Patterns of larval source distribution and mixing in early life stages of Pacific cod (*Gadus macrocephalus*) in the southeastern Bering Sea

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ABSTRACT

Effective and sustainable management depends on knowledge of spawning locations and their relative contributions to marine fish populations. Pacific cod (*Gadus macrocephalus*) in the southeastern Bering Sea aggregate at discrete spawning locations but there is little information on patterns of larval dispersal and the relative contribution of specific spawning areas to nursery habitats. Age-0 Pacific cod from two cohorts (2006 and 2008) were examined to address the following questions: (1) does size, age, and otolith chemistry vary among known capture locations; (2) can variation in elemental composition of the otolith cores (early larval signatures) be used to infer the number of chemically distinct sources contributing to juvenile recruits in the Bering Sea; and (3) to what extent are juvenile collection locations represented by groups of fish with similar chemical histories throughout their early life history? Hierarchical cluster (HCA) and discriminant function analyses (DFA) were used to examine variation in otolith chemistry at discrete periods throughout the early life history. HCA identified five chemically distinct groups of larvae in the 2006 cohort and three groups in 2008; however, three sources accounted for 80-100% of the juveniles in each year. DFA of early larval signatures indicated that there were non-random spatial distributions of early larvae in both years, which may reflect interannual variation in regional oceanography. There was also a detectable and substantial level of coherence in chemical signatures within groups of fish throughout the early life history. The variation in elemental signatures throughout the early life history (hatch to capture) indicates that otolith chemical analysis could be an effective tool to further clarify larval sources and dispersal, identify juvenile nursery habitats, and estimate the contributions of juvenile nursery habitats to the adult population within the southeastern Bering Sea.

Key words: otolith chemistry, Pacific cod, Alaska Peninsula, Bering Sea, juvenile, larval sources

1. Introduction

Knowledge of spawning and nursery locations and their relative contributions to marine fish populations, or stocks, is a fundamental component of sound fisheries management (Begg and Marteinsdottir, 2000; Jonsdottir et al., 2007). However, there are numerous challenges to accurately identifying spawning and nursery areas, evaluating their output, and determining their relative contributions to a population. Demographic, genetic, and otolith structural and chemical approaches have been used to identify discrete aggregations and assess their relative contributions to adult populations (Wilimovsky et al., 1967; Miller et al., 2005; Cunningham et al., 2009; Svedang et al., 2010). Although each method has limitations, a combined approach has the potential to provide corroborative or complementary information on spawning contributions, larval sources, and essential fish habitats.

The Pacific cod fishery in the United States is currently managed as two components, the Gulf of Alaska stock and the larger Bering Sea–Aleutian Islands 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. Additionally, several genetic studies (Grant et al., 1987; Cunningham et al., 2009; Spies 2012) have observed isolation by distance across the species range throughout the North Pacific Ocean but no distinct boundaries within or between the current management areas.

Pacific cod annually aggregate at discrete spawning locations throughout the Aleutian Islands, around the Pribilof Islands, north of Unimak Island, and along the shelf break near Zhemchug Canyon (Neidetcher et al., 2013). The degree of fidelity to each of these sites and the extent to which each of these spawning regions contribute to the Bering Sea population is unknown, and there is additional potential for larval Pacific cod to be transported from the Gulf of Alaska into the Bering Sea through the Unimak Pass (Siddon et al., 2011). Additionally, tagging studies of adult fish indicate that the Unimak Pass–Alaska Peninsula region may support the majority of spawning activity for Bering Sea Pacific cod (Shimada and Kimura, 1994). Pacific cod spawn demersal, non-adhesive eggs. Surveys of reproductive status of adults during winter in the Bering Sea from 2005 to 2007 indicate that spawning begins in February or early March and extends through early to mid-April (Neidetcher et al., 2013). Positively buoyant larvae hatch between 3 - 4 mm standard length (SL), are collected in surface waters, and transform into juveniles at 25 - 35 mm SL. In the Bering Sea, larvae have been most commonly collected from March to August along the Alaska Peninsula and the southeastern portion of the shelf, which is also when the majority of sampling effort has occurred (Matarese et al., 2003). Juveniles are most abundant in coastal waters along the Alaska Peninsula but also occur in pelagic waters over the broad continental shelf (Hurst et al., *in review*). However, little is known regarding patterns of larval dispersal, the relative contribution of specific spawning areas to the widely distributed nursery habitats, or the contribution of those nurseries to the adult population. Given that Pacific cod are fished on their spawning grounds, it is important to identify the factors that influence the abundance, distribution, and connectivity of stocks and to evaluate whether particular spawning sources are more critical than others in sustaining the productivity of populations within the Bering Sea.

Tracking larvae from spawning to settlement is challenging, particularly in widely distributed marine species. Small size and high rates of mortality make external tagging techniques impractical due to the large number of tagged individuals needed to ensure sufficient numbers are recovered (Jones et al., 1999, 2009; Almany et al., 2007). 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), and more recent parentage approaches require representative sampling of parents and offspring which is not feasible in many marine species (Planes et al., 2009; Saenz-Aguledo et al., 2009; Christie, 2010). Isotopic and elemental analyses of otoliths have shown promise as a means to investigate spatial structure in fishes on ecological time scales and have been used to examine natal sources (Thorrold et al., 2001; Barbee and Swearer, 2007) and dispersal patterns in marine fishes (Swearer et al., 1999). This approach is feasible because the chemical composition of otoliths reflects the physical and chemical properties of the ambient water. When 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, we used otolith structure and chemistry of juvenile Pacific cod to evaluate their potential to provide information on larval sources and early life histories in the southeastern Bering Sea. Specifically, we addressed the following questions: (1) do size, age, and otolith chemistry of age-0 Pacific cod vary among known capture locations; (2) can variation in elemental signatures in otolith cores (early larval signatures) be used to infer the number of chemically distinct sources contributing to juvenile recruits in the Bering Sea; and (3) to what extent are juvenile collection locations represented by groups of fish with similar chemical histories throughout their early life history, which could indicate cohesive dispersal patterns?

2. Methods

2.1. Study design

Juvenile (age-0) Pacific cod from 2 cohorts were collected throughout the southeastern Bering Sea to address the research questions presented above. First, we compared juvenile size and age among collection locations. Second, we examined the elemental signatures at the outer edge of the otoliths to evaluate the extent of spatial variation in elemental composition and assess the ability to classify fish to collection location based on otolith edge signatures. Third, we examined elemental signatures in the otolith core of those same juveniles, which represent their early larval stage, using a combination of hierarchical cluster and discriminant function analyses to identify chemically distinct groups of larvae. Fourth, we determined the spatial distribution of juveniles with distinct early larval elemental signatures. Finally, we evaluated how consistent otolith elemental signatures were during discrete periods of the early life history to provide an indication of potential mixing within the study area.

2.2. Sample collection

Juvenile Pacific cod were collected throughout their range in the southeastern Bering Sea (Hurst et al., 2012) from August-September 2006 and 2008 during the Alaska Fisheries Science Center's (AFSC) annual Bering-Aleutian Salmon International Survey (BASIS). Over 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 codend liner (see Farley et al., 2005). Additional samples in both years were collected in the Bristol Bay region with a 3-m beam trawl (Cooper et al., 2014). Based on

115 extensive sampling in 2012, fish collected with beam trawls in Bristol Bay and along the Alaska
116 Peninsula are only slightly larger than fish collected with a surface trawl in deeper waters over
117 the shelf (beam trawl: 66.6 ± 7.1 mm; surface trawl: 64.3 ± 9.2 mm TL), suggesting that summer
118 collections are not influenced by a size-dependent habitat shift. Juveniles were frozen after
119 capture and transported to the AFSC's Fisheries Behavioral Ecology laboratory in Newport,
120 Oregon, for analysis. In both years, fish were selected from six trawl sites that covered the
121 distribution of juveniles collected in the Bering Sea (Hurst et al., 2012); three samples were
122 collected along the western, middle and eastern Alaska Peninsula (AP-W; AP-M; AP-E,
123 respectively) and three samples were collected farther north over the western, middle and eastern
124 Shelf (Sh-W; Sh-M; Sh-E; Fig. 1).
125 Additionally, in May of each sampling year, larval Pacific cod were collected in the
126 vicinity of Unimak Pass in an attempt to relate the elemental signatures of otolith cores from fish
127 collected as juveniles to signatures of larvae from a major known spawning region. However,
128 those larvae averaged 19 d old with a mean hatch date of May 1. Therefore, it appears that the
129 survey, which targets walleye pollock (*Gadus chalcogrammus*), does not coincide with the peak
130 spawning of Pacific cod (Neidetcher et al., 2013). Given that the larval collections likely
131 represent only the later spawners, we did not include them in subsequent analyses. Data on the
132 spatial variation in otolith elemental composition of larval Pacific cod are presented in DiMaria
133 (2011).
134
135 *2.3. Age determination*
136 Juveniles were weighed (to 0.01 mg) and measured (standard length SL, to 1.0 mm), and
137 both sagittal otoliths were removed using standard methods to minimize contamination (Miller,

2009). Otoliths were photographed under a dissecting microscope and their length (the longest axis) and width (the longest perpendicular axis) were measured using image analysis software (ImagePro®). The left otolith was 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 3M™ tri-mite Wetordry™ paper (240-1200 grit).

Narimatsu et al. (2007) validated the daily formation of otolith increments in Pacific cod at 10°C up to 120 d post-hatch. Additionally, we examined the otoliths of juvenile Pacific cod captured and transported to the AFSC laboratory in Newport, Oregon. At approximately 8-9 months old, fish were measured and held at 9°C for 40 d prior to being euthanized. We enumerated 41.2 ± 1.7 SD (n = 6) increments after the presumed handling stress check, providing additional validation of daily increment formation during the juvenile stage. For this study, a subsample of fish was aged from each year. Juveniles were pooled across sampling sites, divided into size bins, and 15% of fish with the clearest otolith preparations within each size bin were identified for ageing (Table 1). A composite image (core to edge) of polished otoliths was acquired using a compound microscope at 400× magnification and used to age fish. Age was estimated by enumerating increments after the hatch check, the presence and size of which was also reported by Narimatsu et al. (2007). We used standard methods for increment analysis, which included careful acquisition of otolith images at the proper focal depth, evaluation of increment optical clarity and uniformity of increment width, and the collection of replicate counts (Beamish and Fournier, 1981; Dougherty, 2008). The average age of each fish (from multiple, independent counts that varied by <10%) was used for analysis. Ages of all other fish <85 mm SL in each year were estimated from best-fit polynomial models relating age of the subsampled fish 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). Because the age of juveniles >85 mm appeared underestimated by the best-fit models, all fish ≥ 85 mm were aged directly from increment counts. Hatch date and average somatic growth rate were calculated for all juveniles assuming a mean size at hatch of 4.6 mm SL (Laurel et al., 2008).

2.4. Otolith elemental composition

Otolith composition (Li, Mg, Ca, Mn, Cu, Zn, Sr, Ba, and Pb) 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 ratios were converted based on measurements of National Institute of Standards and Technology (NIST) 612 standard glass and are presented as molar ratios (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% for Mg, Mn, Zn, Sr and Ba, respectively.

The left sagittal otolith, previously sectioned and polished for aging, was used for elemental analysis. The otolith sections 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 single transect from the core to anterior-dorsal edge; the laser was set at a pulse rate of 2 Hz with a 100-µm spot moving at 100 µm·sec^{-1}.

184 To collect otolith elemental data, the laser was set at a pulse rate of 7 Hz with a spot of 50 µm
185 moving at 2 µm·sec^{-1}.

186 The transect of elemental data was converted to a temporally-resolved life history profile
187 of otolith elemental composition from hatch to capture. First, the length of each transect from the
188 otolith core to the edge was measured. Distance along each transect was converted to calendar
189 date based on fish age and date of capture assuming uniform increment deposition. Second, we
190 identified a 10-d section at the start of each transect, near the core, to characterize the "early
191 larval" signature. Otolith primordia are characterized by elevated Mn:Ca (Brophy et al., 2004;
192 Ruttenberg et al., 2005); therefore, we excluded any portion of the initial transect with sharply
193 elevated Mn in order to isolate the post-hatch, early larval period. Third, four discrete stanzas of
194 50 d were extracted from each individual life history profile. The stanzas reflect specific days of
195 each year (2006: DOY75-125, 126-175, 176-225, 226-275 and 2008: DOY21-70, 71-120, 131-
196 180, 191-240). Stanzas began earlier in 2008 because juveniles hatched earlier in 2008 than
197 2006. Finally, the outermost 10-d section of the transect ("edge") characterized otolith elemental
198 composition immediately prior to capture. The elemental signature of each fish during each
199 transect period was described by those elements consistently above background levels, including
200 Mg, Mn, Sr, and Ba (all normalized to Ca).
201
202 *2.5 Statistical analyses*

203 Differences in the size- and age-at-capture, hatch date, and average somatic growth rate
204 among collection locations were evaluated with Kruskal-Wallis tests. For all multivariate
205 analyses, otolith elemental ratios were examined for normality (P-P plots), homogeneity of

variance (Levene's test), and equality of variance-covariance matrices (Box M plots), and all variables were either natural log or square-root transformed.

2.5.1. Spatial variation in juvenile otolith edge elemental signatures

The variation in otolith edge elemental composition, representing the last 10 d prior to capture, was examined to evaluate the extent of spatial variation in otolith composition. Given observed temporal variation in otolith chemistry within sites (see Elsdon et al. 2008 for review) and the narrow temporal window of sample collection (all samples collected as juveniles within 13-d and 23-d periods in 2006 and 2008, respectively), variation in elemental composition at the otolith edge reflected spatial changes in water mass characteristics as opposed to temporal or ontogenetic patterns. Multivariate Analysis of Variance (MANOVA) was used to evaluate differences among years and collection locations. The Discriminant Function Analysis (DFA) "leave-one-out" procedure (jack-knife) was used to assign fish to their collection site based on the individual otolith edge signatures. We also compared observed classifications to chance using the kappa statistic ($\kappa = P_o - P_e / 1- P_e$), where P_o = observed proportion, P_e = expected proportion based on chance, with $\kappa = 1.0$ indicating perfect agreement, and $\kappa = 0.0$ indicating assignments no better than chance (Cohen, 1960).

2.5.2. Early larval signatures of juvenile Pacific cod recruits

Two complementary analyses were used to examine the relationships between early larval signatures and capture location of the juveniles (Tanner et al., 2012). To determine the number of chemically-distinct larval groups, hierarchical cluster analysis (HCA, Statistica v12) was applied to the early larval signatures (corresponding to the first 10 d of life). HCA assumes

no *a-priori* knowledge of the number of distinct groups included in the sample and makes no assumptions of the relationship between identified groups and capture locations. The HCA was run using a Euclidean (Pythagorean) distance measure and the Ward's linkage method. The resulting dendrogram was scaled to a standardized scale (dlink/dmax*100) to provide a percentage of information remaining. The number of chemically distinct groups was determined by pruning the dendrogram at the location where the number of branches was stable (i.e., longer branch lengths that indicated stable clusters). These chemically distinct groups are subsequently referred to as "clusters". The relative abundance and spatial distribution of fish based on these clusters was plotted as a function of their relative contribution to each juvenile collection site.

In a separate analysis, linear DFA was applied to the early larval otolith signatures. This analysis evaluated the degree to which fish were assigned to their collection site based on otolith elemental composition during the first 10 d of life. Correct assignment of a fish to a collection site based on DFA of early larval signatures indicates that their signature is similar to those of other juveniles collected at the same site, not that the fish were physically at that site during the larval period. High rates of assignment of juveniles to their actual collection site based on the early larval signature would be an indication of coherence in elemental signatures from hatch to juvenile collection, potentially indicating coherence in dispersal patterns. Conversely, poor classification would be indicative of high mixing among source locations or insufficient spatial variation in elemental signatures to differentiate among sources.

The combined analysis using both HCA and DFA provides complementary information on variation among the early larval signatures. The HCA makes no assumption about sources and, thus, provides insight on the spatial distribution of chemically-distinct larval signatures regardless of collection location. The DFA assigns fish to one of the collection locations based

on the early larval signature, which provides quantitative estimates for the similarity within sites (percent correctly assigned and posterior probabilities of assignments). Additionally, we can determine if there is a non-random spatial pattern associated with the assignments, i.e., are fish that are not classified to their collection site more commonly assigned to adjacent sites?

2.5.3. Otolith chemical variation across the early life history: evaluation of temporal cohesiveness

To determine if juvenile fish collected in different regions of the Bering Sea display similar patterns of variation in otolith chemistry throughout their early life history, we conducted separate DFAs on the elemental signatures of fish at the four discrete stanzas during the first year of life. Whereas the DFA of the early larval signatures compared elemental composition of fish at the same ontogenetic point (10 d post-hatch) independent of birth dates, these analyses, based on the temporally-resolved life history profiles, examine patterns in elemental composition for specific time periods through the first year of life. If fish consistently group together based on otolith chemistry during their early life history, it would be evidence that they have experienced similar patterns of dispersal and/or similar water chemistry through the early life history. We completed separate linear DFAs for each of the four discrete stanzas characterized in each year (see *Otolith elemental composition*). Given variable hatch dates, sample sizes in the first stanza were smaller (2006 n = 131 and 2008 n = 110) than in the remaining stanzas (2006 n = 161 and 2008 n = 149) and one site (Sh-W) was excluded from the first stanza in 2006 due to small sample size (n = 3). As noted earlier, assignment of fish to their collection site based on DFA of an earlier life history period indicates similarity in otolith elemental signatures to other fish

collected at that site, not that the fish were physically at the collection site during the earlier life history period.

4. Results

4.1. Spatial variation in juvenile Pacific cod

In both 2006 and 2008, some differences were observed in biological characteristics of juveniles collected throughout the southeastern Bering Sea (Table 1). In 2006, juveniles from AP-W and AP-M were smaller (average of 8-19 mm) and younger (average of 25-41 d) than juveniles from the other sites. In 2008, juveniles from Sh-W and AP-E were smaller (average of 5-24 mm) and younger (22-40 d) than juveniles from the other sites (Kruskal-Wallis ANOVA, $P < 0.001$). Additionally, in 2008 average somatic growth rate of juveniles was slightly greater at AP-M (0.34 vs. 0.29-0.30 at the other sites: $P = 0.001$). However, there were no clear geographic patterns of variation in these metrics within years. Overall, juveniles hatched later (mean date = March 20 vs. February 13; $P < 0.001$) and grew faster (mean = 0.375 vs. 0.301 $mm \cdot day^{-1}$; $P < 0.001$) in 2006 compared with 2008. The overall coefficient of variation in size across all individuals within each year was ≤16% and the majority of juveniles were within 60 d of each other in age (92% and 80% of the juveniles in 2006 and 2008, respectively).

There was significant spatial variation in the elemental ratios at the otolith edge of juvenile cod collected throughout the southeastern Bering Sea in both 2006 and 2008, with significant interactions between site and year for all elemental ratios ($F_{1,5} > 10.0$, $P < 0.01$). Therefore, separate analyses were completed for each year. In 2006, Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca at the edge of the juvenile otoliths varied among sites ($F_{5,155} > 16.0$, $P < 0.01$). Similar results were observed in 2008 ($F_{5,142} > 10.0$, $P < 0.01$). In both years, Ba:Ca and Mn:Ca were

297 higher along the Alaska Peninsula than the Shelf sites. Mg:Ca and Sr:Ca varied among sites but
298 not in a systematic fashion (Fig. 2).

299 Juveniles were classified to collection site based on the elemental signatures at the outer
300 edge of their otoliths with relatively high accuracy in both years (Table 2). In 2006, juveniles
301 were assigned to their collection site 74.5% (±10.6% SD) of the time ($\kappa = 0.69$). Juveniles
302 collected at Sh-E, AP-E, and AP-W had the highest classification success (≥80%) and Sh-M had
303 the lowest (61%). In 2008, overall classification accuracy was lower (54.2 ± 17.7% SD) but still
304 substantially greater than chance alone ($\kappa = 0.45$). Juveniles collected at Sh-W and AP-M had the
305 highest jackknifed classification success (>67%) and Sh-E had the lowest (32%). Posterior
306 assignment probabilities for correct assignments ranged from 0.36 to 1.00 with a mean of 0.76
307 (±0.20) in 2006 and 0.33 to 0.99 with a mean of 0.66 (±0.18) in 2008.
308
309 *4.2. Early larval signatures of juvenile Pacific cod recruits*
310 *4.2.1. Hierarchical Cluster Analysis*
311 Variation in composition (Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca) of the early larval elemental
312 signature was used to identify chemically distinct groups (i.e., presumptive sources) of sampled
313 juveniles using HCS. Clusters with branch lengths that accounted for >25% of the total
314 information were identified, and final clusters incorporated ≥75% of the total information. We
315 identified five chemically distinct clusters of early larval signatures in 2006 and three clusters in
316 2008 (Fig. 3 & 4; note that the sources contributing to each cohort are arbitrarily coded and
317 source 1 in 2006 is not equivalent to source 1 in 2008). In 2006, three main sources each
318 accounted for 26-27% of the juveniles collected throughout the southeastern Bering Sea. The
319 remaining two sources accounted for 12% and 7% of the juveniles. Juveniles from the five

sources were dispersed across most sites; however, the AP-M site had the largest contribution from a single source (60%) and included only four of the five sources. Ba:Ca and Mg:Ca varied across all five clusters (Scheffé test for homogeneous groups, $P < 0.05$) whereas Sr:Ca and Mn:Ca characterized Cluster 4 and Mg:Ca characterized Cluster 5. In 2008, two sources each accounted for 39% of the juveniles with the remaining sources accounting for 22%. Ba:Ca varied among all three clusters ($P < 0.05$), whereas differences in Sr:Ca were marginally significant ($P = 0.06$). Given that there were no significant differences in Mg:Ca and Mn:Ca among clusters, the analysis was re-run with only Ba:Ca and Sr:Ca and the same clusters were identified.

Although three clusters accounted for 80-100% of fish in both years, there were five clusters identified in 2006 compared with three clusters in 2008. The variable number of larval clusters between years could be due to: (1) the contribution of larvae from additional geographic areas, or sources, in 2006; or (2) greater spatial variation in water chemistry in 2006, resulting in greater spatial variation in early larval signatures. Interestingly, there was spatial structure to the distribution of early larval clusters. In 2006, the distribution of clusters displayed an east-west gradient with more similar cluster distributions observed at Sh-W and AP-W, Sh-M and AP-M, and Sh-E and AP-E. In 2008, clusters were more evenly distributed spatially although the westward collection locations were most similar to each other (Sh-W and AP-W).

There were few differences in biological characteristics among the early larval clusters in either year. In 2006, hatch date and somatic growth rate did not differ among the sources (Table 3, Kruskal-Wallis ANOVA, $P > 0.10$). Juveniles in Cluster 1 were slightly larger, on average, than the other four clusters and Cluster 5 had the lowest mean age. In 2008, none of the biological characteristics differed among the clusters (Table 3, Kruskal-Wallis ANOVA, $P > 0.10$).

343

344 *4.2.2. Discriminant function analysis*

345 We also classified juveniles to a collection location based on their early larval otolith
346 signature using DFA to evaluate the similarity within and among collection locations and for
347 comparison with the cluster analysis (Table 4). There were distinct spatial patterns associated
348 with individual assignments. In 2006, 48.7% (± 18.4 SD) of the juveniles were classified to their
349 collection location based on their early larval otolith signature (n = 161, 6 sites), which is greater
350 than chance alone (κ =0. 39). There was also a clear spatial pattern associated with "mis-
351 assignments", with the majority of the individuals that were not assigned to their collection
352 location being assigned to the site directly north or south between shelf and Alaska Peninsula
353 collection sites (Fig. 5). In 2008, juveniles were again classified to their collection location at a
354 rate greater than expected by chance (41.0% ± 25.2% SD, n = 149, 6 sites, κ = 0.33). However,
355 the pattern of "mis-assignments" was different in 2008 with the majority of individuals that were
356 not assigned to their collection location being grouped with the closest cross-shelf location,
357 except for individuals collected at the westernmost locations (Sh-W and AP-W) (Fig. 5).

358

359 *4.3. Otolith chemical variation across the early life history: evaluation of temporal cohesiveness*

360 We completed DFAs for each of the four life history stanzas that encompassed the early
361 life history, which compares juveniles during the same time of year. An average of 57% (±21%
362 SD) and 52% (±20% SD) of the juveniles were assigned to their actual collection location across
363 stanzas in 2006 and 2008, respectively (Fig. 6). The percentage of individuals assigned to their
364 collection location remained steady or increased during each later life history stanza, ranging

365 from 47.6 to 54.2% in 2006 and 50.5 to 73.6% in 2008 (Fig. 6), with one exception. In 2008 at
366 AP-M, classification declined but was consistently fairly high (84% to 50%).
367
368 **5. Discussion**
369 Our work on juvenile Pacific cod demonstrates the potential for otolith elemental
370 signatures to be used in studies examining dispersal and connectivity within the Bering Sea and
371 with adjacent populations in the Aleutian Islands and Gulf of Alaska while illustrating some of
372 the limitations of this approach when applied to large marine populations. We documented
373 significant spatial variation in otolith elemental signatures of juveniles which could be used to
374 evaluate the productivity of distinct nursery areas. While we were unable to link juvenile fish to
375 known spawning areas due to practical issues of obtaining representative larval samples over the
376 large spatial scales relevant to this population, we identified three to five chemically distinct
377 larval source signatures in each year, suggesting the contribution of multiple spawning locations
378 to the juvenile cohort on the southeastern shelf. In addition, temporally-resolved elemental
379 transects from the otolith core to the edge suggested that collection sites along the eastern and
380 central Alaska Peninsula were comprised of individuals with similar elemental signatures
381 throughout the early life history, potentially indicating similar dispersal trajectories. However,
382 there were differences between years in apparent mixing patterns, which may be associated with
383 differences in prevailing current flows over the Bering Sea shelf.
384 Spatial patterns were also evident in both the HCA and DFA of the early larval
385 signatures. The cluster analysis indicated that there were three dominant, chemically distinct
386 sources to juvenile recruits in the southeastern Bering Sea, while the DFA implied that there may
387 be limited mixing among groups of larvae during their early life history. The spatial distribution

388 of the early larval signatures may reflect interannual variation in regional oceanography. During
389 winter months, when Pacific cod spawn, the Bering Sea shelf water column is vertically well-
390 mixed. Based on sea ice and water temperature conditions, the southeastern Bering Sea in 2006
391 was the first "average" (or "transitional") year after a series of warm years and 2008 was one of
392 the coldest years in several decades (Stabeno et al., 2012). During warmer years, surface currents
393 display stronger northward flow during winter, whereas in cold years the mean surface currents
394 are more westward (Stabeno et al., 1999; 2012). During the very early larval period, larval
395 transport and distribution may reflect dominant oceanographic patterns with more north-south
396 mixing in 2006 (an average year) and more east-west mixing in 2008 (a cold year). By April or
397 May, several persistent fronts are established, structuring the southeastern shelf into a vertically
398 mixed inshore coastal domain (<50 m), a stratified middle domain (50-100 m), and a three-layer
399 outer shelf domain (100-200 m) (Kinder and Schumacher, 1981). The establishment of these
400 frontal structures across the shelf in spring could reduce mixing of older larvae (>30 d old). Our
401 DFA on the life history stanzas support this possibility as juveniles were consistently grouped
402 with fish from their actual collection location throughout their life history at relatively high rates
403 (overall mean = 52% in 2006 and 57% in 2008 compared with ~17% random expectation). Thus,
404 while it appears that all of the identified larval clusters were present across the shelf and there
405 was mixing among sources, there was also a detectable and substantial level of spatial coherence
406 among groups of larvae throughout their early life history, which may reflect variation in
407 regional oceanographic conditions.

408 We also documented significant spatial variation in the otolith edge chemistry of juvenile
409 cod collected throughout the southeastern Bering Sea, highlighting the potential of elemental
410 tracers to examine juvenile habitat use within the Bering Sea. Many studies using otolith

411 chemistry have focused on freshwater, estuarine, and coastal environments where there is often
412 extensive spatial variation in water chemistry due to local variation in watershed geology (e.g.,
413 bedrock, sediment load, and groundwater transport and retention; Elsdon et al., 2008). Despite
414 concerns that open ocean environments can be more homogeneous, numerous studies
415 investigating natal sources (Neubauer at al., 2010), movement patterns (Thorrold et al., 2001),
416 and stock structure (Campana et al., 2000; Jónsdóttir et al., 2007; Miller et al. 2005) of marine
417 fishes have observed variation in otolith chemistry across various spatial scales. In the Gulf of
418 Alaska and eastern Bering Sea, spatial variation in otolith chemistry has been documented in
419 several species, including Pacific halibut (*Hippoglossus stenolepsis*; Gao and Beamish, 2003)
420 and walleye pollock (*Gadus chalcogrammus*; Fitzgerald et al., 2004). The present study
421 documents variation in otolith elemental signatures at a smaller spatial scale of 200-800 km,
422 within the southeastern Bering Sea.

423 Despite the economic importance of Pacific cod, much less is known about the habitat
424 use patterns of juveniles in the Bering Sea than in other parts of their range (Takatsu et al., 2001;
425 Laurel et al., 2009). Juveniles (age-0) are known to inhabit surface and sub-surface waters over
426 the continental shelf (Hurst et al., 2012; Parker-Stetter et al., 2013), but Hurst et al. (*in review*)
427 suggest that coastal waters along the Alaska Peninsula are the primary nursery habitat for this
428 population. The variation observed in elemental signatures throughout the juvenile range in the
429 eastern Bering Sea suggests that otolith chemistry could be an effective tool for tracing
430 contributions of these nursery areas to older "recruits" and the adult population (Thorrold et al.,
431 1998; Gillanders and Kingsford, 2000; Brown, 2006).

432 Otolith incorporation of certain trace elements is related to their availability in the rearing
433 water but can also be influenced by other physical (e.g., temperature) and biological (e.g.,

growth) factors (for reviews see Campana, 1999; Elsdon et al., 2008). Juveniles collected from sites along the Alaska Peninsula (< 50 km from shore) generally had higher otolith edge Ba:Ca and lower Sr:Ca than sites over the open shelf in both years, which may in part be due to variation in temperature and/or freshwater entrainment along the Alaska Peninsula by the Bering Coastal Current. Although DiMaria et al. (2010) demonstrated that otolith Sr:Ca and Ba:Ca were inversely related to temperature from 2-8°C in larval Pacific cod, surface water temperatures varied by less than 1°C across collection sites in each sampling year. Similarly, while variation in growth rates has been shown to affect otolith elemental incorporation in some cases (e.g., Sadovy and Severin, 1992, 1994; Walther et al. 2010), it is unlikely that variation in individual growth rates is a significant factor contributing to the patterns of otolith chemical variation observed in this study. Our observed somatic growth rates were generally homogeneous across collection sites and DiMaria et al. (2010) observed no effect of somatic or otolith growth on elemental composition in larval Pacific cod.

Our ultimate goal was the ability to link individuals to specific spawning regions. Application of otolith elemental signatures can be a powerful approach in such studies (Arkhipkin et al., 2009; Svedang et al., 2010). However, in practice, the ability to assign individuals to specific spawning areas is limited by the ability to sample early life stages across the spawning distribution to create the "source map" (Warner et al., 2005; Standish et al., 2008; Woodson et al., 2013). The large scale of many marine populations and the lack of knowledge of the underlying spatial scale of elemental signature variation have limited robust applications of this approach. In our case, potential recruits to the eastern Bering Sea could come from spawning aggregations spread over ~1000 km of the Bering Sea (Neidetcher et al., 2013) as well as possible exchange with adjacent populations along the Aleutian Islands archipelago and in the

457 Gulf of Alaska. These spatial scales effectively preclude comprehensive sampling of larvae from
458 all potential spawning areas. As an alternative, some studies have incorporated an "elimination
459 approach" of describing elemental signatures from some known sources and identifying those
460 fish with signatures not corresponding to any described sources (Standish et al., 2008). We
461 attempted to apply this approach using opportunistic collections of larval Pacific cod in the
462 vicinity of Unimak Island, considered to represent the largest spawning aggregation and potential
463 source for most recruits. However, Pacific cod have a protracted spawning season (> 2 months)
464 and larval sampling captured only late-hatching larvae (DiMaria, 2011), whereas juveniles were
465 more representative of the entire spawning season (Neidetcher et al., 2013). This example
466 illustrates the need for temporal as well as spatial matching of natal source sampling in order to
467 accurately assign fish to specific spawning areas (Cook 2011).
468 This is the first study to investigate the contribution of larval sources to juvenile Pacific
469 cod recruits in the southeastern Bering Sea. Given the protracted winter spawning season and
470 wide geographic range, it will be challenging to characterize all larval sources of cod and assign
471 recruits back to specific spawning locations throughout the Bering Sea. Ideally, larval sampling
472 would be expanded both spatially and temporally to better reflect the temporal and spatial extent
473 of spawning and to provide a more representative larval samples. Additionally, given variation in
474 oceanographic conditions, a more comprehensive analysis would incorporate multiple warm and
475 cold years. However, based on two years of data, we provided information on otolith elemental
476 variation throughout the early life history which indicates that there may be spatial separation of
477 larvae across the southeastern Bering Sea. We were also able to assign juveniles to their
478 collection site based on edge chemistry with relatively high accuracy, which further indicates

that otolith analysis has strong potential to evaluate the relative productivity of juvenile nursery habitats within the southeastern Bering Sea.

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Table 1. 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 ($mm \cdot day^{-1}$). See Fig. 1 for collection site locations. Homogeneous groups were determined using multiple comparisons (Kruskal-Wallis ANOVA) and are indicated by the superscripts of the same letter (a, b, c). Juveniles collected in 2006 hatched later (mean date = March 20 vs. February 13; $P < 0.001$) and grew faster (mean = 0.375 vs. 0.301 $mm \cdot day^{-1}$; $P < 0.001$) compared with juveniles collected in 2008.

Site	N	Depth	SL	Age	Hatch Date	Growth Rate
			2006			
Sh-W	27	117	67.1 (58.0-83.0)[a]	172 (141-186)[a]	Mar 11 (Mar 24 – Apr 10)[a]	0.36 (0.32-0.46)[a]
Sh-M	28	78	67.7 (55.0-83.0)[a]	172 (132-213)[a]	Mar 1 (Jan 19 – Apr 9)[a]	0.37 (0.27-0.49)[a]
Sh-E	26	41	64.4 (51.0-83.0)[a]	168 (108-206)[a]	Mar 8 (Jan 29 – May 11)[a]	0.36 (0.28-0.48)[a]
AP-W	28	91	56.4 (50.0-65.0)[b]	138 (90-175)[b]	Apr 14 (Mar 7 – May 31)[b]	0.38 (0.34-0.55)[a]
AP-M	26	63	57.3 (50.0-66.0)[b]	143 (95-190)[b]	Apr 8 (Feb 20 – May 25)[b]	0.38 (0.28-0.50)[a]
AP-E	26	46	76.0 (57.0-111.0)[a]	179 (145-202)[a]	Mar 17 (Feb 22 – Apr 20)[a,b]	0.40 (0.34-0.59)[a]
			2008			
Sh-W	27	73	58.6 (50.0-72.0)[a]	185 (112-238)[a]	Mar 23 (Jan 31 – June 6)[a]	0.30 (0.23-0.47)[a]
Sh-M	25	65	71.6 (64.0-79.0)[b]	234 (200-260)[b]	Jan 29 (Dec 12 – Mar 5)[b]	0.29 (0.25-0.30)[a]
Sh-E	28	41	71.3 (65.0-80.0)[b]	229 (206-254)[b]	Feb 2 (Jan 8 – Feb 25)[b,c]	0.29 (0.26-0.33)[a]
AP-W	18	80	66.5 (55.0-80.0)[a,b]	209 (165-250)[a]	Feb 14 (Jan 8 – Mar 31)[b,c]	0.30 (0.29-0.36)[a,b]
AP-M	28	35	82.3 (58.5-100.5)[b]	231 (186-256)[b]	Jan 28 (Jan 3 – Mar 14)[b]	0.34 (0.29-0.48)[b]
AP-E	23	35	65.9 (55.0-74.5)[a]	209 (139-239)[a]	Feb 18 (Jan 18 – Apr 29)[b,c]	0.29 (0.29-0.43)[a]

Table 2. Discriminant function analysis (DFA) results (jack-knifed percentage) for juvenile Pacific cod collected in 2006 and 2008 based on otolith edge elemental composition. See Fig. 1 for collection site locations. Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca were used in DFA in 2006 and 2008.

	Sh-W	Sh-M	Sh-E	AP-W	AP-M	AP-E	Total n
2006 sites (total n = 160)							
Sh-W	66.67	18.52	0.00	11.11	3.70	0.00	27
Sh-M	17.86	60.71	7.14	7.14	7.14	0.00	28
Sh-E	3.85	3.85	88.46	0.00	3.85	0.00	26
AP-W	10.71	3.57	3.57	82.14	0.00	0.00	28
AP-M	7.69	3.85	11.54	0.00	69.23	7.69	26
AP-E	12.00	0.00	0.00	0.00	8.00	80.00	25
2008 sites (total n = 149)							
Sh-W	74.07	11.11	0.00	11.11	0.00	0.00	27
Sh-M	28.00	40.00	28.00	4.00	0.00	0.00	25
Sh-E	10.71	35.71	32.14	17.86	0.00	0.00	28
AP-W	11.11	11.11	16.67	44.44	11.11	5.56	18
AP-M	0.00	0.00	3.57	3.57	67.86	25.00	28
AP-E	0.00	0.00	0.00	0.00	34.78	60.87	23

Table 3. Biological characteristics for the clusters juvenile Pacific cod collected across the southeastern Bering Sea in 2006 and 2008. The clusters were identified using hierarchical cluster analysis of the early larval otolith elemental signature. Mean (range), untransformed values for size at capture (SL, mm), age (d), hatch date, and average somatic growth rate ($mm \cdot day^{-1}$). Homogeneous groups were determined using multiple comparisons (Kruskal-Wallis ANOVA) and are indicated by the superscripts of the same letter (a, b).

Cluster	N	SL	Age	Hatch Date	Growth Rate
			2006		
1	43	68.5 (55.0-111.0)[a]	172 (132-206)[b]	Mar 11 (Jan 29 – Apr 19)[a]	0.37 (0.28-0.59)[a]
2	44	62.4 (55.0-83.0)[b]	158 (95-202)[b]	Mar 24 (Feb 15 – Apr 20)[a]	0.37 (0.32-0.48)[a]
3	20	67.8 (51.0-83.0)[a,b]	162 (126-198)[b]	Mar 19 (Feb 18 – May 26)[a]	0.39 (0.34-0.48)[a]
4	12	65.8 (57.0-111.0)[a,b]	159 (104-202)[b]	Mar 25 (Feb 15 – May 14)[a]	0.39 (0.34-0.48)[a]
5	42	61.9 (50.0-66.0)[b]	155 (90-213)[a]	Mar 24 (Jan 19 – Jun 1)[a]	0.38 (0.27-0.55)[a]
			2008		
1	58	69.3 (50.0-72.0)[a]	217 (112-260)[a]	Feb 12 (Jan 3 – June 5)[a]	0.30 (0.25-0.47)[a]
2	58	70.7 (64.0-79.0)[a]	218 (139-256)[a]	Feb 11 (Jan 3 – Apr 29)[a]	0.29 (0.23-0.48)[a]
3	33	68.1 (65.0-80.0)[a]	212 (151-255)[a]	Feb 19 (Jan 4 – Apr 17)[a]	0.30 (0.28-0.43)[a]

Table 4. Discriminant function analysis (DFA) results (jack-knifed percentage) for juvenile Pacific cod collected in 2006 and 2008 based on otolith elemental composition during the early larval period. Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca were used in DFA in 2006 and 2008.

	Sh-W	Sh-M	Sh-E	AP-W	AP-M	AP-E	Total n
2006 sites (n = 161)							
Sh-W	40.74	3.70	3.70	29.63	3.70	40.74	27
Sh-M	0.00	64.29	3.57	0.00	25.00	0.00	28
Sh-E	11.54	7.69	23.08	23.08	0.00	11.54	26
AP-W	17.86	0.00	10.71	67.86	0.00	17.86	28
AP-M	0.00	26.92	0.00	0.00	61.54	0.00	26
AP-E	19.23	3.85	30.77	7.69	3.85	19.23	26
2008 sites (n = 149)							
Sh-W	40.74	14.81	40.74	0.00	3.70	0.00	27
Sh-M	24.00	36.00	28.00	8.00	4.00	0.00	25
Sh-E	28.57	25.00	42.86	0.00	3.57	0.00	28
AP-W	33.33	38.89	22.22	0.00	0.00	5.56	18
AP-M	0.00	0.00	0.00	0.00	78.57	21.43	28
AP-E	0.00	0.00	0.00	0.00	52.17	47.83	23

Figure Captions

Figure 1. Major surface circulation patterns (arrows), known spawning aggregations (stippled shapes) and collection sites of juvenile Pacific cod throughout the southeastern Bering Sea. In each year, collection sites were chosen to maximize the spatial distribution of juveniles collected during the BASIS surveys. Northern collection locations are, from left to right, Shelf-West (Sh-W), Shelf-Middle (Sh-M), and Shelf-East (Sh-E); southern collection locations are, from left to right, Alaska Peninsula-West (AP-W), Alaska Peninsula-Middle (AP-M), Alaska Peninsula-East (AP-E). Circulation patterns are based on Stabeno et al. 1999; spawning aggregations are based on Neidetcher et al. (2013).

Figure 2. Otolith chemistry of otolith edge (last 10 d of life) for juvenile Pacific cod collected in the southeastern Bering Sea. Mean (±SE) for Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca are presented. The sites included are from three locations along the Bering Sea Shelf and the Alaska Peninsula. West = W; Middle = M; East = E. See Fig. 1 for site locations.

Figure 3. Distribution of clusters of juvenile Pacific cod recruits collected throughout the southeastern Bering Sea in 2006 (a) and 2008 (b). Juveniles were clustered using their early larval otolith elemental signature and hierarchical cluster analysis (HCA, Statistica v12). Note that the sources contributing to each cohort are arbitrarily coded: source 1 in 2006 is not equivalent to source 1 in 2008.

Figure 4. Elemental composition of clusters of juvenile Pacific cod identified based on their early larval otolith signature (10 d post-hatch) collected in the southeastern Bering Sea. Mean (±SE) for Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca are presented. Clusters were identified using hierarchical cluster analysis of the early larval otolith elemental signature.

Figure 5. Classification (DFA; jack-knifed) of juvenile Pacific cod based on otolith elemental composition during their very early life history (10 d post-hatch) in 2006 and 2008. Pie charts represent the proportion of fish from each collection location that was assigned to that location based on the early larval otolith signature. The proportion assigned to their actual collection location is identified by the separated slice with an "*" (mean = 42% in 2006 and 49% in 2008). The arrows indicate the location where the greatest proportion of the individuals that were not assigned to their collection location was assigned. Note no fish were correctly assigned to the AP-W site in 2008.

Figure 6. Classification (DFA; jack-knifed) of juvenile Pacific cod based on otolith elemental composition throughout their early life history in 2006 and 2008. Four discrete stanzas of 50 d were extracted from each individual life history profile. The stanzas reflect specific days of each year (2006: DOY75-125, 126-175, 176-225, 226-275 and 2008: DOY21-70, 71-120, 131-180, 191-240). "NA" indicates that the site was not included due to small sample sizes. "0" indicates classification success.

Figure 1

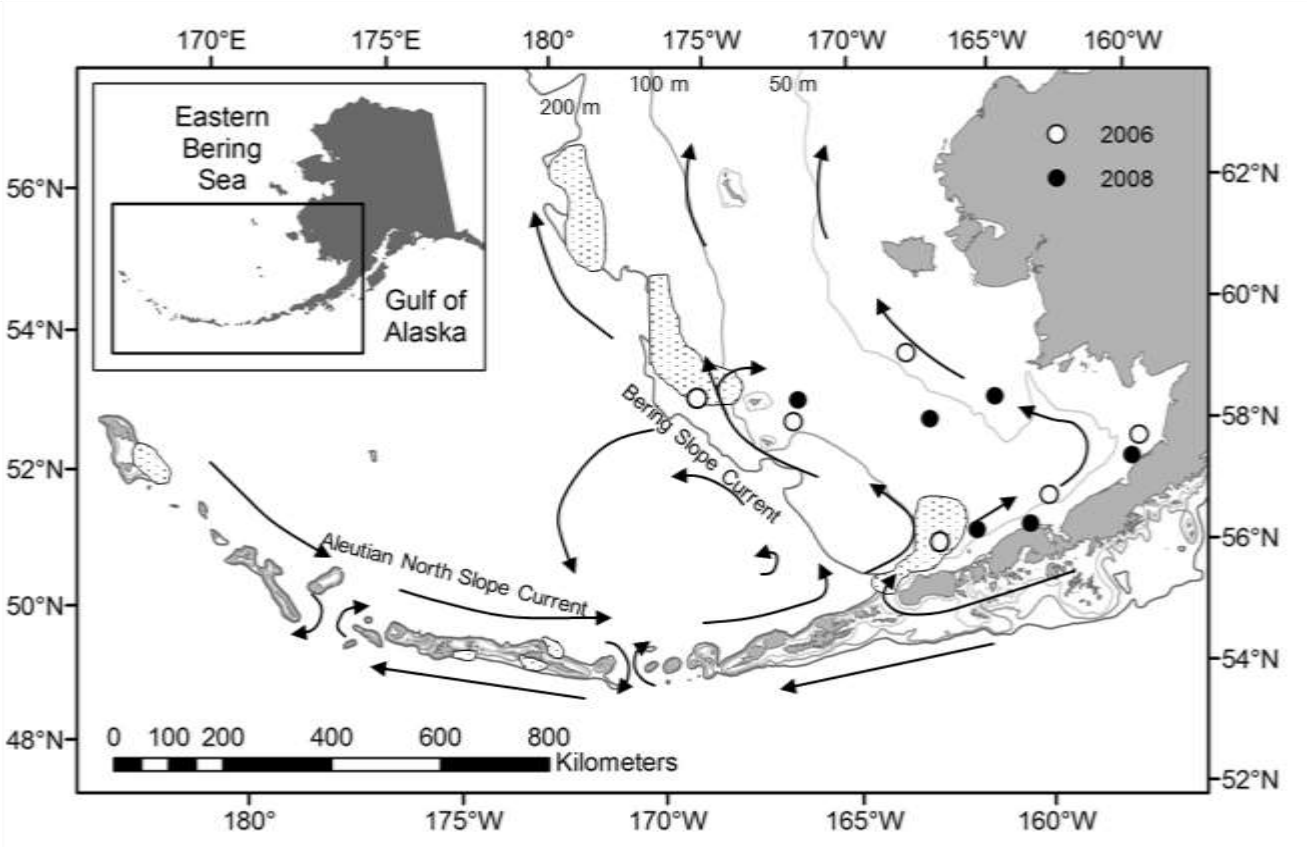

170°E
175°E
180°
175°W
170°W
165°W
160°W
62°N
60°N
58°N
56°N
54°N
52°N
50°N
48°N
200 m
100 m
50 m
2006
2008
Eastern Bering Sea
Gulf of Alaska
Bering Slope Current
Aleutian North Slope Current
0 100 200 400 600 800
Kilometers

Figure 2.

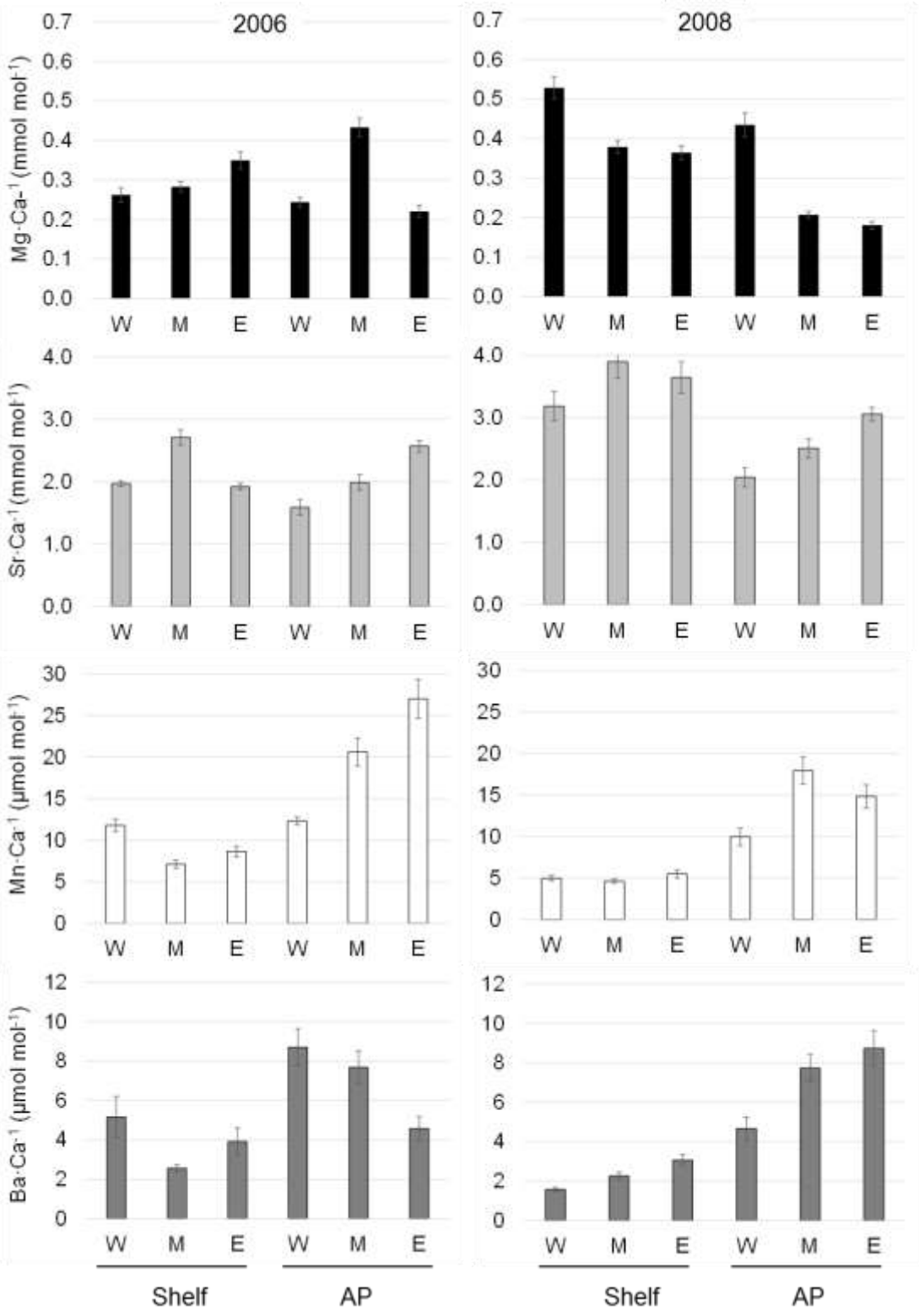

2006
2008
Mg·Ca-1 (mmol mol-1)
Sr·Ca-1 (mmol mol-1)
Mn·Ca-1 (µmol mol-1)
Ba·Ca-1 (µmol mol-1)
W M E W M E
Shelf
AP

Figure 3.

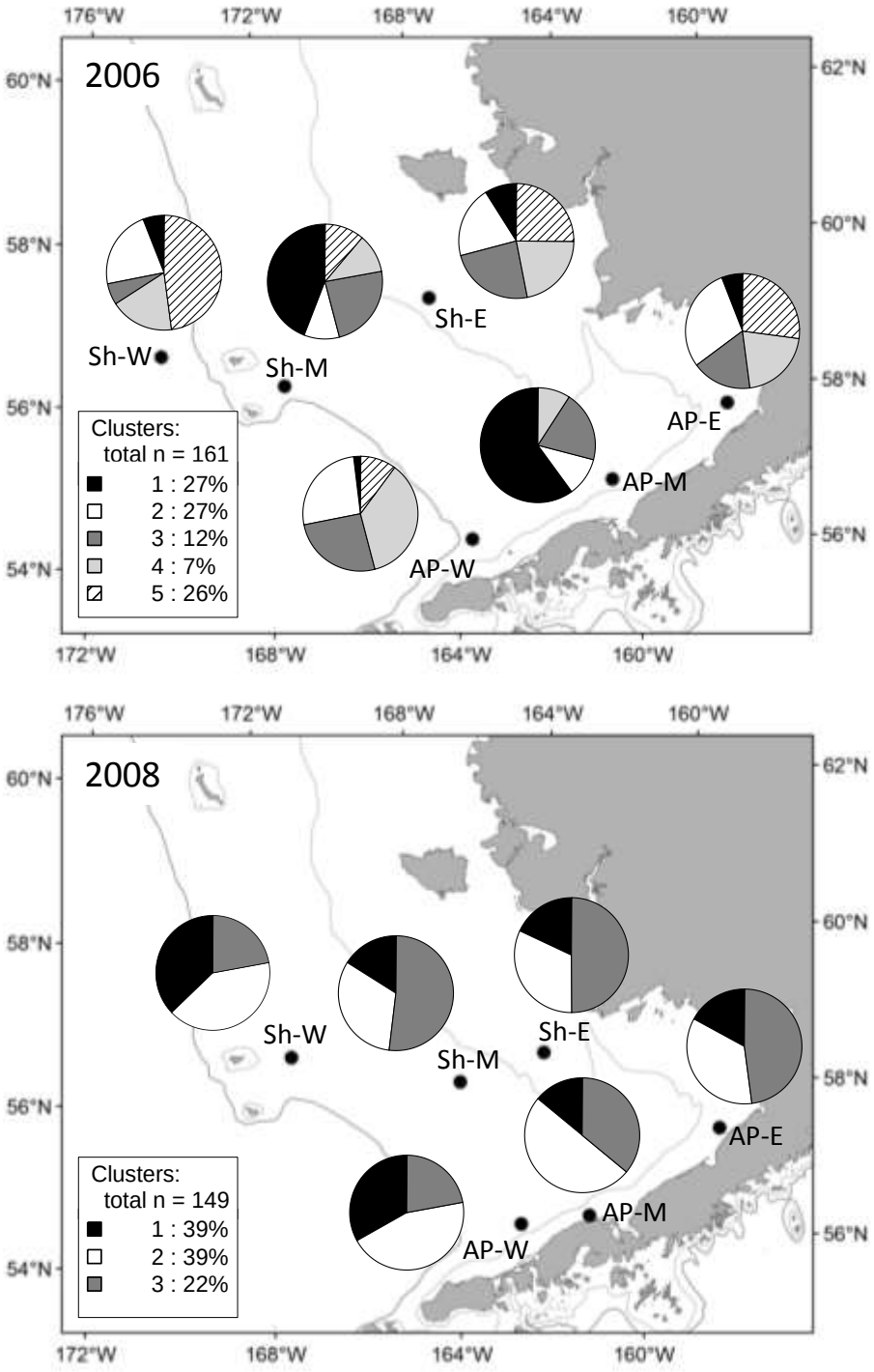

2006
176°W
172°W
168°W
164°W
160°W
60°N
58°N
56°N
54°N
62°N
60°N
58°N
56°N
Sh-W
Sh-M
Sh-E
AP-E
AP-M
AP-W
Clusters:
total n = 161
1 : 27%
2 : 27%
3 : 12%
4 : 7%
5 : 26%
2008
Sh-W
Sh-M
Sh-E
AP-E
AP-M
AP-W
Clusters:
total n = 149
1 : 39%
2 : 39%
3 : 22%

Figure 4.

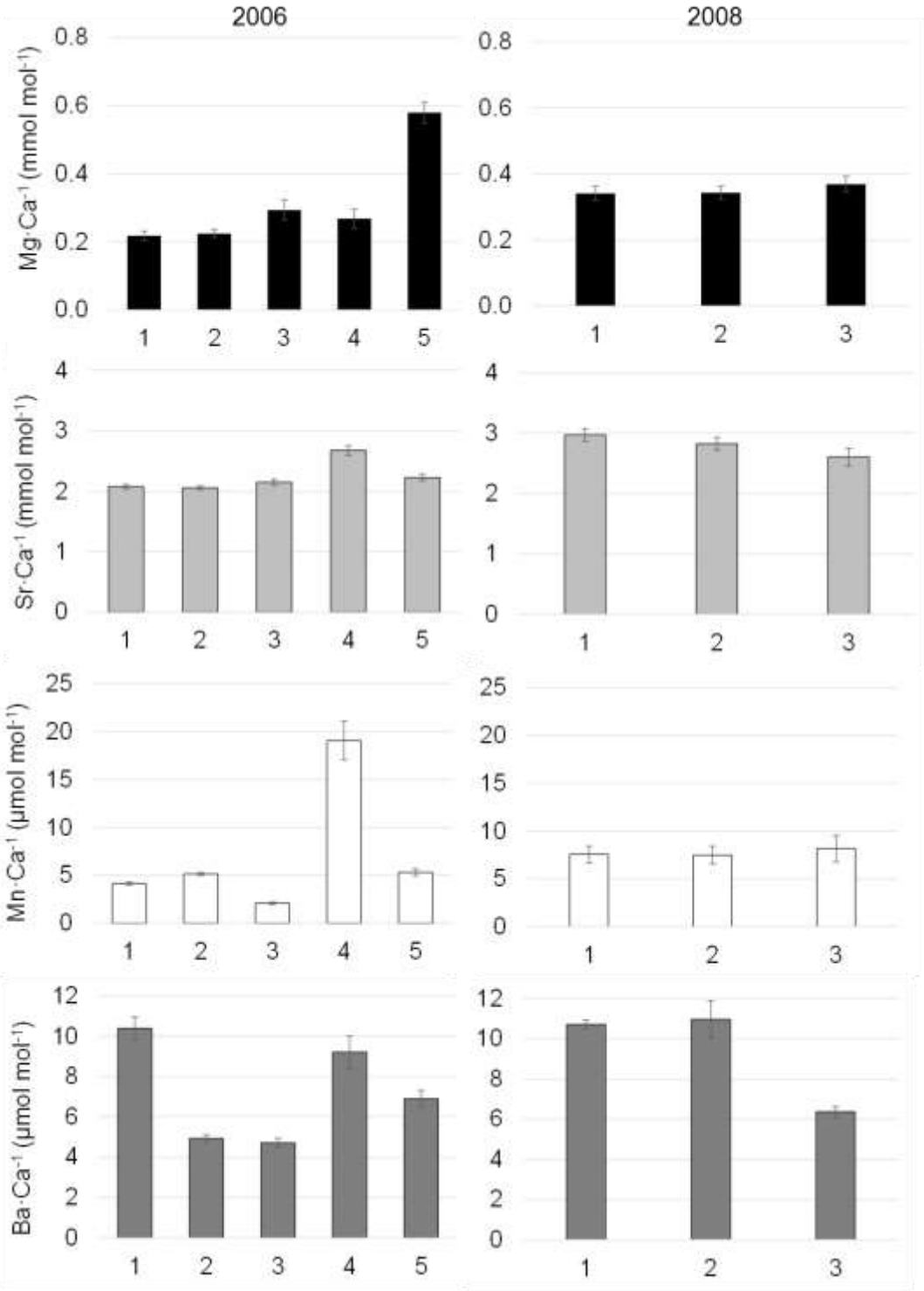

2006
2008
Mg·Ca⁻¹ (mmol mol⁻¹)
Sr·Ca⁻¹ (mmol mol⁻¹)
Mn·Ca⁻¹ (µmol mol⁻¹)
Ba·Ca⁻¹ (µmol mol⁻¹)

Figure 5.

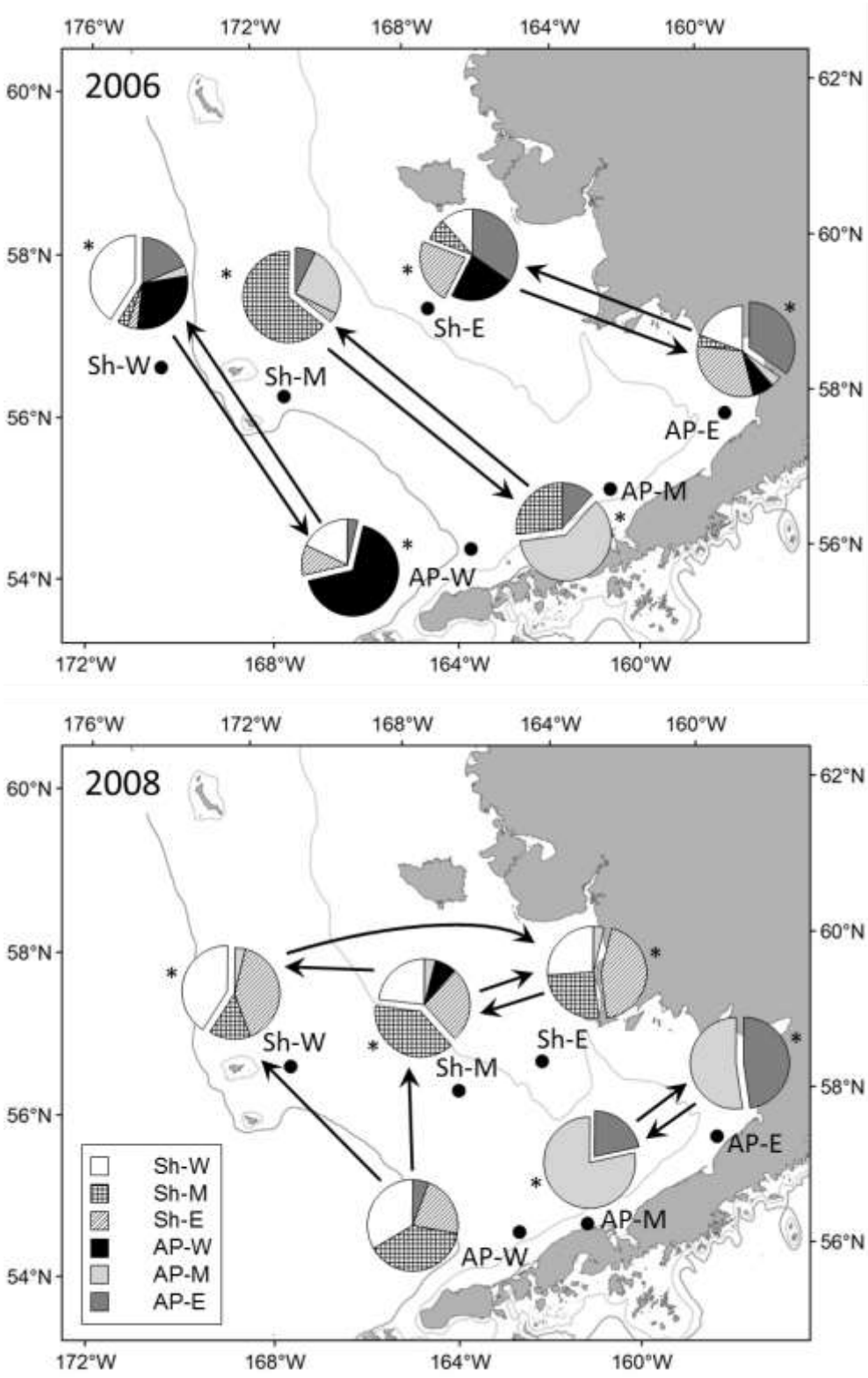
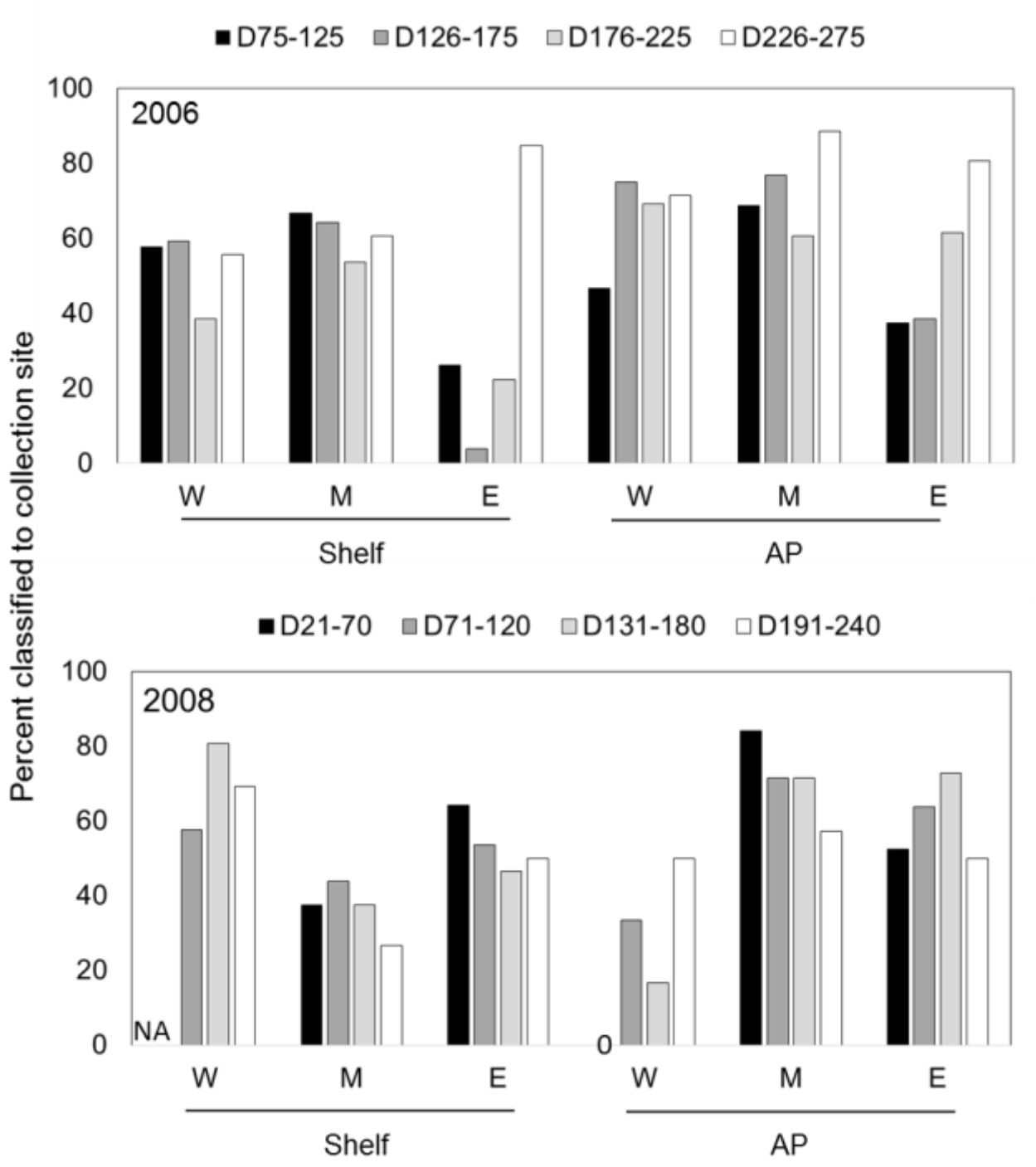

2006
2008
176°W
172°W
168°W
164°W
160°W
60°N
58°N
56°N
54°N
62°N
Sh-W
Sh-M
Sh-E
AP-W
AP-M
AP-E
Sh-W
Sh-M
Sh-E
AP-W
AP-M
AP-E

Figure 6.


D75-125
D126-175
D176-225
D226-275
2006
100
80
60
40
20
0
W
M
E
W
M
E
Shelf
AP
Percent classified to collection site
D21-70
D71-120
D131-180
D191-240
2008
100
80
60
40
20
0
NA
0
W
M
E
W
M
E
Shelf
AP