Abstract—The broad distribution of Pacific sardine (Sardinops sagax) along the Pacific coast of North America makes it difficult for fisheries managers to identify regional stocks of this dominant small pelagic species. An investigation of morphometric characteristics of otoliths of Pacific sardine across most of their range revealed regional differences in populations. In a survey of over 2000 otoliths, all ages (with an emphasis on age-1 recruits) were compared. Principal components analysis, multivariate analysis of variance, and a novel method derived from regression and residuals calculations, termed perimeter-weight profiles (PWPs), revealed otolith similarities and differences. The results of the different approaches to statistical comparisons did not always agree. Sardine otoliths from Mexican waters were generally lighter and more lobate than those from U.S. and Canadian populations. Age-1 otoliths from northern California in 2006-07 tended to be heavier and smoother than those from other areas, including year-class cohorts from southern California. Comparisons of age-groups and year-classes of northern California otoliths with the use of the PWP models indicated significant trends in year-to-year patterns. In conjunction with other established indices of population structure, otolith PWPs are a useful tool for identifying local and regional stocks of Pacific sardine and may help distinguish populations of other fish species as well.

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# Otolith morphometrics and population structure of Pacific sardine (*Sardinops sagax*) along the west coast of North America

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The distribution of Pacific sardine (Sardinops sagax) along approximately 5000 km of the Pacific coast of North America-an area spanning waters of Mexico, U.S.A., and Canada-poses an international challenge to understanding population structure and managing the fishery (Fig. 1; southeast Alaska not shown). The three countries regulate commercial fishing of this often dominant small, pelagic species under management plans based on annual stock assessments, but knowledge of sardine spawning, recruitment, and migratory habits is incomplete (Lo et al., 2010). After a peak in biomass of 3.6 million metric tons (t) in 1936, the commercial fishery collapsed in the 1940s and 1950s, possibly owing to overfishing or climatic changes in the California Current system (Norton and Mason, 2005; http://www.nmfs. noaa.gov/fishwatch/species/sardine. htm [accessed June 2011]). The population began to rebound in the 1970s, and a peak in biomass of 1.7 million t was recorded in 2000. The present existence of three North American stocks has been proposed (reviewed by Smith, 2005): 1) a stock along the California coast that migrates to the Pacific Northwest (Oregon to southeast Alaska; Wing et al., 2000); 2) a stock along the Pacific coast of Baja California, Mexico; and 3) a stock within the Gulf of California. Radovich (1982) proposed further dividing the California stock into northern and far northern races.

Despite a variety of methods, including egg, larval, and adult surveys, fish morphometrics, vertebral counts, tagging, and genetic, parasitic, and otolith studies, investigators have been unable to assign specific attributes and unique characteristics to identify regional stocks since the populations rebounded (Hedgecock et al., 1989; Grant and Bowen, 1998; Pereyra et al., 2004; Félix-Uraga et al., 2005; Smith, 2005; Lo et al., 2005, 2010; Valle and Herzka, 2008; Baldwin, 2010; Dorval et al., 2011). Further clues to stock structure might be found in more detailed surveys of the morphometry and microchemistry of sardine otoliths.

Since 1993 when a study of Atlantic cod (Gadus morhua) showed growth rates significantly correlated with otolith shape (Campana and Casselman, 1993), morphometric analysis has been used as a tool to detect stock structure and interannual variability in a number of fish species, including Pacific sardine (Félix-Uraga et al., 2005) and other Clupeiformes (Somarakis et al., 1997; Turan, 2000; Torres et al., 2000; Gonzalez-Salas and Lenfant, 2007; Burke et al., 2008). Linear measurements between landmark points (truss analysis), calculated geometries (e.g., circularity), and two-dimensional (Fourier series) shape analysis of otoliths are methods typically employed.

Otolith attributes are expressed under the control of genetic, physiological, and environmental factors.

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Studies of tank-reared fish have led to insights into how specific environmental influences affect otolith shape and size. In some cases, feeding condition may affect growth and otolith morphology (Fletcher, 1995; Strelcheck et al., 2003; Gagliano and McCormick, 2004; Hüssy, 2008). Temperature influenced otolith size in tank-reared fish (Høie et al., 1999) and was inferred to regulate otolith growth in natural populations of *Merluccius* spp. and *Coelorhynchus* spp. (Lombarte and Lleonart, 1993; Bolles and Begg, 2000). Otolith morphometry, however, did not vary significantly with temperature or feeding condition in the Japanese flounder (*Paralichthys olivaceus*) (Katayama and Isshiki, 2007).

Pacific sardine inhabit coastal waters of a broad temperature range, from <10°C in Oregon and Washington (Emmett et al., 2005) to >25°C in southern Baja California (Félix-Uraga et al., 2004, 2005). Using temperatureat-catch data and otolith morphometry, Félix-Uraga et al. (2004, 2005) showed that the proposed north-south migration patterns of sardines supported the idea of three stocks in Baja California. The southern, warmwater otoliths were most differentiated from the rest, especially from those taken in coldest water catches. The authors performed multivariate discriminant analysis on over 1000 otoliths, using four straight linear measurements. Although they revealed statistical significance, the results showed a high degree of overlap between collection sites (Ensenada and Bahía Magdalena, ca. 500 km apart). That study did not address the cold-water stocks in the United States and Canada.

Our overall goal was to evaluate the efficacy of otolith morphometrics as a tool to identify Pacific sardine stocks for fishery management. One specific purpose of this investigation was to compare age-1 otoliths throughout their geographic range to detect regional differences and similarities by using several statistical approaches. Because sardine otoliths elongate asymmetrically as they grow, we applied a novel statistical approach that accommodated such changes, with the use of perimeter-weight profiles (PWPs), in addition to multivariate analysis of variance (MANOVA), principal component analysis (PCA), and generalized linear modeling (GLM). A second objective was to compare regional and age-related attributes in northern and southern California cohorts of recruits over multiple years. This research paralleled 1) a multiyear study of southern California sardine in the live bait industry, 2) an investigation of the microchemistry (trace elements and stable isotopes) of a subset of otoliths from the same multiyear study, and 3) genetic analysis of sardine tissues (Dorval et al., 2011; B. Javor, unpubl. data).

## Materials and methods

## Collections

Either whole fish or otoliths were collected from sites between Vancouver Island, British Columbia, Canada,



Map of the North American collection sites for Pacific sardine (*Sardinops sagax*) for determination of coastwide population structure from otolith morphometrics. Details of the collections are given in Table 1.

and the Gulf of California, Mexico (Table 1, Fig. 1). Samples from Canada, the Pacific Northwest, and Mexico were provided by other researchers investigating those populations. Samples from California were either specifically targeted in our study or were collected as part of regular port sampling by the California Department of Fish and Game (CDFG). No standard length (SL) or fish weight data were available for many of the otoliths obtained from archived CDFG collections.

The collections were divided into seven geographic groups that reflect oceanographic or political boundaries relevant to national fisheries: 1) Canada (Can); 2) Pacific Northwest (PNW), which includes Oregon and Washington; 3) Northern California (Monterey [Mon]); 4) Southern California Bight (SoCal), 32°–35°N, which includes San Diego (SD) and Los Angeles; 5) Ensenada (Ens); 6) Bahía Magdalena (BMag); and 7) Gulf of California (Gulf). Humboldt Bay (Hum, region 2\3 between the Pacific Northwest and Monterey) and Port Hueneme (PH, region 3\4 between northern and southern California) were considered to be transitional zones based on oceanographic features.

# **Otolith measurements**

Sardine sagittal otoliths are asymmetric and lend themselves to measurements between multiple land-



mark points on the perimeter and through the primordium. The perimeter may develop rounded, irregular, or dentate protuberances, particularly along the ventral side, such that some otoliths are relatively smooth and others are relatively lobed (Fig. 2, A and B). Sagittal otoliths composed of vaterite, which are always clear and highly lobate, were omitted from the study.

We used both left and right otoliths. Age was determined by the method of Yaremko (1996). Age-1 otoliths weigh 0.73–1.30 mg based on our unpublished aging studies. After having been cleaned in distilled water, otoliths were dried, weighed on a Cahn C-33 microbalance (Thermo Electron Corp., Marietta, OH) with 0.005 mg accuracy and photographed with a reference scale for measuring otolith dimensions with Image-Pro Plus, vers. 4.5.1 or 6.3 software (Media Cybernetics, Inc., Bethesda, MD). The length (segment AB, Fig. 2C) was determined first, from the midpoint on the rostrum tip through the primordium to the posterior edge. Three segments perpendicular to the length included the width through the primordium, segment CE, and CE subsegment C-C' (a measure of the gap between the rostrum and antirostrum [point C]). Point D was the notch between the rostrum and the antirostrum. Other measured segments included AC, AD, BC, and BD. In addition to weight, the measurements included eight straight linear dimensions, perimeter, and area. The autotrace function of the software determined the perimeter and area.

#### Northern vs. southern California sardine populations

A synoptic study for 2006–07 of age-1 cohorts that turned age-2 during the spring of the calendar year was conducted to compare regional differences in sardine otoliths from Monterey Bay and from San Diego, about 700 km apart. Sardine from both regions presumably share the same spawning area offshore from central

# Table 1

Dates and regions for collections of Pacific sardine (*Sardinops sagax*) from north to south. The number of otoliths obtained per site is given in Figure 4. Areas with two region numbers (e.g., 2\3) were considered transitional regions. DFO=Fisheries and Oceans; SWFSC=Southwest Fisheries Science Center; NWFSC=Northwest Fisheries Science Center; CDFG=California Department of Fish and Game; CICIMAR=Centro Interdisciplinario de Ciencias Marinas; CICESE=Centro de Investigación Científica y de Educación Superior de Ensenada.

Region no. and area	Year	Collections	Provider
1 Canada	2003	Vancouver I., 4 dates (adults)	C. Hrabek, DFO
	2005	Vancouver I., 1/28/05 area 24 (age 0)	C. Hrabek, DFO
2 Pacific Northwest	2003	Cruise FR0307 (3/03), 4 trawls	SWFSC
	2003	Cruise MF0313 (11/03), 6 trawls	R. Emmett, NWFSC
	2004	Cruise FR0403 (3/04), 3 trawls	SWFSC
	2010	Columbia River plume, 5/12 and 5/25	R. Emmett, NWFSC
2\3 Humboldt Bay	1996	Port samples, 3 dates	CDFG
3 Monterey	1996 - 97	Port samples, 8 dates	CDFG
	2006 - 97	Port samples, 21 dates	CDFG
	2008	Port samples, 4 dates	CDFG
3\4 Port Hueneme	2007	Port samples, 7 dates	CDFG
4 Los Angeles	1995 - 2003	Port samples, March-April (age 1)	CDFG
4 San Diego	2003-09	Bait receiver, monthly samples	SWFSC
5 Ensenada	1991 - 92	Port samples, spring and fall	CDFG
6 Bahía Magdalena	2004	Spring and fall, 4 dates	R. Félix-Uraga, CICIMAR
7 Gulf of California	2006	February and December	Y. Ríos, CICESE

and southern California (Lo et al., 2005). However, sea surface temperatures are markedly different at the two areas. The average annual temperature range in Monterey Bay at Pacific Grove is 11.8°-14.5°C, whereas 20 km south on the open coast, strong upwelling drives the temperatures lower (10°-13°C annual range; Breaker, 2005). The mean annual temperature range at the Scripps Institution of Oceanography pier in La Jolla (San Diego) is 13.9°-20.0°C (www.nodc.noaa.gov/dsdt/ cwtg, accessed September 2010), whereas 23 km offshore from San Diego the temperatures are about 1°C warmer (www.calcofi.org, accessed September 2010). We also included sardine captured in 2007 near Port Hueneme (region 3\4), a landing in the Southern California Bight about midway between Monterey (region 3) and San Diego (region 4). Because regions 3 and 4 sardine reach a birthday during April, collections of cohorts during a calendar year are indicated as age 0–1, age 1–2, and age 2-3. Each sample set included 19-25 fish per collection, and both left and right otoliths were used when possible.

# Statistical analysis

For coast-wide comparisons, several statistical approaches were used to ascertain patterns and regional characteristics of otoliths: principal components analysis (PCA), multivariate analysis of variance (MANOVA), and a method based on analysis of residuals described below. PCA was used initially to select the four most important otolith dimensions for the MANOVA and calculations of residuals. PCA and associated MANOVA statistics were applied only to age-1 otoliths (0.73–1.30 mg) because

this age group was collected from all regions, whereas younger juveniles and older adults were not available from all areas. The coefficient of the characteristic vector of the product of contrast sum-of-square cross-product (SSCP) matrix (H) and the inverse of the error SSCP matrix (E) were used to determine the influential measurement among four variables. These selected measurements (length, area, perimeter, and weight) were then standardized (i.e., the correlation matrix rather than the covariance matrix) to be used in the MANOVA to test for possible differences in otolith dimensions with six orthogonal contrasts of individual regions or clusters of regions by using the Wilks's lambda test of significance: C1, regions 1–2 vs. 3–7; C2, region 1 vs. 2; C3, regions 3-5 vs. 6-7; C4, regions 3 vs. 4-5; C5, region 4 vs. 5; and C6, region 6 vs. 7. PCA and MANOVA were conducted with S-Plus (TIBCO Software, Palo Alto, CA) or SAS (SAS Institute, San Diego, CA) software.

In addition to MANOVA, we designed a method based on the residuals calculated from regression equations for measured otolith features in order to express morphometric data for comparisons with average data in simple models. Three regression equations with the use of the four most important dimensions determined by PCA (perimeter vs. area, perimeter vs. length, and weight vs. length) were derived from a data set of 2213 otoliths from all ages of sardine and all regions, whereas the MANOVA was performed for age-1 fish only. By applying these equations to the observed measurements of each otolith, the expected average perimeter and weight were calculated from the otolith area or length. The differences between observed and calculated measurements (residuals) were employed to identify regional characteristics. According to the null hypothesis, 50% of the measurements for otoliths from a region should fall above the regression line and 50% below it if there is no regional bias for otolith perimeter or weight. We tested that hypothesis using the following equation expressed as a percentage:

$$PWP = \frac{\sum Z_i}{n},$$
(1)

where  $Z_i = 1$  if the observed measurement is greater than the calculated value from the regression line (otherwise scored as 0); and n = the total number in the sample set.

We termed the results "perimeter-weight profiles," or PWPs.

PWPs reported this way correlated well with residuals expressed as plus or minus values in mm or mg. The correlation coefficients determined by comparing PWP (%) vs. average residuals (mm or mg) for 61 sample sets from San Diego collected monthly for over five years were the following: perimeter based on area, 0.876; perimeter based on length, 0.889; and weight based on length, 0.920. PWPs provide an advantage for categorizing the data on residuals, particularly when there is a wide spread of values and when the average residuals fall near zero.

Statistical significance of the three PWP calculations between geographic areas was determined by three-way chi-square tests and by using likelihood-ratio tests (or log linear model) with  $G^2$  statistics (log-linear analysis, http://faculty.vassar.edu/lowry/abc.html, accessed September 2010). For the ABC chi-square matrix, we used pairs of values (i.e.,  $2 \times 2 \times 2$ ): A=two locations; B=the number of otoliths above and the number below the regression line that describes the model otolith for each kind of measurement; and C=two kinds of measurements (PWP perimeter derived from the otolith area and PWP weight derived from the otolith length). Significance values (P) were determined from the  $G^2$ statistic for AB(C) that represented the AB interaction when the AC and BC interactions were removed. It can be obtained by constructing a separate AB table for each level of C, calculating a separate  $G^2$  measure for each AB table, and then summing the results.

Comparisons between northern and southern California sardine otoliths were conducted several ways. A GLM with logistic link was used to examine the possible differences between PWP for perimeter based on area (P/A), perimeter based on length (P/L), and weight based on length (W/L) for regions 3 and 4 (Monterey and San Diego, location effect) using cohorts collected in 2006 and 2007 (year effect):

$$g(PWP) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2, \qquad (2)$$

where g(PWP) is a logistic link function of the population proportion for each of the three equations (P/A), (P/L), and (W/L); and

$$g(PWP) = \log(PWP / (1 - PWP)), \qquad (3)$$

where  $x_1$  and  $x_2$  are categorical variables:  $x_1=0$  for 2006 and 1 for 2007, and  $x_2=0$  for Monterey and 1 for San Diego. The last term ( $\beta_3$ ) is the interaction term. When the coefficient  $\beta_3$  was significant, the GLM was performed to test the location effect for each year with  $x_2$ as the only independent variable.

Because the multiyear data collected from Monterey area included more than one age, the GLM was also used to test age and year effect on otoliths sampled during 2006–07 by using the same methods described for Equations 2 and 3. The only difference between these two GLM applications was that here  $x_2$  is the age category:  $x_2=0$  for age-0 fish and 1 for age 1–2 fish. The coefficient  $\beta_2$  was applied to measure the age effect, whereas in the previous GLM,  $x_2$  was the indicator for the location.

## Results

#### Coast-wide survey

**PCA and MANOVA** When otoliths of all ages and from all regions were compared, most measurements were highly correlated (coefficients $\geq$ 0.90, *n*=2309 otoliths; data not shown). Length, perimeter, and area had the highest correlation coefficients (0.98–0.99). Otolith weight strongly correlated with length, perimeter, and area (0.94–0.98), and fish standard length similarly correlated with those four otolith features (0.95–0.97). When correlations were conducted for each of the seven areas, no regional patterns were detected (data not shown).

For the PCA of age-1 otoliths with all eleven measurements, the otolith dimensions (except C–C') had nearly equivalent PC1 coefficients (data not shown). When only the four most important dimensions were compared by PCA (area, length, perimeter, and weight), PC1 explained 86% of the variance and the coefficients were similar (Table 2). PC1 was the only component with an eigenvalue >1. These samples represented aggregated collections by region for all dates and provided one otolith per pair. When PCA was conducted with both otoliths per pair, the results were nearly identical (results not shown).

MANOVA on these four variables based on the correlation matrix indicated otolith sizes were not the same for all regions despite the selection of a single age class (0.73–1.30 mg, nearly a two-fold difference within the class). MANOVA results showed that all regions were not the same, and each of the six tested regional contrasts were significantly different (P<0.05) (Table 3). In three of the six regional contrasts, perimeter, or perimeter and weight together, contributed the most to the differences. Length was generally the least influential factor for any of these contrasts.

Although differences between widely spaced collection areas might be expected (contrasts 1, 3, and 4), the reasons for the significant differences between neighboring regions that share spawning or oceanographic features (contrasts 2, 5, and 6) were not apparent. Small sample sizes may have biased some of the results. Age 0–1 otoliths from the northernmost areas were not well represented: n=30 from Canada (region 1), all from a single collection date; and n=20 from the Pacific Northwest (region 2). However, sample size might not explain why southern California and Ensenada (regions 4 and 5) otoliths were significantly different, and why Bahía Magdalena and Gulf of California (regions 6 and 7) were dissimilar.

We initially conducted PCA of over 1100 otoliths by aggregating all ages in a region, from juveniles to adults, which resulted in size-biased, significant differences within and between regions (data not shown). Otoliths from regions 1 and 2, the only areas with large adults in the collections, differed from all other regions. This response derived from the overall shape differences between young and older otoliths (Fig. 2). In order to compare otoliths of all sizes in collections that had different distributions of sizes, another approach was required.

**Perimeter-weight profiles (PWPs)** The regression lines between pairs of otolith features for sardine of all ages and regions were linear (perimeter vs. length) or curvilinear (perimeter vs. area, and weight vs. length) (Fig. 3). The regression equations used for calculating PWPs and their correlation coefficient  $(R^2)$  values for these features are as follows:

 $\begin{array}{l} Perimeter \ (based \ on \ area) = \\ -0.2250 \ area^2 + 3.1559 \ area + 1.9071, \ R^2 = 0.968 \quad (4) \\ Perimeter \ (based \ on \ length) = \\ 2.6808 \ length + 0.118, \ R^2 = 0.975 \quad (5) \end{array}$ 

## Table 2

Summary of principal components (comp) analyses of age-1 sardine (*Sardinops sagax*) otolith measurements based on the four most important features of length, area, perimeter, and weight. One otolith was examined per fish. The numbers of otoliths per region are as follows: region 1 (30), region 2 (20), region 3 (86), region 4 (280), region 5 (87), region 6 (36), region 7 (150), total (689).

	Importance of components				
	Comp 1	Comp 2	Comp 3	Comp 4	
Standard deviation	1.86	0.59	0.32	0.29	
Proportion of variance	0.864	0.088	0.026	0.022	
Cumulative proportion	0.864	0.953	0.978	1.000	
Eigenvalues	3.46	0.35	0.10	0.08	
Coefficients					
Length	0.514	-0.229	0.732	0.384	
Area	0.520			-0.850	
Perimeter	0.496	-0.540	-0.639	0.233	
Weight	0.468	0.810	-0.220	0.276	

$$\begin{array}{l} Weight \ (\text{based on length}) = \\ (length^{2.2429}) \times (0.1054), \\ R^2 = 0.966, \ \text{for otoliths <3 mm} \\ Weight \ (\text{based on length}) = \\ 0.2709 \ length^2 - 0.605 \ length + 0.6084, \\ R^2 = 0.947, \ \text{for otoliths >3 mm} \end{array}$$

PWPs showed several distinct regional and age patterns, particularly between northern California (regions 2\3 and 3, Humboldt Bay and Monterey) and regions 5–7

# Table 3

Data from a coast-wide survey of the four most important Pacific sardine (*Sardinops sagax*) otolith dimensions (length, area, perimeter, and weight) determined by principal component analysis and multivariate analysis of variance to test the hypothesis of no overall region effects and in each of the six orthogonal contrasts of individual regions and clusters of regions. The coefficient of the characteristic vector of the product of contrast sum-of-square cross-product (SSCP) matrix ( $\mathbf{H}$ ) and the inverse of the error SSCP matrix ( $\mathbf{E}$ ) were used to determine the influential measurement among the four variables. The results include characteristic roots and vectors of  $\mathbf{E}$ -1 $\mathbf{H}$ . Significance (*PR*>*F*) was <0.0001 for no region effect and all contrasted regions. Denom.=denominator.

	Wilks's	Wilks's					Characteristic vector Standardized measurements			
Contrasted regions Hypothesis: no effect	lambda value	<i>F</i> value	No. of df	Denom. df	Characteristic root	Percent	Length	Area	Perimeter	Weight
No region effect	0.61	15.13	24	2370	0.359	64.15	0.010	-0.003	0.055	-0.032
1: 1–2 vs. 3–7	0.94	10.02	4	679	0.071	100	0.010	-0.074	0.081	0.010
2: 1 vs. 2	0.92	14.61	4	679	0.059	100	-0.010	0.057	0.019	-0.033
3: 3–5 vs. 6–7	0.90	19.86	4	679	0.042	100	-0.170	-0.007	0.042	0.025
4: 3 vs. 4–5	0.93	12.06	4	679	0.116	100	0.001	0.042	0.029	-0.044
5: 4 vs. 5	0.90	19.74	4	679	0.086	100	0.011	-0.011	0.019	-0.026
6: 6 vs. 7	0.96	7.16	4	679	0.117	100	-0.026	0.039	-0.055	0.045

in Mexico (Fig. 4, Table 4). Most of the otoliths were from late age-0 and age-1fish, except those indicated as being from juvenile (all age-0) and adult ( $\geq 2$  years) fish. Values close to 50% indicate the collection was close to the average of the entire population. Samples in Figure 4 were aggregated by collection area regardless of date, except for two sets: juveniles from region 2 separated by collection period, 2003–04 vs. 2010; and region 4 (San Diego) monthly otolith collections separated into 2006–07 and 2009–10 sets.



#### Figure 3

Pacific sardine (*Sardinops sagax*) otolith dimension relationships for 2213 otoliths from all ages and regions: (**A**) perimeter vs. area; (**B**) perimeter vs. length; and (**C**) weight vs. length relationships with two regression lines shown. The power equation best described otoliths <3 mm in length, and the polynomial equation best described larger otoliths. The regression equations are described in the text.

PWPs for Mexican sardine otoliths (regions 5-7) were distinct. Otolith weights from the three areas of collection were less than the predicted average. Perimeters of southern Baja California otoliths from Bahía Magdalena (pooled from spring and fall samples in 2004) and the Gulf of California (pooled from January and December, 2006 samples) were markedly lobed. Overall, the PWPs of sardine otoliths from region 6 and 7 resembled each other in the chi-square tests, unlike the results of the MANOVA described in Table 3. Region 5 otoliths had a PWP weight signature resembling the more southern fish and a PWP perimeter signature similar to that of southern California sardine. The chisquare test of the PWP factors indicated that region 5 otoliths were different (P=0.0002) from more southern sardine in regions 6 and 7.

Sardine otoliths from regions 1, 2 (2003–04 collection), and 4 resembled each other. These sets included both juveniles and adults. By contrast, region 1 and 2 otoliths were significantly different in the MANOVA presented in Table 3. Northern California otoliths (regions  $2\backslash3$  and 3) were moderately similar to each other (P=0.03). The strongest similarities determined in the chi-square tests were between regions 2 and 4, and between regions 6 and 7 (P>0.8).

Correlation coefficients between the residuals (observed minus average values, ±mm or mg) for each

#### Table 4

Similarities in perimeter-weight profiles (PWPs) in the coast-wide survey of Pacific sardine (*Sardinops sagax*) determined by three-way chi-square tests contrasting perimeter based on area, perimeter based on length, and weight based on length; n=2213 otoliths. All otoliths were age 1–2 except those in collections described as juveniles (age-0) and adults (>age-2). Regions are shown in Figure 1. Region 2, 2003–04 collection; region 3, 1996–97 collection; region 4, San Diego collection. Dates of other collections are given in Table 1. Where indicated with +, the collections were aggregated.

Contrasted ages and regions	Р
Region 1: juveniles vs. adults	0.0351
Region 2: juveniles vs. adults	0.3985
Region 1 vs. 2	0.1882
Region 1 adults vs. region 2 adults	0.0226
Region 1 vs. 4	0.2039
Region 2 vs. 3	< 0.0001
Region 2 vs. 4	0.8025
Region 3 vs. 3\4	< 0.0001
Region 3 vs. 2\3	0.0347
Region $3 + 2 \setminus 3$ vs. regions $6 + 7$	< 0.0001
Region 4 vs. 3\4	< 0.0001
Region 4 vs. 5	< 0.0001
Region 5 vs. 6 + 7	0.0002
Region 6 vs. 7	0.8781



of the three PWP factors, compared for each otolith (n=2213), were largely similar across the seven geographic areas. They were positive between the two ways of conducting perimeter calculations (0.682) and negative or neutral between perimeter (P/A and P/L) and weight calculations (-0.382 and 0.071) (data not shown).

#### Northern vs. southern California sardine populations

The PWP perimeters calculated from otolith area and length in monthly or semimonthly collections were significantly different for Monterey and San Diego (regions 3 and 4) sardine in 2006–07 (Fig. 5, A and B). Monterey otoliths tended to have smoother perimeters. Distinctions between predicted and observed otolith weights for the two sites were not apparent (Fig. 5C). Fish standard lengths (SL) and condition factors were similar for the two sites as were the regressions for SL vs. otolith weight and SL vs. otolith length (data not shown). Because somatic and otolith growth rates were similar in the cohorts at the two locations, differences in otolith perimeters were likely due to environmental factors.

The Port Hueneme (region 3\4) samples in 2007 had perimeter profiles more like those of San Diego otoliths with the area-based regression and widely ranging perimeter attributes similar to both Monterey Bay and San Diego otoliths with the length-based regression. There was no distinction between weight profiles for the three sites during the same time period. The most salient feature of the 13 Port Hueneme collections over a five-month period was their nonuniformity.

A GLM with logistic link was used to examine the possible difference between PWPs for perimeter based on area (P/A), perimeter based on length (P/L), and weight based on length (W/L) between Monterey and San Diego (location effect) and between years 2006 and 2007 (year effect) (Table 5). For both cases of perimeter (P/A and P/L), the interaction term  $(year \times age)$  was significant, and therefore two separate GLMs were performed to test for possible location effects, each for 2006 and 2007. The location effect for each of the two years was significant (P < 0.001) with the difference between locations being greater in 2007 than in 2006. For the GLM analysis of PWP for W/L, location and year effects were not significant. Thus, only PWP perimeters (P/A and P/L) were dissimilar between regions 3 and 4 for these two years.

Fish age and collection year were factors for PWPs of sardine captured in Monterey. In a multi-year survey, age-1 and older otoliths tended to be smoother and heavier than average, but year-to-year PWPs were somewhat inconsistent (Fig. 6). Age-0 otolith PWPs did not show a predictable pattern that resembled that of older fish. The GLM was used to test age effect (age 0 and age 1–2) and year effect (2006 and 2007) for the three PWP factors P/L, P/A, and W/L of the region 3



Perimeter-weight profiles (PWPs) of late age-0 and age-1 Pacific sardine (Sardinops sagax) otoliths from Monterey (region 3), San Diego (region 4), and Port Hueneme (region  $3\setminus4$ ), 2006–07. (A) Perimeter based on area. (B) Perimeter based on length. (C) Weight based on length. The dashed line at 50% PWP represents the population average (i.e., 50% of the residuals lie above the regression line) as determined by the regression equations.

data in Figure 6. The age:year interaction term was not significant for perimeter (both P/L and P/A); therefore no further GLM was performed (Table 6). The age effect was significant for perimeter (both P/A and P/L); the year effect was significant for only P/A and not for P/L. For the GLM of W/L, the age and year interaction term was significant.



Multiyear comparisons of perimeter-weight profiles (PWPs) of Pacific sardine (*Sardinops sagax*) otoliths from Monterey (region 3). (A) Profiles by collection year and age (number of otoliths indicated). The dashed line at 50% PWP represents the population average (i.e., 50% of the residuals lie above the regression line) as determined by the regression equations. The number of otoliths is indicated above each set. (B) Averages by age: age-0 (mean of 3 years) and ages 1–2 (mean of 5 years). Each collection was normalized to 100 otoliths to remove year-based bias. Error bars are  $\pm 95\%$  confidence intervals.

## Discussion

# PCA and PWP methods of comparison

We compared otolith characteristics from a large collection of Pacific sardine sampled from most of their North American range, which was divided into seven regions between Canada and the Gulf of California. Regional similarities and differences determined with MANOVA and perimeter-weight profile comparisons did not agree consistently with each other.

One of the inherent problems with this kind of survey is that sardine otoliths elongate asymmetrically as they grow. Including all sizes of otoliths in any statistical analysis resulted in significant differences between northern stocks (where large adult sardine are commercially captured and few juveniles were found) and

# Table 5

General linear model (GLM) for year ( $\beta_1$ ) and location ( $\beta_2$ ) interactions effects of region 3 and 4 sardine (*Sardinops sagax*) otolith measurements for each perimeter-weight profile (PWP) factor: perimeter based on area, perimeter based on length, and weight based on length. Because the interaction term ( $\beta_3$ ) was significant, GLM was performed for both 2006 and 2007 for the three PWP factors. Significance: \*=P<0.005, \*\*=P<0.001.

	Coefficient estimate	Standard error	<i>z</i> -value	Р	Significance
Perimeter based on area, coefficients					
(Intercept)	-0.9163	0.1479	-6.195	$5.82 \times 10^{-10}$	***
Location $(\beta_1)$	0.6286	0.1874	3.354	$7.97 \times 10^{-4}$	***
Year $(\beta_2)$	-0.4773	0.2006	-2.380	0.0173	*
Location:year $(\beta_3)$	0.8760	0.2513	3.486	$4.91 \times 10^{-4}$	***
Perimeter based on length, coefficients					
(Intercept)	-1.0515	0.1525	-6.894	$5.42 \times 10^{-12}$	***
Location	0.8035	0.1909	4.209	$2.57 \times 10^{-5}$	***
Year	-0.2702	0.2021	-1.337	0.1813	
Location:year	0.7360	0.2526	2.914	0.00357	**
Weight based on length					
(Intercept)	0.1791	0.1993	0.899	0.3763	
Location	0.0164	0.2620	0.063	0.9506	
Year	0.4879	0.2617	1.864	0.0724	
Location:year	-0.4265	0.3450	-1.236	0.2263	
Weight based on length after excluding interaction term					
(Intercept)	0.3226	0.1650	1.956	0.0599	
Location	-0.2305	0.1718	-1.342	0.1897	
Year	0.2427	0.1722	1.410	0.1690	
2006 Perimeter based on area, coefficients					
(Intercept)	-0.9163	0.1479	-6.195	$5.82 \times 10^{-10}$	***
Location	0.6286	0.1874	3.354	$7.97{ imes}10^{-4}$	***
2007 Perimeter based on area, coefficients					
(Intercept)	-1.3936	0.1355	-10.286	$2.00 \times 10^{-16}$	***
Location	1.5046	0.1674	8.988	$2.00 \times 10^{-16}$	***
2006 Perimeter based on length, coefficients					
(Intercept)	-1.0515	0.1525	-6.894	$5.42 \times 10^{-12}$	***
Location	0.8035	0.1909	4.209	$2.57{ imes}10^{-5}$	***
2007 Perimeter based on length, coefficients					
(Intercept)	-1.3218	0.1326	-9.965	$2.00 \times 10^{-16}$	***
Location	1.5395	0.1654	9.310	$2.00 \times 10^{-16}$	***

southern stocks (where large adults are rarely captured). These dissimilarities are likely due to age differences. Within the size-class defined to comprise mostly age-1 otoliths, the dimensional relationships spanned a smaller range, but they could have biased the results if all regions did not have a similar distribution of otolith sizes. The results may have also been biased by the relatively small number of age-1 representatives from regions 1 and 2. Any statistical analysis is most reliable when sample sizes are large and balanced (Osborne and Costello, 2004).

The predictability of dimensions of an average sardine otolith of any length or area was the premise for developing the PWP method to compare sets of otoliths as an alternative approach for analyzing the data. PWPs gave a picture of regional signatures and temporal trends within and between year classes. Juvenile otoliths from northern California (and in 2010, the Pacific Northwest) showed a regional signature as predominantly heavy and smooth, whereas juvenile otoliths from their southernmost distribution were predominantly light and lobate. Multiyear surveys showed trends in age-specific profiles for Monterey otoliths.

The PWP method, which permitted the comparison of individual otolith features, revealed unique profiles among Mexican sardine. Region 5 otoliths appeared to have weight characteristics of the more southerly region 6 and 7 populations and perimeter characteristics of region 4 southern California sardine. Migratory movements that could account for the uniformity of region 6

# Table 6

General linear model (GLM) for year and age effect in region 3 (Monterey) Pacific sardine (*Sardinops sagax*) otoliths for each perimeter-weight profile (PWP) factor: perimeter based on area, perimeter based on length, and weight based on length. Significance: \*=P<0.005, \*\*=P<0.005, \*\*=P<0.001.

Perimeter based on area(Intercept) $-0.0757$ $0.1471$ $-0.515$ $0.6069$ Year $-0.4729$ $0.2869$ $-1.648$ $0.0994$ Age $-0.8406$ $0.2086$ $-4.029$ $5.60 \times 10^{-5}$ Year:age $-0.0045$ $0.3501$ $-0.013$ $0.9898$ Perimeter based on area after excluding interaction term $-0.0749$ $0.1335$ $-0.561$ $0.575$ Year $-0.4759$ $0.1644$ $-2.894$ $3.80 \times 10^{-3}$ Age $-0.8422$ $0.1676$ $-5.026$ $5.01 \times 10^{-7}$ Perimeter based on length $-0.8422$ $0.1676$ $-5.026$ $5.01 \times 10^{-7}$	***
$\begin{array}{cccccc} (Intercept) & -0.0757 & 0.1471 & -0.515 & 0.6069 \\ Year & -0.4729 & 0.2869 & -1.648 & 0.0994 \\ Age & -0.8406 & 0.2086 & -4.029 & 5.60 \times 10^{-5} \\ Year:age & -0.0045 & 0.3501 & -0.013 & 0.9898 \\ \end{array}$	***
Year $-0.4729$ $0.2869$ $-1.648$ $0.0994$ Age $-0.8406$ $0.2086$ $-4.029$ $5.60 \times 10^{-5}$ Year:age $-0.0045$ $0.3501$ $-0.013$ $0.9898$ Perimeter based on area after excluding interaction term $-0.0749$ $0.1335$ $-0.561$ $0.575$ Year $-0.4759$ $0.1644$ $-2.894$ $3.80 \times 10^{-3}$ Age $-0.8422$ $0.1676$ $-5.026$ $5.01 \times 10^{-7}$ Perimeter based on length $-0.8422$ $-0.676$ $-0.13 \times 10^{-7}$	***
Age Year:age $-0.8406$ $0.2086$ $-4.029$ $5.60 \times 10^{-5}$ Perimeter based on area after excluding interaction term (Intercept) $-0.0749$ $0.1335$ $-0.561$ $0.575$ Year Age $-0.4759$ $0.1644$ $-2.894$ $3.80 \times 10^{-3}$ Perimeter based on length $-0.8422$ $0.1676$ $-5.026$ $5.01 \times 10^{-7}$	***
Year:age $-0.0045$ $0.3501$ $-0.013$ $0.9898$ Perimeter based on area after excluding interaction term(Intercept) $-0.0749$ $0.1335$ $-0.561$ $0.575$ Year $-0.4759$ $0.1644$ $-2.894$ $3.80 \ge 10^{-3}$ Age $-0.8422$ $0.1676$ $-5.026$ $5.01 \ge 10^{-7}$ Perimeter based on length	
Perimeter based on area after excluding interaction term $-0.0749$ $0.1335$ $-0.561$ $0.575$ Year $-0.4759$ $0.1644$ $-2.894$ $3.80 \ge 10^{-3}$ Age $-0.8422$ $0.1676$ $-5.026$ $5.01 \ge 10^{-7}$ Perimeter based on length $-0.8422$ $-0.676$ $-0.1 \ge 10^{-7}$	
$\begin{array}{cccc} (Intercept) & -0.0749 & 0.1335 & -0.561 & 0.575 \\ Year & -0.4759 & 0.1644 & -2.894 & 3.80 \ x \ 10^{-3} \\ Age & -0.8422 & 0.1676 & -5.026 & 5.01 \ x \ 10^{-7} \\ \end{array}$ Perimeter based on length	
Year $-0.4759$ $0.1644$ $-2.894$ $3.80 \ge 10^{-3}$ Age $-0.8422$ $0.1676$ $-5.026$ $5.01 \ge 10^{-7}$ Perimeter based on length	
Age $-0.8422$ $0.1676$ $-5.026$ $5.01 \times 10^{-7}$ Perimeter based on length	**
Perimeter based on length	***
(Intercept) -0.3606 0.1494 -2.413 0.0158	*
Year -0.1880 0.2881 -0.652 0.5142	
Age $-0.6909$ $0.2135$ $-3.236$ $1.21 \times 10^{-3}$	**
Year:age -0.0823 0.3520 -0.234 0.8152	
Perimeter based on length after excluding interaction term	
(Intercept) -0.3458 0.1352 -2.557 0.0106	*
Year -0.2431 0.1658 -1.467 0.1424	
Age $-0.7212$ $0.1701$ $-4.239$ $2.24 \times 10^{-5}$	***
Weight based on length	
(Intercept) 0.9391 0.1636 5.742 9.36×10 <sup>-9</sup>	***
Year -0.2670 0.2995 -0.892 0.3726	
Age $-0.7601$ $0.2115$ $-3.593$ $3.27 \times 10^{-4}$	***
Year:age 0.7549 0.3475 2.173 0.0298	*
Weight based on length, 2006	
(Intercept) $0.9391  0.1636  5.742  9.36 \times 10^{-9}$	***
Age $-0.7601$ $0.2115$ $-3.593$ $3.27 \times 10^{-4}$	***
Weight based on length, 2007	
(Intercept) $0.6721  0.2509  2.679  7.39 \times 10^{-3}$	
Age -0.0051 0.2757 -0.019 0.9851	**

and 7 otoliths and the mixed characteristics of region 4 and 5 otoliths were described by Félix-Uraga et al. (2005) in their study of Baja California populations. The results from our study support the generally accepted belief that southern Baja California sardine represent a distinct stock.

The PWP method clearly differentiated region 3 and 4 (Monterey and San Diego) cohorts collected in 2006– 07. The mixed results of the Port Hueneme samples compared with Monterey and San Diego sardine could indicate that Port Hueneme is a zone of overlap where representatives are carried south by the cool California Current and others are transported north by the warm Southern California Countercurrent.

The PWP method has limitations for assigning fish to stocks or environments. Similar PWPs between region 2 and 4 age-1 sardine, but not region 3 fish, do not necessarily indicate a common stock, although it is generally believed adult sardine in California migrate north during the summer (Smith, 2005; Lo et al., 2010). Likewise, dissimilar perimeter profiles between age-0 and age-1 sardine otoliths from Monterey do not necessarily indicate two regions of origin. Some attributes of the PWP method need further study. For example, it is not obvious why the two equations to model otolith perimeters correlated differently with the weight model.

# Factors affecting otolith morphometrics

In previous studies of otolith morphology, length, area, and perimeter were often the most important characteristics that defined fish stocks (Bolles and Begg, 2000; Torres et al., 2000; DeVries et al., 2002; Cardinale et al., 2004). When included in such studies, otolith weight was also an important factor (Tuset et al., 2006; Jónsdóttir et al., 2006). In a morphometric study of Pacific sardine otoliths from Baja California, Mexico, Félix-Uraga et al. (2005) used length and other linear dimensions, but not area, perimeter, or weight. Because these factors were the most important in the first principal component that explained most of the variance in the present investigation, a re-evaluation of those otoliths might refine the results of the earlier study. However, those otoliths were permanently mounted on slides in clear resin that precluded weighing them and obtaining sharp digital images to measure area and perimeter with the autotrace tool of the image-processing software (R. Félix-Uraga<sup>1</sup>).

Both temperature and growth rate can be factors influencing otolith shape. In a study of two stocks of silver hake (*Merluccius bilinearis*), fish in the northern stock grew slower, probably due to colder temperatures, and their otoliths were subsequently larger (i.e., older) than those from southern-stock fish of the same standard length (Bolles and Begg, 2000). Similar phenomena have been noted in other fish (summarized by Strelcheck et al., 2003). Hüssy (2008) showed higher food consumption resulted in a higher number of lobes in otoliths of juvenile Atlantic cod (*Gadus morhua*).

In our 2006–07 synoptic study of region 3 and 4 cohorts, we found no apparent growth differences between recruits captured near Monterey and San Diego, although the higher percentage of smoother otoliths in Monterey was notable. Juveniles from both locations are believed to come from central and southern California coastal spawning grounds (Lo et al., 2005). An obvious explanation for the differences in otolith morphometrics between the two regions may be temperature.

Temperature has been tested and shown to affect otolith growth characteristics in other species. Positive effects of temperature on otolith weight have been observed in red drum (Sciaenops ocellatus) (Hoff and Fuiman, 1993) and herring (Clupea harengus) (Fey, 2001). Flounder (Paralichthys olivaceus) otoliths showed no significant difference in marginal coarseness between wild fish and experimental fish maintained at 15°, 20°, and 25°C (Katayama and Isshiki, 2007). Hoff and Fuiman suggested that otolith growth was more directly affected by metabolic rate than by growth rate. Our unpublished data indicate that otolith weights and perimeters of sardine reared in tanks at different temperatures are dissimilar to those same variables in otoliths of wild-caught sardine of the same age and they likely reflect artifacts of aquaculture that may be independent of water temperature.

Temperature may affect sardine otolith morphometrics in wild populations, but other factors may modify them. An improved survey to address possible temperature effects would compare all sardine sizes from their environmental ranges. Sardine do not regularly spawn successfully in the Pacific Northwest (McFarlane and Beamish, 2001; Emmett et al., 2005; Lo et al., 2010), and therefore further study to elucidate the environmental or growth factors that contribute to regional differences in otolith morphometrics in cold ocean environments would be hampered by the availability of samples of young fish. Likewise, older sardine are not usually collected in warmer Mexican waters.

## Conclusion

Results from MANOVA indicated there were regional differences in age-1 otoliths between regions or clusters of regions when nearly 700 fish were compared. Based on comparisons with average otoliths of the same length or area, PWPs of young and adult sardine otoliths coupled with MANOVA and chi-square tests showed differences among some regions, as well as significant similarities. This investigation provided further evidence that S. sagax populations in their southernmost distribution in Mexican waters are a distinct stock from U.S. and Canadian populations (Félix-Uraga et al., 2004, 2005; Smith, 2005). Sardine from Ensenada (region 5) shared otolith features with both southern and northern stocks. Age-1 otoliths from northern California (region 3) tended to be heavier and smoother than those from other areas. Some regions showed variations in PWPs between years. PWPs were useful for describing relationships between and within local and regional sardine stocks and age cohorts. PWPs can be applied as a tool for understanding residence, migration, and population connectivity when used in combination with otolith chemistry, aging, genetics, and other traditional measures of population structures for sardine and other species of fish.

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