of Pacific cod (*Gadus macrocephalus*) recruits  
614 in the southeastern Bering Sea. Masters Thesis, Oregon State University, Corvallis, OR,  
615 123 p.
- 616 DiMaria, R.A., Miller, J.A., Hurst, T.P., 2010. Temperature and growth effects on otolith  
617 elemental chemistry of larval Pacific cod, *Gadus macrocephalus*. *Environ. Biol. Fish.* 89,  
618 453-462.
- 619 Doyle, M.J., Picquello, S.J., Mier, K.L., Spillane, M.C., Bond, N.A., 2009. Larval fish abundance  
620 and physical forcing in the Gulf of Alaska, 1981-2003. *Prog. Oceanogr.* 80, 163-187.
- 621 Elsdon, T.S., Gillanders, B.M., 2003. Reconstructing migratory patterns of fish based on  
622 environmental influences on otolith chemistry. *Rev. Fish Biol. Fish.* 13, 219-235.
- 623 Elsdon, T.S., Gillanders, B.M., 2004. Fish otolith chemistry influenced by exposure to multiple  
624 environmental variables. *J. Exp. Mar. Biol. Ecol.* 313, 269-284.
- 625 Elsdon, T.S., Gillanders, B.M., 2005. Alternative life-history patterns of estuarine fish: barium in  
626 otoliths elucidates freshwater residency. *Can. J. Fish. Aquat. Sci.* 62, 1143-1152.
- 627 Elsdon, T.S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C.M., Limburg, K.E., Secor,  
628 D.H., Thorrold, S.R., Walther, B.D., 2008. Otolith chemistry to describe movements and  
629 life-history parameters of fishes: hypotheses, assumptions, limitations and inferences.  
630 *Oceanogr. Mar. Biol.* 46, 297-330.
- 631 Gillanders, B.M., 2002. Connectivity between juvenile and adult fish populations: do adults  
632 remain near their recruitment estuaries? *Mar. Ecol. Prog. Ser.* 240, 215-223.
- 633 Gillanders, B.M., Kingsford, M.J., 2000. Elemental fingerprints of otoliths of fish may  
634 distinguish estuarine 'nursery' habitats. *Mar. Ecol. Prog. Ser.* 201, 273-286.
- 635 Hall, D.K., Giffen, B.A., Chien, J.Y.L., 2005. Changes in the Harding Icefield and the  
636 Grewingk-Yalik Glacier Complex, 62nd Eastern Snow Conference, Waterloo, ON,  
637 Canada.
- 638 Hetzinger, S., Halfar, J., Zack, T., Mecking, J.V., Kunz, B.E., Jacob, D.E., Adey, W.H., 2013.  
639 Coralline algal barium as indicator for 20th century northwestern North Atlantic surface  
640 ocean freshwater variability. *Sci. Rep.* 3, 1-8.
- 641 Hurst, T.P., Cooper, D.W., Duffy-Anderson, J.T., Farley, E.V., 2014. Contrasting coastal and  
642 shelf nursery habitats of Pacific cod in the southeastern Bering Sea. *ICES J. Mar. Sci.* 72,  
643 515-527.
- 644 Hurst, T.P., Cooper, D.W., Scheingross, J.S., Seale, E.M., Laurel, B.J., Spencer, M.L., 2009.  
645 Effects of ontogeny, temperature, and light on vertical movements of larval Pacific cod  
646 (*Gadus macrocephalus*). *Fish. Oceanogr.* 18, 301-311.
- 647 Izzo, C., Doubleday, Z.A., Grammer, G.L., Disspain, M.C.F., Ye, Q., Gillanders, B.M., 2017.  
648 Seasonally resolved environmental reconstructions using fish otoliths. *Can. J. Fish.*  
649 *Aquat. Sci.* 74, 23-31.
- 650 Jochum, K.P., Weis, U., Stoll, B., Kuzmin, D., Yang, Q.C., Raczek, I., Jacob, D.E., Stracke, A.,  
651 Birbaum, K., Frick, D.A., Gunther, D., Enzweiler, J., 2011. Determination of reference

- 652 values for NIST SRM 610-617 glasses following ISO guidelines. *Geostand. Geoanal.*  
653 *Res.* 35, 397-429.
- 654 Johnson, S.W., Neff, A.D., Thedinga, J.F., Lindeberg, M.R., Maselko, J.M., 2012. Atlas of  
655 nearshore fishes of Alaska: a synthesis of marine surveys from 1998 to 2011. NOAA  
656 Tech. Memo., 261 p.
- 657 Kent, A.J.R., Ungerer, C.A.A., 2006. Analysis of light lithophile elements (Li, Be, B) by laser  
658 ablation ICP-MS: comparison between magnetic sector and quadrupole ICP-MS. *Am.*  
659 *Mineral.* 91, 1401-1411.
- 660 Laurel, B.J., Knoth, B.A., Ryer, C.H., 2016. Growth, mortality, and recruitment signals in age-0  
661 gadids settling in coastal Gulf of Alaska. *ICES J. Mar. Sci.* 73, 2227-2237.
- 662 Laurel, B.J., Ryer, C.H., Knoth, B., Stoner, A.W., 2009. Temporal and ontogenetic shifts in  
663 habitat use of juvenile Pacific cod (*Gadus macrocephalus*). *J. Exp. Mar. Biol. Ecol.* 377,  
664 28-35.
- 665 Laurel, J., Stoner, A.W., Ryer, C.H., Hurst, T.P., Abookire, A.A., 2007. Comparative habitat  
666 associations in juvenile Pacific cod and other gadids using seines, baited cameras and  
667 laboratory techniques. *J. Exp. Mar. Biol. Ecol.* 351, 42-55.
- 668 Linehan, J.E., 2001. Predation risk of age-0 cod (*Gadus*) relative to depth and substrate in coastal  
669 waters. *J. Exp. Mar. Biol. Ecol.* 263, 25-44.
- 670 McCune, B., Grace, J.B., 2002. Data transformations, *Analysis of Ecological Communities.*  
671 MjM Software Design, Gleneden Beach, OR, pp. 67-79.
- 672 McGarigal, K., Cushman, S., Stafford, S., 2000. *Multivariate statistics for wildlife and ecology*  
673 *research.* Springer, New York.
- 674 Miller, J.A., 2011. Effects of water temperature and barium concentration on otolith composition  
675 along a salinity gradient: implications for migratory reconstructions. *J. Exp. Mar. Biol.*  
676 *Ecol.* 405, 42-52.
- 677 Miller, J.A., DiMaria, R.A., Hurst, T.P., 2016. Patterns of larval source distribution and mixing  
678 in early life stages of Pacific cod (*Gadus macrocephalus*) in the southeastern Bering Sea.  
679 *Deep-Sea Res. Pt. II* 134, 270-282.
- 680 Mueter, F.J., Norcross, B.L., 2002. Spatial and temporal patterns in the demersal fish community  
681 on the shelf and upper slope regions of the Gulf of Alaska. *Fish. Bull.* 100, 559-581.
- 682 Mundy, P.R., 2005. *The Gulf of Alaska: Biology and Oceanography.* University of Alaska  
683 Fairbanks, Alaska Sea Grant College Program, Fairbanks, AK.
- 684 Narimatsu, Y., Hattori, T., Ueda, Y., Matsuzaka, H., Shiogaki, M., 2007. Somatic growth and  
685 otolith microstructure of larval and juvenile Pacific cod *Gadus macrocephalus*. *Fish. Sci.*  
686 73, 1257-1264.
- 687 Ormseth, O.A., Rand, K.M., DeRobertis, A., 2017. Fishes and invertebrates in Gulf of Alaska  
688 bays and islands: Results from inshore ecosystem surveys in 2011 and 2013. NOAA  
689 Tech. Memo., 140 p.
- 690 Pearce, N.J.G., Perkins, W.T., Westgate, J.A., Gorton, M.P., Jackson, S.E., Neal, C.R., Chenery,  
691 S.P., 1997. A compilation of new and published major and trace element data for NIST  
692 SRM 610 and NIST SRM 612 glass reference materials. *Geostandards Newsletter* 21,  
693 115-144.
- 694 R Core Team, 2016. *R: A language and environment for statistical computing.* R Foundation for  
695 Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

- 696 Rand, K.M., Munro, P., Neidetcher, S.K., Nichol, D.G., 2014. Observations of seasonal  
697 movement from a single tag release group of Pacific cod in the eastern Bering Sea. *Mar.*  
698 *Coast. Fish.* 6, 287-296.
- 699 Rugen, W.C., Matarese, A.C., 1988. Spatial and temporal distribution and relative abundance of  
700 Pacific cod (*Gadus macrocephalus*) larvae in the western Gulf of Alaska. U.S. Dep.  
701 Commer., NWAFC Processed Report 88-18. 53 p.
- 702 Shimada, A.M., Kimura, D.K., 1994. Seasonal movements of Pacific cod, *Gadus*  
703 *macrocephalus*, in the eastern Bering Sea and adjacent waters based on tag-recapture  
704 data. *Fish. Bull.* 92, 800-816.
- 705 Spies, I., 2012. Landscape genetics reveals population subdivision in Bering Sea and Aleutian  
706 Islands Pacific cod. *Trans. Am. Fish. Soc.* 141, 1557-1573.
- 707 Spies, R.B., 2007. Long-term ecological change in the northern Gulf of Alaska. Elsevier,  
708 Amsterdam.
- 709 Stabeno, P.J., Bond, N.A., Hermann, A.J., Kachel, N.B., Mordy, C.W., Overland, J.E., 2004.  
710 Meteorology and oceanography of the Northern Gulf of Alaska. *Cont. Shelf Res.* 24, 859-  
711 897.
- 712 Stanley, R.R.E., Bradbury, I.R., DiBacco, C., Snelgrove, P.V.R., Thorrold, S.R., Killen, S.S.,  
713 2015. Environmentally mediated trends in otolith composition of juvenile Atlantic cod  
714 (*Gadus morhua*). *ICES J. Mar. Sci.* 72, 2350-2363.
- 715 Stanley, R.R.E., DiBacco, C., Thorrold, S.R., Snelgrove, P.V.R., Morris, C.J., Gregory, R.S.,  
716 Campana, S.E., Bradbury, I.R., 2016. Regional variation in otolith geochemistry of  
717 juvenile Atlantic cod (*Gadus morhua*) in coastal Newfoundland. *Can. J. Fish. Aquat. Sci.*  
718 73, 1507-1519.
- 719 Stoner, A.W., Laurel, B.J., Hurst, T.P., 2008. Using a baited camera to assess relative abundance  
720 of juvenile Pacific cod: field and laboratory trials. *J. Exp. Mar. Biol. Ecol.* 354, 202-211.
- 721 Sturrock, A.M., Hunter, E., Milton, J.A., Johnson, R.C., Waring, C.P., Trueman, C.N., Leder, E.,  
722 2015. Quantifying physiological influences on otolith microchemistry. *Methods Ecol.*  
723 *Evol.* 6, 806-816.
- 724 Sturrock, A.M., Trueman, C.N., Milton, J.A., Waring, C.P., Cooper, M.J., Hunter, E., 2014.  
725 Physiological influences can outweigh environmental signals in otolith microchemistry  
726 research. *Mar. Ecol. Prog. Ser.* 500, 245-264.
- 727 VanLooy, J., Forster, R., Ford, A., 2006. Accelerating thinning of Kenai Peninsula glaciers,  
728 Alaska. *Geophys. Res. Lett.* 33.
- 729 Waite, J.N., Mueter, F.J., 2013. Spatial and temporal variability of chlorophyll-a concentrations  
730 in the coastal Gulf of Alaska, 1998-2011, using cloud-free reconstructions of SeaWiFS  
731 and MODIS-Aqua data. *Prog. Oceanogr.* 116, 179-192.
- 732 Walther, B.D., Thorrold, S.R., Olney, J.E., 2008. Geochemical signatures in otoliths record natal  
733 origins of American shad. *Trans. Am. Fish. Soc.* 137, 57-69.
- 734 Wehrmann, L.M., Formolo, M.J., Owens, J.D., Raiswell, R., Ferdelman, T.G., Riedinger, N.,  
735 Lyons, T.W., 2014. Iron and manganese speciation and cycling in glacially influenced  
736 high-latitude fjord sediments (West Spitsbergen, Svalbard): Evidence for a benthic  
737 recycling-transport mechanism. *Geochim. Cosmochim. Acta* 141, 628-655.
- 738 Weingartner, T., 2007. The physical environment of the Gulf of Alaska, in: Spies, R.B. (Ed.),  
739 Long-term Ecological Change in the Northern Gulf of Alaska. Elsevier, Amsterdam, pp.  
740 12-47.
- 741 Zar, J.H., 1999. Biostatistical Analysis, 4th ed. Prentice Hall, Upper Saddle River, NJ.



742 Zimmerman, M., Reid, J.A., Golden, N.E., 2016. Using smooth sheets to describe groundfish  
743 habitat in Alaskan waters, with specific application to two flatfishes. *Deep-Sea Res. Pt. II*  
744 132, 210-226.

745

746 **Table 1.** Region, bay, site, date, number of seine sets within each site, numbers of Pacific cod  
 747 (*Gadus macrocephalus*) otoliths collected, and fish fork lengths (FL; range and mean  $\pm$  1 SD).

Region	Bay	Site	Season	Collection date	n sets	n otoliths	FL range (mm)	Mean FL (mm)
Western	Kiliuda Bay	Dungie	summer	Aug 7	2	52	53-88	67 ( $\pm$ 9)
			summer	Aug 8	1	11	56-105	85 ( $\pm$ 19)
			fall	Oct 17	1	19	80-125	99 ( $\pm$ 14)
		Shearwater	summer	Aug 8	2	27	55-85	68 ( $\pm$ 10)
			fall	Oct 16-17	2	28	78-115	94 ( $\pm$ 8)
			Port Dick	Swan	summer	Aug 13	2	45
	Taylor	summer		Aug 12	1	22	60-125	86 ( $\pm$ 18)
	Waterfall	fall		Oct 26	2	19	92-124	103 ( $\pm$ 9)
	Izhut Bay	Kitoi		fall	Oct 21	1	21	89-135
		Saposa	fall	Oct 21	1	15	81-103	91 ( $\pm$ 6)
Eastern	Islas Bay	Fjordselheim	summer	Jul 15	2	36	52-86	66 ( $\pm$ 8)
			summer	Jul 14	1	25	50-77	66 ( $\pm$ 7)
			summer	Jul 14	2	19	52-86	63 ( $\pm$ 9)
	Salisbury Sound	Kane	fall	Sep 22	1	24	87-144	116 ( $\pm$ 15)

748 **Table 2.** Results of quadratic discriminant analysis (DA) for age-0 Pacific cod (*Gadus*  
 749 *macrocephalus*) collected from the Gulf of Alaska, based on otolith elemental early July  
 750 signatures. Correct classifications (indicated by bold italics) and misclassifications to region  
 751 (western and eastern GOA) and bay (arranged west to east: KB = Kiliuda Bay, IZ = Izhut Bay,  
 752 PD = Port Dick, IB = Islas Bay, SS = Salisbury Sound) from separate jack-knife cross-validation  
 753 procedures are shown in terms of numbers of fish, with total percentage correctly classified.

754

		<i>Region-level DA</i>		<i>predicted</i>		
		Western	Eastern	Western	Eastern	% correct
<i>observed</i>	Western	<b>192</b>			56	77
	Eastern		21		<b>75</b>	78

		<i>Bay-level DA</i>					<i>predicted</i>
		KB	IZ	PD	IB	SS	
<i>observed</i>	KB	<b>57</b>	31	1	13	27	44
	IZ	8	<b>17</b>	--	3	5	52
	PD	2	--	<b>82</b>	2	--	95
	IB	6	4	1	<b>55</b>	7	75
	SS	7	7	--	2	<b>7</b>	30

755

756

757 **Supplementary Table 1.** Values of temperature (°C), salinity, and dissolved oxygen (DO;  
 758  $\mu\text{mol/kg}$ ) measured at 5 m depth in each bay within 1-2 days of fish collection (mean  $\pm$  1 SD in  
 759 bays where multiple CTD casts were taken). See Figure 1 for CTD cast locations.

	Temperature	Salinity	DO
<i>Summer</i>			
Kiliuda Bay	10.55 ( $\pm$ 0.14)	32.16 ( $\pm$ 0.21)	261.24 ( $\pm$ 7.91)
Port Dick	12.28 ( $\pm$ 0.17)	29.55 ( $\pm$ 0.13)	295.63 ( $\pm$ 13.26)
Islas Bay	12.26	31.88	281.02
<i>Fall</i>			
Kiliuda Bay	7.81 ( $\pm$ 0.01)	31.81 ( $\pm$ 0.03)	262.51 ( $\pm$ 5.12)
Izhut Bay	7.59 ( $\pm$ 0.05)	31.98 ( $\pm$ 0.04)	248.58 ( $\pm$ 16.38)
Port Dick	8.58	28.09	251.95
Salisbury Sound	10.72	29.64	214.44

760

761

762 **Supplementary Table 2.** Results of linear regressions between elemental ratios measured at the  
 763 outer edges of age-0 Pacific cod (*Gadus macrocephalus*) otoliths and environmental variables  
 764 (temperature, °C; salinity; dissolved oxygen, DO, µmol/kg) measured near time of capture at 5 m  
 765 depth.

Elemental ratio	Variable	$R^2$	$F_{1,346}$	$P$ value	$r$
B:Ca	Temp	0.15	59.64	<0.001	0.38
Mg:Ca	Temp	0.43	255.67	<0.001	0.65
Mn:Ca	Temp	0.02	7.33	0.007	0.14
Zn:Ca	Temp	0.07	25.17	<0.001	0.26
Sr:Ca	Temp	0.39	225.20	<0.001	-0.63
Ba:Ca	Temp	0.04	14.16	<0.001	0.20
Pb:Ca	Temp	<0.01	0.07	0.798	0.01
B:Ca	Salinity	0.01	2.52	0.114	0.09
Mg:Ca	Salinity	0.07	24.01	<0.001	0.26
Mn:Ca	Salinity	0.09	34.97	<0.001	-0.30
Zn:Ca	Salinity	0.03	12.46	<0.001	0.19
Sr:Ca	Salinity	0.13	53.13	<0.001	-0.37
Ba:Ca	Salinity	0.25	113.24	<0.001	0.50
Pb:Ca	Salinity	<0.01	0.01	0.919	-0.01
B:Ca	DO	0.13	52.25	<0.001	0.36
Mg:Ca	DO	0.06	21.90	<0.001	0.24
Mn:Ca	DO	<0.01	0.00	0.983	<0.01
Zn:Ca	DO	0.01	3.59	0.059	0.10
Sr:Ca	DO	0.12	45.60	<0.001	-0.34
Ba:Ca	DO	<0.01	0.12	0.733	0.02
Pb:Ca	DO	<0.01	0.17	0.681	-0.02

766

767 **Figure Captions**

768 **Fig. 1.** Pacific cod (*Gadus macrocephalus*) collection areas in the Gulf of Alaska. Top left panel  
769 shows bays (bull's eye symbols) within larger regions of interest; remaining panels show  
770 sampling locations in each bay with respect to site. Circles represent summer collection  
771 locations, triangles represent fall collection locations, and stars represent locations where fish  
772 were collected in both seasons. Crosshair symbols represent CTD casts that were closest to each  
773 fish collection location.

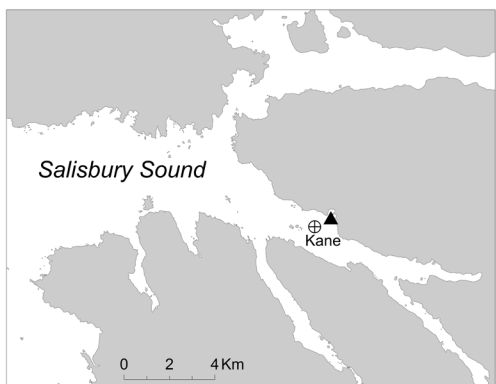
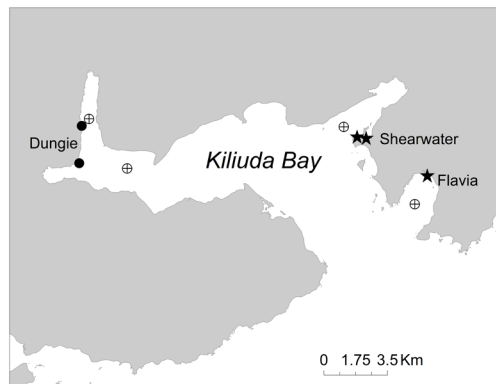
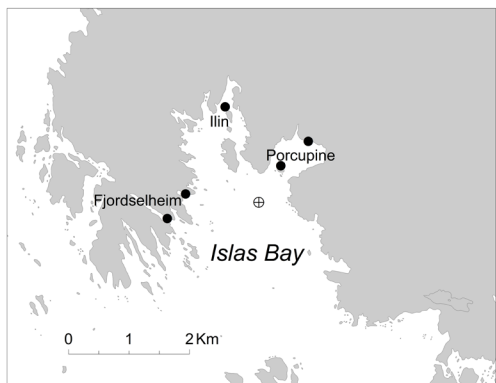
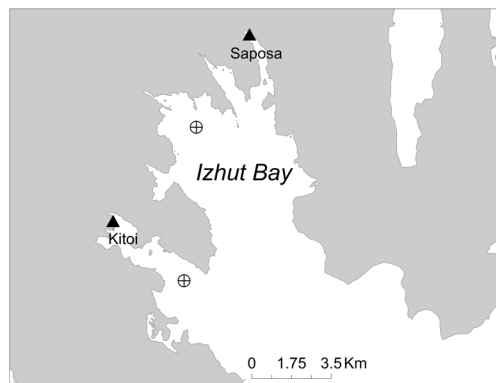
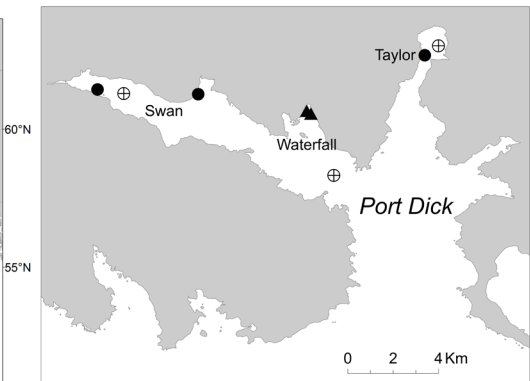
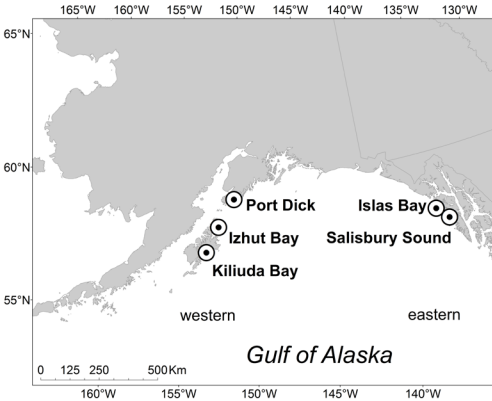
774 **Fig. 2.** Laser tracks measured for trace element composition in juvenile Pacific cod (*Gadus*  
775 *macrocephalus*) otoliths.

776 **Fig. 3.** Boxplots showing elemental ratios measured from the early July segment of the life  
777 history transects of age-0 Pacific cod (*Gadus macrocephalus*) otoliths. Horizontal bars indicate  
778 median values for each site, the top and bottom of each box represent the 25th and 75th  
779 percentiles, respectively, and hatched lines represent values within 1.5 times the interquartile  
780 range. Circles represent values greater than 1.5 times the interquartile range. Sites are arranged  
781 from west to east and bays are indicated by the following abbreviations and colors: KB = Kiliuda  
782 Bay (yellow), IZ = Izhut Bay (blue), PD = Port Dick (green), IB = Islas Bay (orange), SS =  
783 Salisbury Sound (red). Regions (western and eastern Gulf of Alaska) are divided by the vertical  
784 bars.

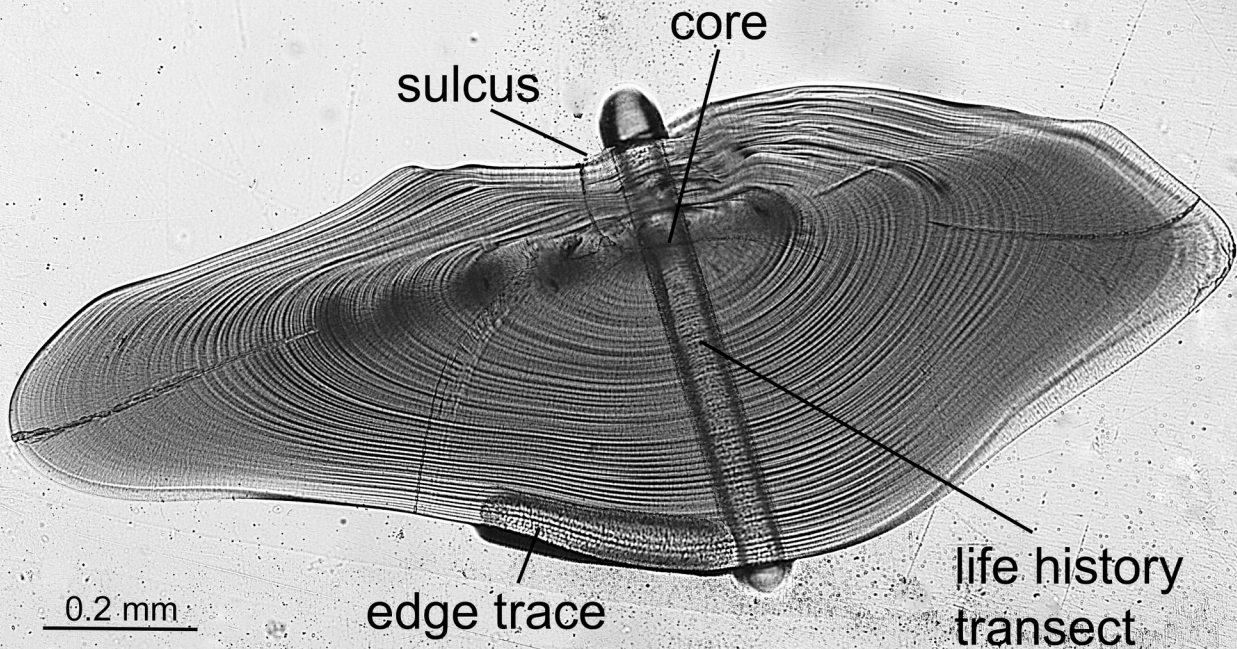
785 **Fig. 4.** Differences between Pacific cod (*Gadus macrocephalus*) otolith edge trace elemental  
786 ratios measured in summer (light gray) and fall (dark gray) in Kiliuda Bay (KB) and Port Dick  
787 (PD). Horizontal bars indicate median values, the top and bottom of each box represent the 25th  
788 and 75th percentiles, respectively, and hatched lines represent values within 1.5 times the  
789 interquartile range. Circles represent values greater than 1.5 times the interquartile range.

790 **Fig. 5.** Relationships between transformed elemental ratios measured at the edge of age-0 Pacific  
791 cod (*Gadus macrocephalus*) otoliths and temperature (closed circles), salinity (open circles), and  
792 dissolved oxygen content (closed triangles). Solid lines indicate significant ( $P < 0.05$ ) linear  
793 regressions.

794 **Fig. 6.** Quadratic discriminant analysis based on age-0 Pacific cod (*Gadus macrocephalus*)  
795 otolith multi-elemental signatures from early July, used to predict membership to nursery bays in  
796 the Gulf of Alaska (IB = Islas Bay, IZ = Izhut Bay, KB = Kiliuda Bay, PD = Port Dick, SS =  
797 Salisbury Sound). Axes represent the first two factors with the percent of variance explained by  
798 each in parentheses. Ellipses represent 95% confidence intervals about the centroids (black dots)  
799 for each grouping.







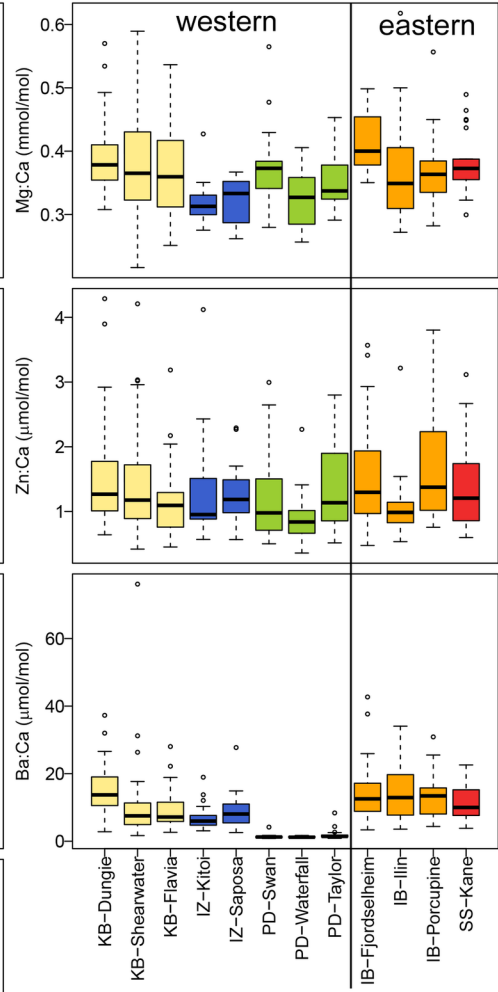
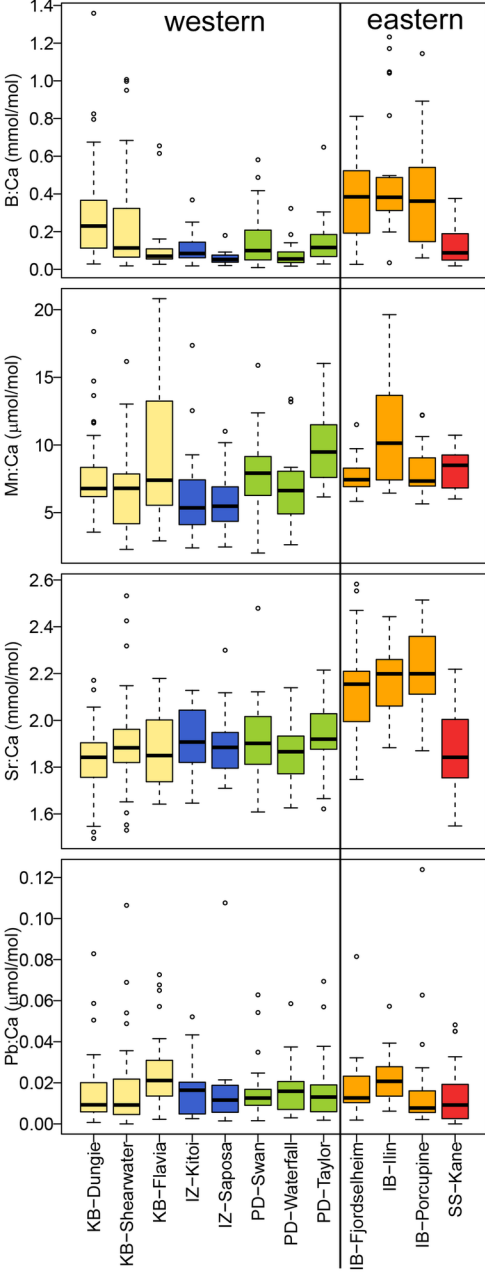
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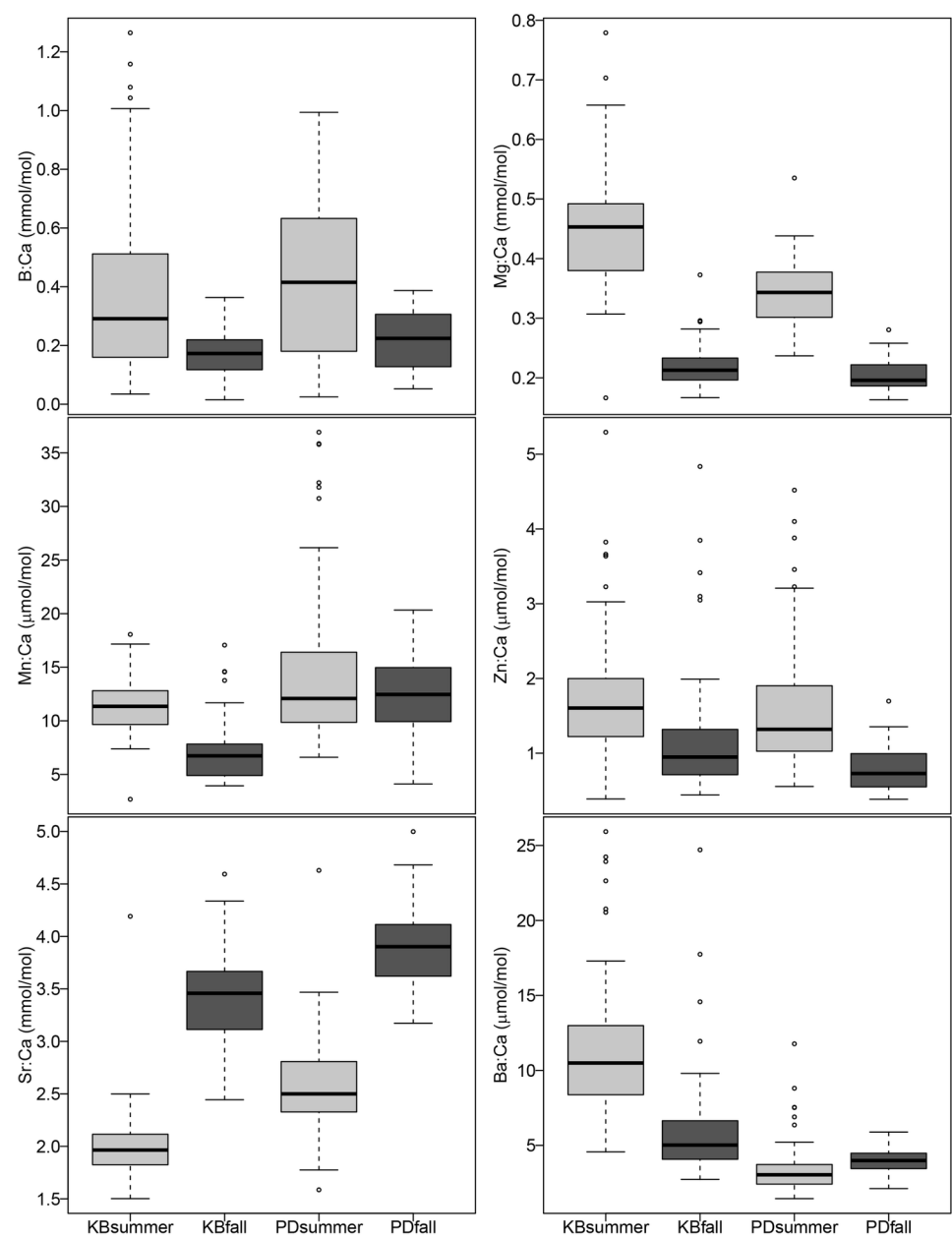
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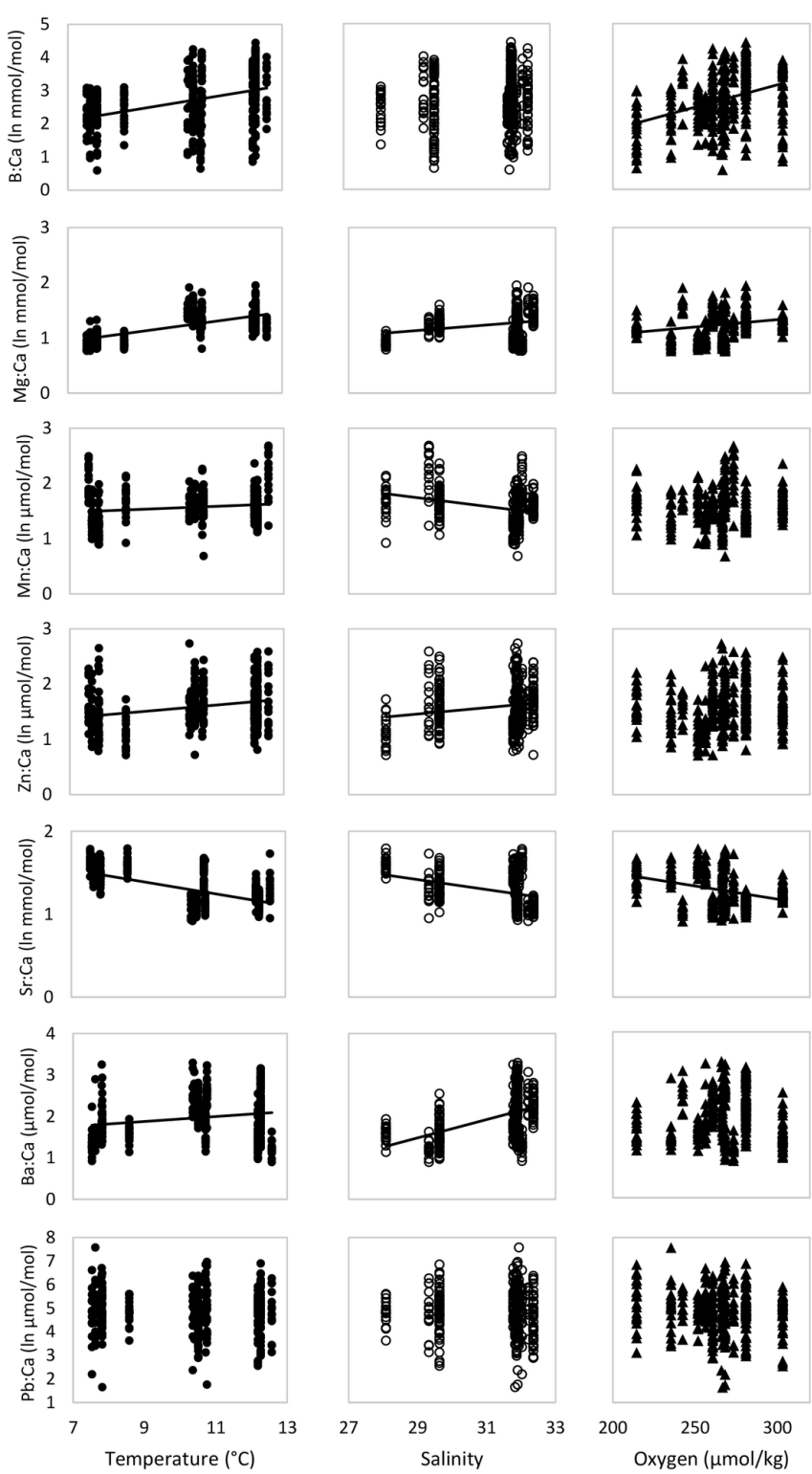
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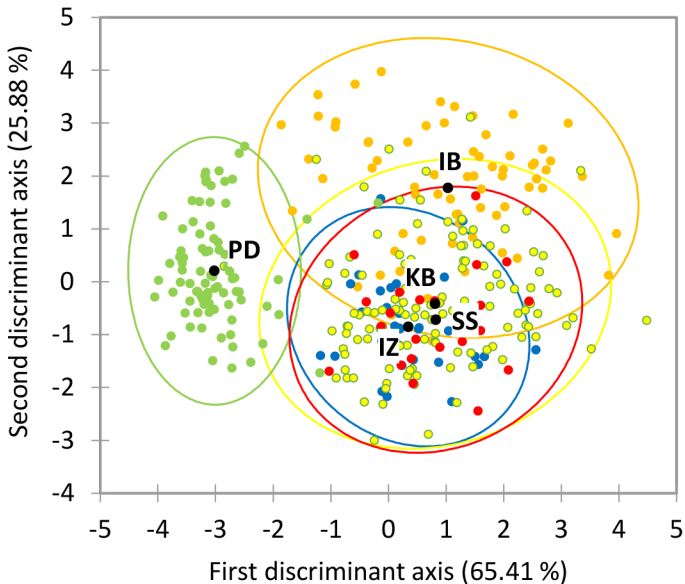
edge trace

life history  
transect









● IB ● IZ ● KB ● PD ● SS ● Centroids