

Abstract—The population structure of walleye pollock (*Theragra chalcogramma*) in the northeastern Pacific Ocean remains unknown. We examined elemental signatures in the otoliths of larval and juvenile pollock from locations in the Bering Sea and Gulf of Alaska to determine if there were significant geographic variations in otolith composition that may be used as natural tags of population affinities. Otoliths were assayed by using both electron probe microanalysis (EPMA) and laser ablation inductively coupled plasma mass spectrometry (ICP-MS). Elements measured at the nucleus of otoliths by EPMA and laser ablation ICP-MS differed significantly among locations. However, geographic groupings identified by a multivariate statistical approach from EPMA and ICP-MS were dissimilar, indicating that the elements assayed by each technique were controlled by separate depositional processes within the endolymph. Elemental profiles across the pollock otoliths were generally consistent at distances up to 100 μm from the nucleus. At distances beyond 100 μm , profiles varied significantly but were remarkably consistent among individuals collected at each location. These data may indicate that larvae from various spawning locations are encountering water masses with differing physicochemical properties through their larval lives, and at approximately the same time. Although our results are promising, we require a better understanding of the mechanisms controlling otolith chemistry before it will be possible to reconstruct dispersal pathways of larval pollock based on probe-based analyses of otolith geochemistry. Elemental signatures in otoliths of pollock may allow for the delineation of fine-scale population structure in pollock that has yet to be consistently revealed by using population genetic approaches.

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Elemental signatures in otoliths of larval walleye pollock (*Theragra chalcogramma*) from the northeast Pacific Ocean*

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The “stock” concept is a central tenet of modern fisheries science because it represents the fundamental management unit of marine fisheries (Begg and Waldman, 1999). This emphasis, in turn, places a premium on accurate identification of groups of fish whose population statistics are largely independent of other groups. However, stock identification has often proved problematic in marine fishes. For instance, the stock structure of walleye pollock (*Theragra chalcogramma*) across the North Pacific Ocean has been a topic of investigation for many years. Early studies were based on phenotypic characteristics of pollock, such as meristics and morphometrics (Serobaba, 1977; Hinckley, 1987; Temnykh, 1994). Other studies have focused on genotypic markers, such as DNA and allozyme analyses (Grant and Utter, 1980; Mulligan et al., 1992; Shields and Gust, 1995). These approaches resulted in only the broadest characterization of pollock stock structure but have been able to distinguish populations from the eastern and western Pacific (Bailey et al., 1999). Quasi-isolated subpopula-

tions may be at least demographically isolated on smaller spatial scales. For instance, within the Gulf of Alaska, spawning pollock aggregate at specific locations in Shelikof Strait, Prince William Sound, and in the Shumagin Islands region (Bailey et al., 1999). However, the extent of larval dispersal from the spawning sites and the degree of spawning site fidelity of adult pollock to these locations remains unknown.

The difficulties associated with determining stock structure in fishes are essentially the same ones that currently limit our ability to determine population connectivity in marine systems (Thorrold et al., 2002). Tag-recapture studies using tags have limited applicability in the case of pollock. Adults are located deep in the water column and are sensitive to barotrauma during the process of being caught, brought to the surface, and tagged. Traditional population genetic approaches may be similarly

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ineffective because of the low level of exchange required to maintain genetic homogeneity, at least over ecological time scales, and the low level of genetic drift associated with large populations (Waples, 1998; Hellberg et al., 2002). However, preliminary studies have indicated that otolith geochemistry may prove to be a useful natural tag of population structure in walleye pollock (Severin et al., 1995). Otoliths are accretionary crystalline structures located within the inner ear of teleost fish. They are formed through concentric additions of alternating protein and aragonite layers around a central nucleus. The use of otoliths as natural geochemical tags is contingent on the metabolically inert nature of the otolith and the fact that once deposited, otolith material is neither resorbed nor metabolically reworked (Campana and Neilson, 1985; Campana, 1999). The chemical composition of otoliths also reflects to some degree the physicochemical characteristics of the ambient water (Bath et al., 2000). If the water where pollock reside has distinct oceanographic characteristics, then many of the elements incorporated into the otoliths should differ among locations. Migrations between water masses at some age will, therefore, be recorded in the chemical composition of the otolith at the appropriate daily increment. Natural geochemical signatures in otoliths may therefore be useful markers of environmental history throughout the life of the individual and in turn, fish stock composition (e.g., Campana et al., 1995).

The use of geochemical signatures in otoliths as natural tags requires accurate and precise assays of otolith composition. Electron probe micro-analysis (EPMA) has been commonly used for probe-based analyses of otolith chemistry (Gunn et al., 1992). However, detection limits of approximately 100 $\mu\text{g/g}$ limit the technique to a relatively small number of minor elements in otoliths, including Na, Cl, K, and Sr (Campana et al., 1997). Most of the elements measured by EPMA are probably controlled by physiological rather than environmental factors, which may limit their usefulness in stock identification studies (Campana, 1999). Nonetheless, a number of researchers using EPMA have reported geographic differences in otolith chemistry (e.g., Thresher et al., 1994). More recently, attention has focused on inductively coupled plasma mass spectrometry (ICP-MS) to assay elements that are typically below the detection limits of EPMA. Laser ablation ICP-MS uses focused Nd:YAG or excimer lasers to ablate specific locations on the otolith. The vaporized material is then swept up by a carrier gas into a plasma torch and analyzed by mass spectrometry. Limits of detection of the technique are typically on the order of 0.1–1 $\mu\text{g/g}$, allowing for quantification of several elements that cannot be assayed by using EPMA including Mg, Mn, Ba, and Pb (Thorrold et al., 1997; Thorrold and Shuttleworth, 2000). These observations led Campana et al. (1997) to conclude that EPMA and laser ablation ICP-MS were complementary and that there is little overlap in the elements that are accurately measured by the two techniques. Yet few studies of otolith geochemistry have attempted to use both approaches on the same samples.

The objectives of this study are to determine if larval walleye pollock from different geographic localities can be distinguished based on elemental signatures in their otoliths. By analyzing sagittal otoliths with both EPMA and laser ablation ICP-MS, we hoped to identify greater differences among locations than would have been possible by using either technique in isolation. If successful, the study may provide a powerful tool for determining stock structure and tracing migration pathways of walleye pollock in the north Pacific. These data could then be used by managers of one of the world's largest single species fisheries to direct the sustainable harvest of this considerable natural resource.

Materials and methods

All fish used in the study were collected in the spring and summer of 1999 from Alaska Fisheries Science Center research cruises in the Bering Sea and Gulf of Alaska (Fig. 1, Table 1). Fish of birth year 1999 were collected within three months of spawning time to minimize the likelihood of larval transport from other regions. In the case of the Yakutat samples, fresh juvenile pollock were removed from Pacific cod guts. Samples were collected only when the pollock were readily identifiable and heads were intact. Otoliths showed no visible sign of degradation from digestive processes. Juvenile pollock were frozen whole and transported to the laboratory for otolith removal.

Otoliths were removed from the fish and mounted on petrographic slides in LR White resin (acrylic, hard-grade). Larval otoliths were ground on one side to expose the nucleus by using 500-grit paper and were polished with 0.25- μm grit diamond paste. Juvenile otoliths were ground and polished in the sagittal plane on both sides to maximize clarity of the nucleus during microanalysis.

Electron probe microanalysis

After having been polished, the otoliths were cleaned with Formula 409® and coated with a 30-nm layer of carbon. They were subsequently analyzed with a Cameca SX-50 electron microprobe equipped with four wavelength dispersive spectrometers. A 15keV, 10 nA, 4- μm diameter beam was used for all analyses. Counting times, standards, detection limits, and analytical errors are summarized in Table 2. Although Mg was analyzed in all otoliths, in most cases it was below detection limits and was therefore not used in the statistical analysis.

Laser ablation ICP-MS

After having been ground and polished, otolith sections were decontaminated before elemental analysis by using laser ablation ICP-MS. Sections were rinsed in ultrapure water, scrubbed with a nylon brush in a solution of ultrapure H_2O , triple rinsed with ultrapure 1% HNO_3 ,

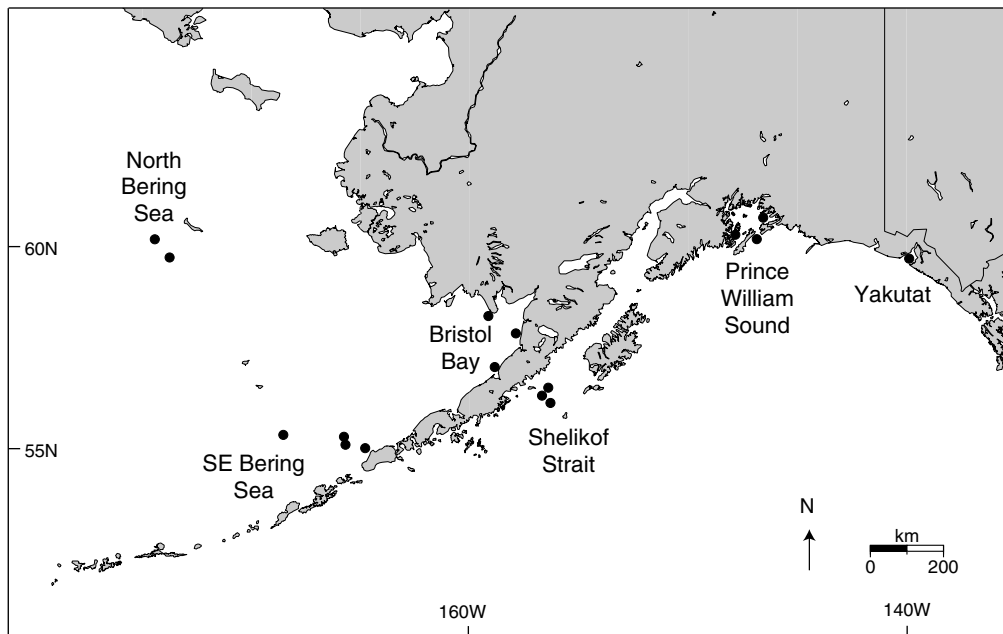


Figure 1

Locations of sampling sites for larval and juvenile walleye pollock (*Theragra chalcogramma*) in the Gulf of Alaska and Bering Sea.

sonified for 5 minutes in ultrapure H₂O, and finally triple rinsed again with Milli-Q water. The section was dried under a positive flow hood for 24 hours and stored in a polyethylene bag.

Elemental analyses were conducted with a Finnigan MAT Element2 magnetic sector field ICP-MS and Merchantek EO LUV266X laser ablation system (Thorrold and Shuttleworth, 2000). Instrument set-up was similar to that outlined by Günther and Heinrich (1999). An Ar gas stream was used to carry ablated material from the laser cell to the ICP-MS. The carrier gas was then mixed with the Ar sample gas and a wet aerosol (1% HNO₃) in the concentric region of the quartz dual inlet spray chamber. The wet aerosol was supplied by a self-aspirating PFA micro-flow (20 μ L/min) nebulizer attached to a CETAC ASX100 autosampler. Diameter of the 266-nm laser beam was nominally 5 μ m, repetition rate was 5 Hz, and the scanning rate was set at 5 μ m/sec.

A typical run for an individual otolith consisted of a blank sample (1% HNO₃ only), a standard sample, five laser samples, and then another blank and standard. The number of laser samples in a run ranged from 5 to 15, depending upon the size of the otolith. All laser runs began with a 70 μ m \times 70 μ m raster, centered on the otolith nucleus. The laser software was then used to trace out concentric lines, 720 μ m in length and approximately 40 μ m apart, which followed the contour of individual growth increments from the raster to the otolith edge. This approach produced reasonably high spatial resolution (30–50 μ m) for life history scans across otoliths while allowing sufficient acquisition time to maintain measurement precision.

We examined Mn/Ca, Sr/Ca, and Ba/Ca ratios in the pollock otoliths by monitoring ⁴⁸Ca, ⁵⁵Mn, ⁸⁶Sr, and ¹³⁸Ba. Quantification followed the approach outlined by Rosenthal et al. (1999) for precise element/Ca ratios using sector field ICP-MS (Thorrold et al., 2001). Quality control was maintained by assaying a dissolved aragonite standard (Yoshinaga et al., 2000) every five samples. The standard was introduced at the appropriate time by moving the autosampler probe from the solution containing the 1% HNO₃ to the standard solution, while maintaining the carrier gas flow through the ablation cell. Elemental mass bias was calculated by reference to known values of the standard, and a correction factor was then interpolated and applied to the laser samples bracketed between adjacent standard measurements. Average ($n=40$) within-run precisions (RSD) of the standard measurements were all less than 1% (Mn/Ca: 0.16%, Sr/Ca: 0.16%, and Ba/Ca: 0.33%). Long-term (5-month) estimates of the standard measurements ($n=40$), again uncorrected for changes in mass bias over time, were less precise (Mn/Ca: 5.6%, Sr/Ca: 3.7%, and Ba/Ca: 5.6%). However, laser samples were corrected for changes in mass bias by using the laboratory standard. Precision of the technique was approximately 1% for all the ratios that we measured.

Statistical analyses

All elemental data were initially examined for normality and homogeneity of variance by using residual analysis (Winer, 1971) and were found to conform to the assumptions of ANOVA without the need for data transformation. We therefore assumed that require-

Table 1

Location, collection date, standard length range (mm), and sample sizes (n) of larval and juvenile walleye pollock (*Theragra chalcogramma*) captured from the southeast Bering Sea (SE Bering), North Bering Sea (N Bering), Bristol Bay, Shelikof Strait, Prince William Sound (PWS), and Yakutat, and analyzed by laser ablation ICP-MS (ICP-MS) and electron probe microanalysis (EPMA).

Area	Date	SL range (mm)	n (total)	n (ICP-MS)	n (EPMA)
SE Bering	23 May–27 July 1999	5.3–42.2	117	8	30
N Bering	18–23 July 1999	15.6–30.7	45	9	25
Bristol Bay	22–24 July 1999	85.1–135.7	75	11	28
Shelikof	27–28 May 1999	3.6–7.9	46	—	25
PWS	7 July–19 August 1999	35.2–66.0	11	4	6
Yakutat	15 July, 1999	—	50	6	24

Table 2

Counting times for each element (time [seconds], standards, limits of detection [LOD, %weight, 99% confidence limits] and analytical errors [Error, %weight, 1 standard deviation]) for electron probe microanalysis (EPMA). Detection limits and analytical errors were calculated by following the procedures of Scott et al. (1995). N/A = not applicable.

Element	Time	Standard	LOD	Error
Na	60	Halite (CM Taylor)	0.029	0.023
Mg	60	Osumilite (USNM 143967)	0.019	0.022
P	60	Apatite (Wilberforce)	0.036	0.027
S	60	Gypsum (CM Taylor)	0.023	0.017
Cl	46	Halite (CM Taylor)	0.027	0.015
K	46	Osumilite (USNM 143967)	0.019	0.012
Ca	20	Calcite (NMNH 136321)	N/A	0.245
Sr	120	Strontianite (Smithsonian R-10065)	0.036	0.019

ments for the MANOVA were also met by the data. Among-location differences in the elemental composition of larval pollock in specific regions of the otoliths were compared by using one-factor multivariate analysis of variance (MANOVA) and one-factor analysis of variance (ANOVA). We treated location as a fixed factor in both MANOVA and ANOVA tests. Because of difficulties collecting pollock larvae, we were unable to achieve equal replication of sites within locations. We therefore pooled samples from collections within a location by randomly selecting fish from each location for subsequent analysis. However, the lack of replication at the within-location level necessarily restricted our ability to draw general conclusions concerning spatial variability in otolith composition beyond the samples analyzed in the present study. All *a posteriori* comparisons among locations were performed by using Tukey's honestly significant difference (HSD) test (experimentwise error rate=0.05). Multivariate differences in elemental signatures from the MANOVA were visualized by using canonical discriminant analyses (CDA). All analyses were conducted by using the SAS statistical program (SAS, version 6, 1990, SAS Inst. Inc., Cary, NC).

Comparisons of elemental profiles across otoliths were made with repeated measures ANOVA. We tested the

following null hypotheses: 1) there was no variation in trace element profiles across individual otoliths (i.e., from the nucleus to the edge), 2) there were no differences in mean element concentrations among locations, determined by averaging data across individual otolith profiles, and 3) there were no differences in the pattern of element profiles across otoliths among locations. Otolith profiles with missing values were removed, and therefore we were able to use MANOVA for the repeated measures analysis. The multivariate approach to repeated measures is generally more conservative than univariate repeated measures analysis. However, the multivariate test does not assume sphericity of orthogonal components, requiring only that the data conform to multivariate normality with a common covariance matrix for individual larvae at each location (Littell et al., 1991). The approach still requires that adjacent points on the trajectories be equidistant. Therefore samples from EPMA were assigned to a distance category at intervals of 15 μm (0 μm , 15 μm , 30 μm , 45 μm , 60 μm , 75 μm , and 90 μm) across the otolith, to a distance of 90 μm from the nucleus. Samples were averaged when more than one measurement was available within a distance category. Laser ablation ICP-MS samples were assigned to a distance category at intervals of approxi-

Table 3

EPMA results of one-factor ANOVA (degrees of freedom [df]; sums of squares [SS]; mean square [MS]) at two positions (0–20 μm and 20–45 μm from the nucleus) in otoliths of larval walleye pollock (*Theragra chalcogramma*) collected from six locations: three locations in the Bering Sea (southeast Bering Sea [SB]; North Bering Sea [NB]; Bristol Bay [BB]) and three in the Gulf of Alaska (Prince William Sound [PW]; Shelikof Strait [SH]; and Yakutat [YK]). ***= significant at $\alpha = 0.05$; ns = nonsignificant. *A posteriori* multiple comparisons among locations were conducted by using Tukey's honestly significant difference (HSD). Locations are ordered (left to right) from lowest to highest concentrations, and lines link locations that are not significantly different (experimentwise error rate=0.05).

Element	Source	df	SS	MS	F	P < F	Tukey's HSD
0–20 μm							
Na	Location	5	24.7	4.9	16.37	***	<u>PW BB YK NB SB SH</u>
	Error	113	34.1	3.0×10^{-1}			
P	Location	5	18.3	3.7	14.93	***	<u>BB SH NB SB PW YK</u>
	Error	113	27.7	2.5×10^{-1}			
S	Location	5	2.0	3.9×10^{-1}	5.04	***	<u>PW BB NB YK SH SB</u>
	Error	113	8.8	7.8×10^{-2}			
Cl	Location	5	10.2	2.0	3.93	***	<u>PW NB BB YK SB SH</u>
	Error	113	58.6	5.2×10^{-1}			
K	Location	5	2.4	4.8×10^{-1}	4.01	***	<u>SH NB SB PW BB YK</u>
	Error	113	13.51	1.2×10^{-1}			
Sr	Location	5	7.1	1.4	6.66	***	<u>YK NB BB SH PW SB</u>
	Error	113	24.1	2.1×10^{-1}			
20–45 μm							
Na	Location	4	15.9	4.0	26.66	***	<u>BB PW YK SB NB</u>
	Error	93	13.9	1.5×10^{-1}			
P	Location	4	14.9	3.7	17.07	***	<u>BB NB SB PW YK</u>
	Error	93	20.3	2.2×10^{-1}			
S	Location	4	1.1	2.8×10^{-1}	4.62	***	<u>BB PW SB NB YK</u>
	Error	93	5.7	6.1×10^{-2}			
Cl	Location	4	8.4×10^{-1}	2.1×10^{-1}	1.61	ns	<u>PW SB NB BB YK</u>
	Error	93	12.1	1.3×10^{-1}			
K	Location	4	1.9	4.7×10^{-1}	5.65	***	<u>NB PW BB SB YK</u>
	Error	93	7.7	8.3×10^{-2}			
Sr	Location	4	9.0×10^{-1}	2.2×10^{-1}	3.75	***	<u>NB YK BB PW SB</u>
	Error	93	5.6	6.0×10^{-2}			

mately 40 μm (nucleus, 40–80 μm , 80–120 μm , 120–160 μm , and 160–200 μm) across the otolith, to a distance of 200 μm from the nucleus.

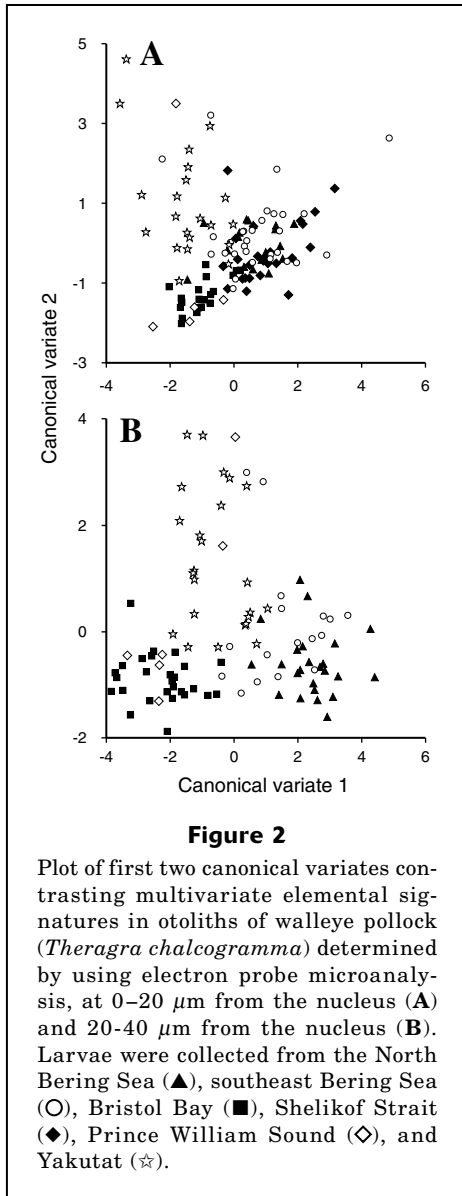
Results

Electron probe microanalysis

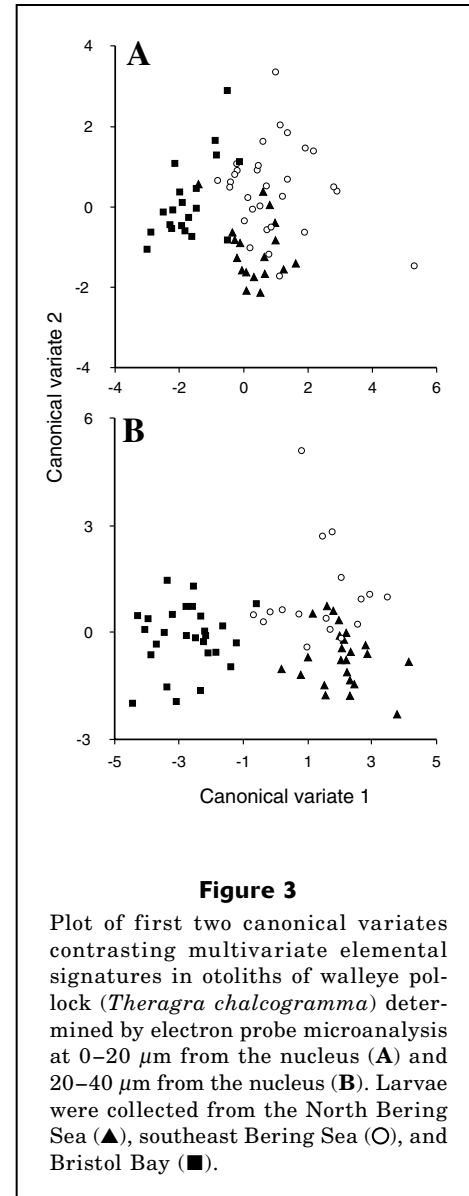
A total of six elements (Na, P, S, Cl, K, and Sr) were quantified in the otoliths of pollock larvae by using EPMA. Average concentrations of the elements ranged from approximately 4 mg/g (otolith weight) for Na to less than 1 mg/g for Cl, S, and K. We found significant differences in the elemental composition both among sampling locations and across positions on the otoliths. Multivariate analyses of elemental signatures revealed significant differences among locations from samples 0–20 μm from the nucleus (MANOVA; Pillai's trace=1.18; $F_{30,560}=5.74$; $P<0.0001$), and 20–45 μm from

the nucleus (MANOVA; Pillai's trace=1.47; $F_{24,364}=8.86$; $P<0.0001$).

Analysis of variance and Tukey's HSD *a posteriori* multiple comparison tests identified the individual elements contributing to differences in the multivariate signatures among locations. All six elements showed significant differences among locations at 0–20 μm from the otolith nucleus (Table 3). Multiple comparisons for each of the elements indicated relatively subtle differences among locations. Phosphorus concentrations were, however, significantly higher in samples from Yakutat than any of the other locations. Results of the ANOVA from samples at distances 20–45 μm from the nucleus were generally comparable with samples closer to the nucleus (Table 3). Although only Cl showed no significant variation among locations (Table 3), multiple comparisons of mean values for each element revealed little geographic patterns among locations. Note that Shelikof Strait samples were removed from this analysis because of a small sample size in this distance category.



We used CDA to visualize multivariate differences among locations in reduced dimensional space. Three groups were readily discernible in a plot of the first two canonical variates (Fig. 2). Samples from the North Bering Sea, the southeast Bering Sea, and Shelikof Strait formed one group separated from Yakutat, Bristol Bay, and Prince William Sound samples along the first canonical variate. The second canonical variate separated Yakutat samples from Bristol Bay and Prince William Sound individuals. Elemental signatures at 20–45 μm from the otolith nucleus were distributed similarly in canonical space to samples from the otolith nucleus (Fig. 2). Three groupings were apparent in the canonical plot, and Bering Sea larvae were separated from Bristol Bay and Prince William Sound samples on canonical variate one, and Yakutat samples were separated from all other locations on canonical variate two. We then



conducted a similar analysis with only samples from the southeast and North Bering Sea and Bristol Bay. Elemental signatures of larvae from the Bering Sea separated from Bristol Bay on canonical variate one. The southeast Bering Sea samples separated from the North Bering Sea along canonical variate two, although not as clearly as with the elemental signatures from the Bering Sea and Bristol Bay (Fig. 3).

Elemental profiles across otoliths varied significantly, as determined by repeated measures ANOVA, among the five locations for Na, P, S, and Sr (Table 4). Both S and Sr concentrations declined from high values at the nucleus to significantly lower values towards the edge of the otolith (Fig. 4). Repeated measures ANOVA also provided a test of the differences among locations when data were averaged over the otolith profiles. Significant differences among locations were detected for

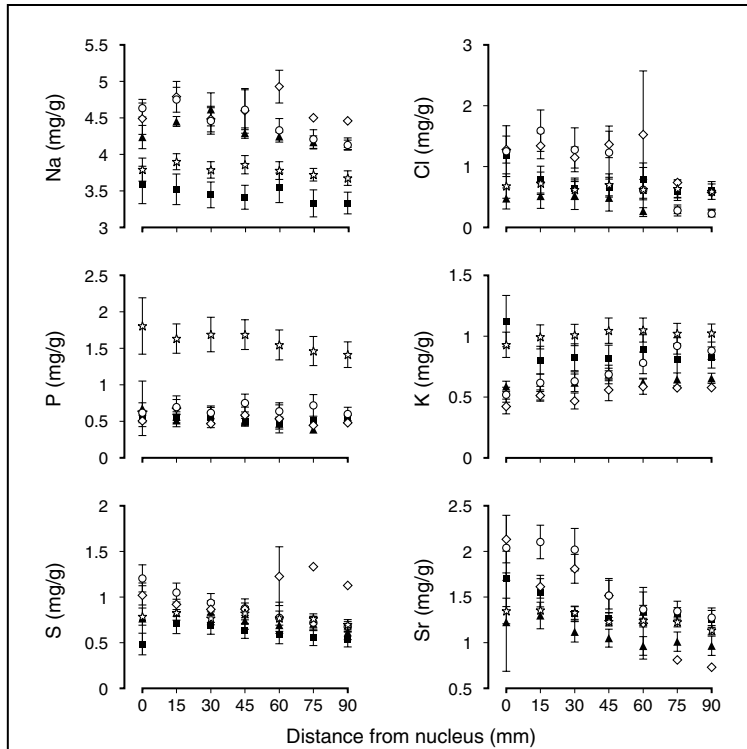


Figure 4

Profiles of elemental concentrations, determined by electron probe microanalysis, from the nucleus out to a distance of approximately 90 μm in the otoliths of larval walleye pollock (*Theragra chalcogramma*) collected from the North Bering Sea (▲), southeast Bering Sea (○), Bristol Bay (■), Prince William Sound (◇), and Yakutat (☆). Individual points are mean (\pm SE) values grouped at 15- μm intervals.

five elements (Sr, K, S, P, and Na). Finally, the interaction term (position \times location) in the repeated measures ANOVA tested the hypothesis that the shape of the elemental profiles differed among locations. There was a significant interaction between profile and location for K.

Laser ablation ICP-MS

We quantified Mn/Ca, Sr/Ca, and Ba/Ca ratios in the otoliths of larval walleye pollock using laser ablation ICP-MS. Both Mn and Ba were found at trace levels in otoliths, with average values of approximately 3 $\mu\text{mol/mol}$ and 6 $\mu\text{mol/mol}$, respectively. Strontium was present in the otoliths at an average concentration of approximately 2.2 mmol/mol. A MANOVA detected significant differences among locations from a raster centered on the nucleus (MANOVA; Pillai's trace=0.85; $F_{12,99}=3.26$; $P<0.0005$), and from the average values of lines 40–80 μm from the nucleus (MANOVA; Pillai's trace=0.99; $F_{12,99}=4.1$; $P<0.0001$).

Univariate ANOVA and *a posteriori* multiple comparisons by using Tukey's HSD revealed that Mn/Ca, Sr/Ca,

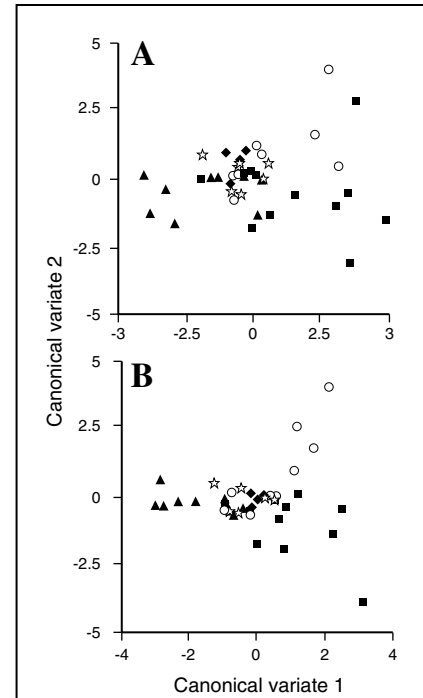


Figure 5

Plot of the first two canonical variates contrasting multivariate elemental signatures in otoliths of walleye pollock (*Theragra chalcogramma*) determined with laser ablation ICP-MS, at 0–40 μm from the nucleus (A) and 40–80 μm from the nucleus (B). Larvae and juveniles were collected from the North Bering Sea (▲), southeast Bering Sea (○), Bristol Bay (■), Prince William Sound (◆), and Yakutat (☆).

and Ba/Ca ratios varied significantly among locations at the otolith nucleus and at positions 40–80 μm from the nucleus (Table 5). Samples from the North Bering Sea had consistently lower Sr/Ca and Ba/Ca ratios than those from the southeast Bering Sea at both positions. However, we noted only subtle differences among the Gulf of Alaska and Bristol Bay samples.

We found a total of three groupings in canonical plots of multivariate elemental signatures from the otoliths of larval walleye pollock (Fig. 5). Samples from the North Bering Sea and Bristol Bay were separated along canonical variate one. A third grouping, including larvae from the southeast Bering Sea, Prince William Sound, and Yakutat, clustered together in the center of the canonical plot. Samples from the nucleus and 40–80 μm outside the nucleus showed very similar geographic patterns.

Repeated measures ANOVA detected significant differences in both Mn/Ca and Ba/Ca profiles from the nu-

Table 4

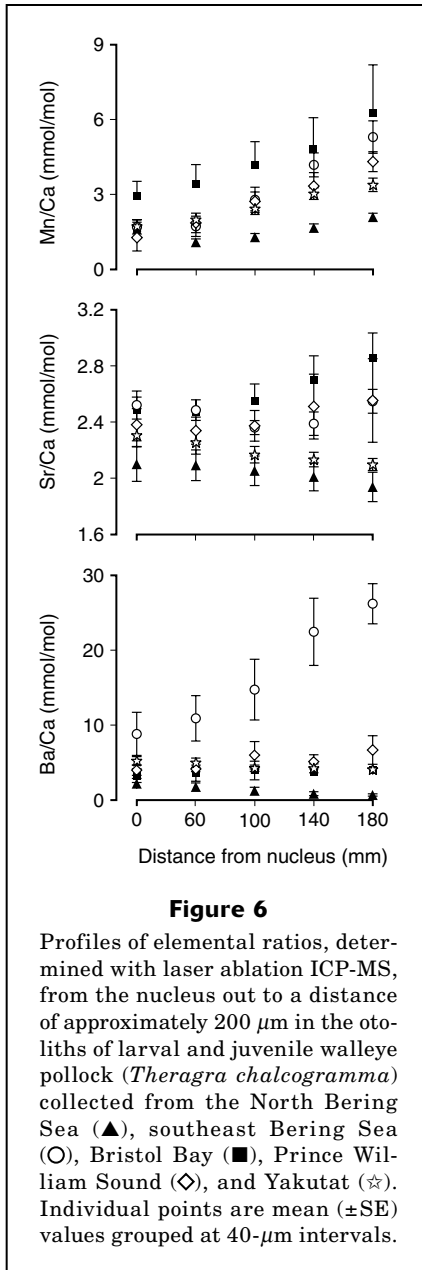
EPMA results from repeated-measures ANOVA of elemental profiles across otoliths from walleye pollock (*Theragra chalcogramma*) larvae collected at five locations in the Bering Sea and the Gulf of Alaska. Within-subject effects (profile and profile×location) tested by using MANOVA (Pillai's trace), and between subjects effect (location) tested by using ANOVA (degrees of freedom [df]; sums of squares [SS]; mean squares [MS]). *** = significant at $\alpha = 0.05$; ns = nonsignificant.

Element	Source	df	Pillai's trace or SS	MS	F	P < F
Na	Profile	6, 41	4.9×10^{-1}		6.67	***
	Profile×location	24, 176	5.3×10^{-1}		1.12	ns
	Location	4	43.8	10.9	10.35	***
	Error	46	48.7	1.1		
P	Profile	6, 40	3.0×10^{-1}		2.91	***
	Profile×location	24, 172	4.6×10^{-1}		0.93	ns
	Location	4	55.0	13.8	8.07	***
	Error	45	76.8	1.7		
S	Profile	6, 41	3.6×10^{-1}		3.92	***
	Profile×location	24, 176	5.3×10^{-1}		1.12	ns
	Location	4	3.8	9.6×10^{-1}	4.56	***
	Error	46	9.7	2.1×10^{-1}		
Cl	Profile	6, 39	1.8×10^{-1}		1.41	ns
	Profile×location	24, 168	3.7×10^{-1}		0.72	ns
	Location	4	8.0	2.0	1.6	ns
	Error	44	55.3	1.3		
K	Profile	6, 41	8.3×10^{-2}		0.62	ns
	Profile×location	24, 176	8.5×10^{-1}		1.98	***
	Location	4	4.3	1.1	2.61	***
	Error	46	19.1	4.1×10^{-1}		
Sr	Profile	6, 40	4.5×10^{-1}		5.51	***
	Profile×location	24, 172	7.7×10^{-1}		1.72	ns
	Location	4	6.4	1.6	3.96	***
	Error	45	18.2	4.0×10^{-1}		

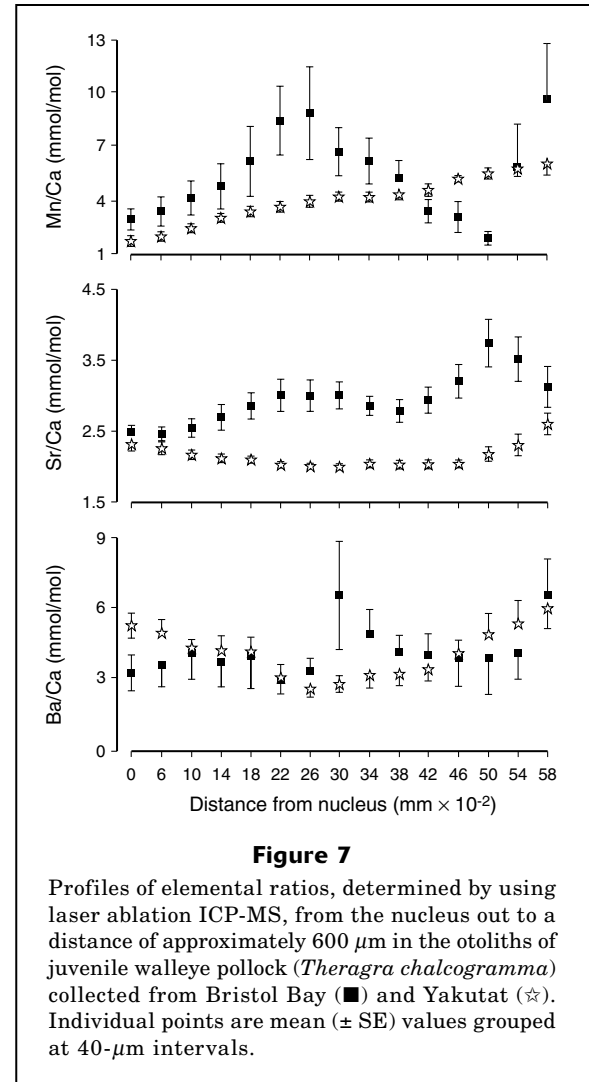
Table 5

Laser ablation ICP-MS results of one-factor ANOVA (degrees of freedom [df]; sums of squares [SS]; mean square [MS]) at two positions (0–40 μm and 40–80 μm from the nucleus) in walleye pollock (*Theragra chalcogramma*) otoliths collected at five locations: the southeast Bering Sea [SB], North Bering Sea (NB), Bristol Bay (BB), Prince William Sound (PW), and Yakutat (YK). ***= significant at $\alpha = 0.05$; ns=nonsignificant. Multiple comparisons among locations were conducted by using Tukey's honestly significant difference (HSD). Locations were ordered (left to right) from lowest to highest ratios; lines link locations that were not significantly different ($\alpha=0.05$).

Element	Source	df	SS	MS	F	P < F	Tukey's HSD
0–40 μm (nucleus)							
Mn/Ca	Locations	4	19.3	4.82	3.5	***	PW NB SB YK BB
	Error	33	45.5	1.38			
Sr/Ca	Locations	4	1.07	0.27	3.43	***	NB YK BB PW SB
	Error	33	2.58	0.08			
Ba/Ca	Locations	4	219	54.8	3.35	***	NB BB PW YK SB
	Error	33	540	16.4			
40–80 μm							
Mn/Ca	Locations	4	30.7	7.68	3.24	***	NB PW SB YK BB
	Error	33	78.3	2.37			
Sr/Ca	Locations	4	0.99	0.25	3.70	***	NB YK PW BB SB
	Error	33	2.21	0.07			
Ba/Ca	Locations	4	397	99.2	5.50	***	NB BB PW YK SB
	Error	33	595	18.0			



nucleus out to a distance of approximately 200 μm in the walleye pollock otoliths (Fig. 6, Table 6). The univariate test of location, averaged over the individual otolith profiles, was significant for both Sr/Ca and Ba/Ca. We also found significant interactions between profile and location for Mn/Ca, Sr/Ca, and Ba/Ca ratios (Table 6). Manganese values increased from the nucleus to the otolith edge at all locations, indicating that the significant interaction was generated by the observation that the profile from the North Bering Sea was considerably flatter than profiles from Bristol Bay and southeast Bering Sea. Strontium trajectories were more dynamic; profiles from some locations increased from the nucleus to the edge (Bristol Bay and Prince Williams Sound),



profiles from other locations decreased (North Bering Sea and Yakutat), and a single location (southeast Bering Sea) showed no obvious trend. Finally, profile variations in Ba/Ca ratios among locations were dominated by a sharp increase in Ba/Ca ratios across the otoliths in the southeast Bering Sea samples. Profiles were effectively horizontal for the other four locations.

Otoliths in walleye pollock collected from Bristol Bay and Yakutat were significantly larger than those from the other four locations. We were, therefore, able to conduct extended profiles in these otoliths out to a distance of approximately 600 μm (Fig. 7). After starting at similar values at the nucleus, Mn/Ca and Sr/Ca profiles from the two locations quickly diverged and appeared to vary largely independently over the remaining time periods. The Ba/Ca profiles also appeared to be varying independently between the two locations, although the relative magnitude of differences between the two locations was smaller than for either Mn/Ca or Sr/Ca profiles.

Table 6

Laser ablation ICP-MS results from repeated-measures ANOVA of elemental profiles across otoliths from walleye pollock (*Theragra chalcogramma*) larvae collected at five locations in the Bering Sea and the Gulf of Alaska. Within-subject effects (profile and profile×location) were tested by using MANOVA (Pillai's trace), and between subjects effect (location) tested by using ANOVA (degrees of freedom [df]; sums of squares [SS]; mean squares [MS]). *** = significant at $\alpha = 0.05$; ns = nonsignificant.

Element	Source	df	Pillai's trace or SS	MS	F	P < F
Mn/Ca	Profile	4, 30	3.0×10^{-1}		17.1	***
	Position×location	16, 132	7.4×10^{-1}		1.9	***
	Location	4	1.99×10^2	49.7	2.49	ns
	Error	33	6.61×10^{-2}	20.1		
Sr/Ca	Profile	4, 30	2.18×10^{-1}		2.09	ns
	Position×location	16, 132	9.19×10^{-1}		2.46	***
	Location	4	1.05×10^1	2.62	6.02	***
	Error	33	1.44×10^1	0.44		
Ba/Ca	Profile	4, 30	6.92×10^{-1}		16.83	***
	Position×location	16, 132	1.04		2.9	***
	Location	4	5.83×10^3	1459	15.25	***
	Error	33	3.16×10^3	95.6		

Discussion

Quantifying dispersal pathways of larval fishes in marine environments is a difficult proposition. Marine fishes typically produce on the order of 10^4 – 10^6 eggs in a single spawning episode. These propagules are quickly dispersed in large volumes of seawater, making recovery of marked individuals difficult even if it were possible to introduce an artificial tag into the larvae at the time of spawning (Jones et al., 1999). Natural geochemical signatures in otoliths offer a useful alternative to artificial tagging approaches (Thorrold et al., 2002). The technique relies upon the assumption that larvae spawned at any given location retain a unique elemental or isotopic signature in their otoliths that can be recovered some time afterwards, and that variations in otolith geochemistry are sufficient to distinguish among geographic locations of interest. We found that elemental signatures in the otoliths of larval walleye pollock differed significantly geographically and with ontogeny. Samples at specific points on the otoliths, at the nucleus, and shortly after hatching, showed very similar patterns of variability, suggesting that the technique will likely be a robust method for identifying natal origins of walleye pollock after suitable groundtruthing of known spawning locations (e.g., Thorrold et al., 2001; Gillanders, 2002).

Elemental signatures in the otoliths of the larval pollock were assayed by using EPMA and laser ablation ICP-MS. Campana et al. (1997) noted that the two techniques were largely complementary in terms of elements that could be reliably assayed, and indeed only Sr was able to be quantified by both instruments in our study. Patterns of geographic variability presumably reflected the elements that were used in generating the multivariate signatures produced by each instrument. Elemental signatures from the EPMA data clustered

into a Bering Sea group that included Shelikof Strait but excluded Bristol Bay, a coastal grouping that included Bristol Bay and Prince William Sound, whereas samples from Yakutat were separated from all other locations. Although sample sizes were smaller for the laser ablation ICP-MS assays, the data identified a grouping of locations in multivariate space that included the southeast Bering Sea, Prince William Sound, and Yakutat, whereas samples from both the north Bering Sea and Bristol Bay were separated from each other and the other locations. The observation that locations did not cluster into similar geographic groupings was probably a function of different mechanisms of elemental incorporation in otoliths. Elements assayed by ICP-MS in the present study substitute for Ca in the aragonitic lattice, and are believed to primarily reflect ambient physicochemical differences among natal locations (Bath et al., 2000; Milton and Chenery, 2001; Bath Martin et al., 2003). However, with the exception of Sr, the elements assayed by EPMA are likely under physiological regulation and therefore probably do not directly reflect either water chemistry or temperature (Campana, 1999). In either case, the application of elemental signatures in otoliths as natural tags of natal origins requires only that the signatures allow accurate classification of the natal origins of an unknown fish. A final caveat is necessary because it remains possible that preservation effects, particularly for labile elements that are not incorporated into the aragonite lattice, may also have contributed to at least some of the differences among locations (Milton and Chenery, 1998; Proctor et al., 1998). If present, such effects would clearly confound attempts to use elemental signatures as a natural tag of natal origins (Thresher, 1999).

It is important to note that although EPMA and laser ablation ICP-MS provided complementary information

on elemental composition, the spatial scale on which the data were gathered was different. Our laser ablation ICP-MS method required that we ablate a $70\ \mu\text{m} \times 70\ \mu\text{m}$ raster, or a $720\text{-}\mu\text{m}$ line, in order to enable sufficient time to generate precise estimates of otolith composition. The EPMA analysis was less destructive than laser ablation ICP-MS, and therefore it was possible to sample individual points at a much finer spatial resolution ($\sim 5\ \mu\text{m}$), albeit with considerably less sensitivity and precision. For instance, using EPMA we were able to sample five points across a transect ending approximately $90\ \mu\text{m}$ from the nucleus. In contrast, only a single raster could be sampled along this profile with laser ablation ICP-MS. Although the diameter of laser probes is approaching that of EPMA, ICP-MS is unlikely to match the spatial resolution of EPMA without further development of truly simultaneous mass analyzers such as time-of-flight ICP mass spectrometry (Mahoney et al., 1996). However, we were able to program the laser probe to trace out growth increments once the otolith radius had reached $120\ \mu\text{m}$ and we found that the total length of a daily ring was approximately $700\ \mu\text{m}$. This finding, in turn, allowed us to construct elemental profiles at reasonable spatial resolution across the otoliths of larval pollock without sacrificing instrument precision by limiting acquisition times. Although it has not been used before with otoliths, our approach provides significant advantages over previous methods of using a raster to create elemental profiles (e.g., Thorrold et al., 1997; Thorrold and Shuttleworth 2000).

Previous work on pollock otolith chemistry was somewhat successful at distinguishing fish from locations in the Bering Sea and the Gulf of Alaska. Severin et al. (1995) used EPMA to sample the outer margin of otoliths from juvenile pollock collected along the Alaska Peninsula in the Gulf of Alaska and in Bristol Bay. We generated elemental profiles across otoliths from the nucleus out to approximately $90\ \mu\text{m}$ for the EPMA samples, and up to $600\ \mu\text{m}$ for the laser ablation ICP-MS assays. The profiles revealed some interesting differences between the elements assayed by each instrument. For instance, only one of the elements (K) from the EPMA analysis showed a significant interaction between profile and location, yet significant profile \times location interactions were detected for Mn/Ca, Sr/Ca, and Ba/Ca ratios with laser ablation ICP-MS. We were also struck by the similarity of profiles from individuals sampled at the same location, as evidenced by the size of standard errors around mean values at specific distances across the otolith. For instance, the extended profiles from pollock collected in Bristol Bay and Yakutat show independent patterns of variation for all three elements from the nucleus out to $600\ \mu\text{m}$. Taken together, these data indicate that larvae from several spawning locations are indeed encountering water masses with differing physicochemical properties through their larval lives, and at approximately the same time. We lack, however, a sufficient understanding of the mechanisms controlling otolith chemistry to be able to relate the profiles to specific properties of different water masses in the

study area. This knowledge will be necessary before it is possible to reconstruct dispersal pathways of larval pollock based on probe-based analyses of otolith geochemistry. Nonetheless, the among-location variability in elemental profiles revealed by both instruments is encouraging and justifies further investigations of otolith geochemistry in larval pollock.

Past attempts at identifying stock structure of walleye pollock in the North Pacific Ocean based on genetic techniques have been inconclusive (Bailey et al., 1999). In the most recent study, Olsen et al. (2002) were unable to distinguish between pollock from the Kamchatka Peninsula and several locations within the Gulf of Alaska based on three polymorphic microsatellite loci. Allozyme and MtDNA markers showed significant differences between North American and Asian populations, and among Gulf of Alaska locations. These data were difficult to reconcile because both markers showed temporal instability within locations. Adult tagging studies shed little light on the population structure of pollock because they address questions of repeat spawning, whereby adult fish return to the same area to spawn in subsequent years, rather than homing to natal spawning locations (Tsugi, 1989). It has proved impossible, except in rare circumstances (Jones et al., 1999), to artificially mark larvae before they are dispersed from spawning grounds, and therefore natural geochemical tags remain the most promising avenue for determining natal origins in walleye pollock. The ability to determine natal origins of individual fish is critical in the case of migratory marine fishes because it allows quantification of population connectivity through straying of adults as well as through larval dispersal (Thorrold et al., 2001). These data, in turn, identify the spatial extent of fish stocks that are demographically isolated or alternatively provide connectivity rates that are necessary to parameterize spatially explicit models if the species is usefully viewed as a metapopulation (Hanski and Gilpin, 1997; Smedbol and Wroblewski, 2002).

In summary, the elemental composition of otolith material deposited during early larval life in walleye pollock differed significantly among locations in the Gulf of Alaska and Bering Sea. These results imply that the larvae originated from different spawning locations, not that they constitute separate stocks. Nonetheless, these data represent the necessary first steps in using elemental signatures in otoliths as natural tags of natal origins in walleye pollock. Elemental profiles across otoliths were also unique to specific locations, suggesting that individuals collected at a location had experienced similar environmental conditions throughout their larval lives. This observation raised the possibility of reconstructing larval dispersal pathways based on high-resolution sampling of otolith chemistry. Although further work is needed to understand the processes influencing elemental uptake in pollock otoliths, we suggest that the potential information available from such studies would be invaluable for effective management of commercial pollock fisheries (Bailey et al., 1999). The approach appears to be particularly appropriate for in-

vestigating the potential existence of fine-scale population structure throughout the species range. Significant fine-scale population structure has been linked to the failure of northern cod stocks to recover from exploitation, even in the face of fishing moratoriums (Frank and Brickman, 2000; Hutchings, 2000). Analogous demographic processes acting in northern cod populations are clearly possible in walleye pollock, given the phylogenetic and life history similarities between the two species. The structure of pollock stock complexes within the major basins of the North Pacific Ocean remains, therefore, a critical gap in the knowledge necessary for the sustainable management of one of the world's largest marine fisheries.

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