COMPOSITION AND INTERANNUAL VARIABILITY IN TRACE ELEMENT PROFILES OF PACIFIC SARDINE OTOLITHS

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ABSTRACT

Temperature is a primary factor separating Pacific sardine *Sardinops sagax* into cold and temperate stocks in the California Current Ecosystem. We collected otoliths of age-1 sardine captured in the spring off central California (1996–97) and in the Southern California Bight (SCB; 1991–92 and 1995–2004) where the cold and temperate stocks presumably overlap during seasonal migrations. To assess whether the sardine had distinguishing chemical characteristics, we compared trace element profiles of otoliths with seawater temperatures to evaluate composition and interannual variability. Ca, Mg, P, Sr, Mn, and Ba in dissolved whole otoliths were analyzed by inductively coupled plasma mass spectrometry. Cohorts from central and southern California differed by collection site in Mg/Ca and P/Ca ratios. Mg/Ca and P/Ca ratios covaried and correlated with seawater temperature in the SCB in most years. High interannual variability in some trace element ratios within sites made it difficult to characterize site-specific otolith profiles. Otolith composition was likely influenced by temperature and local conditions. Stock mixing in the SCB may have contributed to interannual variability as well.

INTRODUCTION

Cooperation between Canada, the United States (US), and Mexico in management of Pacific sardine *Sardinops sagax* (Jenyns 1842) fisheries along the North American coast would be facilitated by knowledge of the distribution of regional stocks of mature and immature sardine along with their spawning habits and migration patterns. The range of Pacific sardine encompasses much of the California Current Ecosystem (CCE, fig. 1). Three stocks are believed to exist with seasonally synchronous, north-south migrations: a cold, northern stock along the California coast that migrates to the Pacific Northwest in the summer as adults to feed; a temperate, southern stock along the Pacific coast of Baja California, Mexico, that migrates to southern California; and a warm, gulf stock within the Gulf of California that migrates to the Pacific coast as far as Bahía Magdalena, Mexico (Félix-Uraga et al. 2004, 2005; Smith 2005).

The biological and environmental factors that divide Pacific sardine into regional stocks are not definitive. Unique genetic profiles within stocks have not been identified (Hedgecock et al. 1989; Grant and Bowen 1998; Pereyra et al. 2004; García-Rodríguez et al. 2011). Other methods that have offered clues to describing regional stocks and migration patterns include: egg, larval, and adult surveys (Lo et al. 2005, 2010, 2011); vertebral counts and tags (Smith 2005); temperature at catch (Félix-Uraga et al. 2004, 2005; Demer et al. 2012; Demer and Zwolinski 2014); analysis of spawning habitats (Reiss et al. 2008; Zwolinski and Demer 2013); fish and otolith morphometric analysis and aging (Javor et al. 2011; Javor 2013; Vergara-Solana et al. 2013); and otolith stable isotope measurements (Valle and Herzka 2008; Dorval et al. 2011; Javor and Dorval 2014).

Trace element composition in immature sardine otoliths could reflect chemical and temperature differences in spawning regions. In US waters, sardine spawn primarily in the spring off central and southern California (Lo et al. 2005, 2010). They spawn in the late fall and winter in the Gulf of California, and in the summer in Bahía Magdalena off southern Baja California (Smith 2005). Seawater temperature of sardine habitats ranges from less than 10°C in the Pacific Northwest (Emmett et al. 2005) to over 25°C in their southern distribution in Mexico (Mitchell et al. 2002; Félix-Uraga et al. 2004, 2005). Temperature is believed to be the primary physical driver of sardine population and individual growth rates, abundance, and large-scale migrations along the North American Pacific coast (Demer et al. 2012; Hill et al. 2014; Dorval et al. 2015).

Fisheries biologists often use otolith trace element composition to identify fish stocks. Factors that influence elemental partitioning into otoliths include temperature, salinity (Hoff and Fuiman 1993; Fowler et al. 1995; Elsdon and Gillanders 2002; Bath Martin and Wuenschel 2006), age, and ontogeny (Begg et al. 1998; Rooker et al. 2001; Brophy et al. 2003; Ruttenberg et al. 2005). Using several experimental approaches to influence trace element chemistry of otoliths in a companion study of juvenile Pacific sardine, Javor and Dorval (2016) demonstrated Mg and P were metastable in the
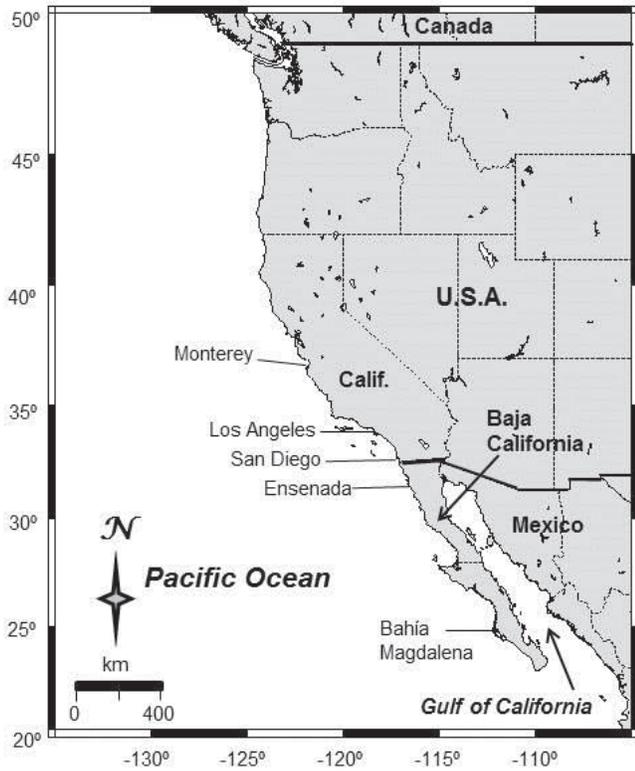
Figure 1. Map of collection sites of *S. sagax*.

otoliths of juveniles over time as they grew, and these two elements covaried in cultured sardine. Mg/Ca and P/Ca ratios decreased with growth in age-0 sardine at 16°–19°C, and Mn/Ca ratios increased.

The overall goal of this study was to use otolith trace element composition to identify and compare cohorts of age-1 Pacific sardine fished off central California (Monterey Bay) and in the Southern California Bight (SCB). The central California sardine belong to the cold stock, while the SCB sardine consist of seasonally influenced, migrating populations from cold and temperate stocks (Félix-Uraga et al. 2005). We approached the broad problem by addressing three specific issues: 1) How similar are trace element profiles of sardine otoliths within and between collection sites in the SCB? 2) How do otoliths of sardine cohorts in the SCB compare to each other and with seawater temperature over multiple years? 3) How do trace element profiles in otoliths of cohorts compare in age-1 sardine captured off central and southern California?

This investigation is a companion report to comparative surveys of oxygen stable isotopes in sardine otoliths (Dorval et al. 2011; Javor and Dorval 2014) and an experimental study of trace element behavior in otoliths of laboratory-reared, juvenile sardine (Javor and Dorval

TABLE 1
Collection sites, dates, and number of otoliths used in this study.
Mn/Ca and Ba/Ca ratios (both µmol mol^{-1}) are noted for each collection.

Site	Date	n	Mn/Ca Avg ± S.E.	Ba/Ca Avg ± S.E.
Monterey Bay	1996	30	2.286 ± 0.105	3.224 ± 0.175
	1997	26	2.353 ± 0.144	4.095 ± 0.211
SCB (Los Angeles)	1995–2003	122		
	1995	17	2.558 ± 0.113	3.701 ± 0.359
	1996	18	2.535 ± 0.177	3.425 ± 0.469
	1997	14	2.797 ± 0.220	4.981 ± 0.449
	1998	14	2.567 ± 0.150	4.333 ± 0.294
	1999	14	2.581 ± 0.181	2.932 ± 0.226
	2000	17	2.475 ± 0.141	3.520 ± 0.269
	2001	12	2.349 ± 0.139	4.012 ± 0.219
	2002	6	2.307 ± 0.157	4.213 ± 0.595
	2003	10	1.842 ± 0.142	3.019 ± 0.475
SCB (San Diego)	2004	10	2.187 ± 0.212	1.919 ± 0.186
Ensenada	1991	30		
	Apr	9	1.587 ± 0.099	0.876 ± 0.116
	Jun	6	2.014 ± 0.135	2.255 ± 1.037
	Oct	8	2.736 ± 0.438	3.151 ± 0.335
	Nov–Dec	7	2.710 ± 0.323	4.179 ± 0.810
	1992	30		
	Apr–May	11	2.338 ± 0.128	5.216 ± 0.755
	Jun	4	2.362 ± 0.292	4.907 ± 0.953
	Aug	5	2.630 ± 0.323	7.338 ± 1.134
	Sep	10	1.947 ± 0.130	6.419 ± 0.673

2016). The present study surveyed trace element composition in otoliths of wild-caught sardine to determine whether this method might be useful for differentiating stocks. It also addressed whether in vivo losses of Mg and P observed in otoliths of cultured juveniles could be detected in otoliths of wild sardine.

MATERIALS AND METHODS

Sources of Pacific Sardine

Fish were sampled each spring, coinciding with their presumed one-year birth date. During winter and spring, Pacific sardine migrate south when the SCB population is believed to consist of cold stock with some temperate stock influence (Félix-Uraga et al. 2005). Sardine otoliths were collected from port samples of commercial fisheries in central California (Monterey Bay) and the SCB (off Los Angeles, San Diego, and Ensenada) by Southwest Fisheries Science Center, California Fish and Wildlife, or Mexican affiliates (Javor and Dorval 2014) (fig. 1 and table 1). The SCB, 34.5° to 31.7°N, is considered here to include Ensenada, which is on the Baja California Peninsula (termed Baja California in this report), Mexico. One otolith of each pair was used for oxygen stable isotope analysis (Dorval et al. 2011; Javor and Dorval 2014), and the other was used for the trace element investigation.

Temperature

We compared otolith trace element ratios with published yearly sea surface temperatures (January–December averages at 5–15 m) for the SCB in US waters, determined from CalCOFI survey data as reported by Hill et al. (2014). The average measured temperature during 1995–2003 was 16.0°C, with annual values ranging from 14.9° to 16.7°C. However, based on oxygen stable isotopes determined in the study of Dorval et al. (2011), the average calculated temperature recorded in the whole otoliths of the 1995–2003 survey was 15.1°C, with the warmest years recorded in the 1996 and 1998 collections (both 16.6°C), and the coldest years recorded in the 1997 and 2002 collections (14.0° and 12.7°C, respectively). Temperatures calculated from oxygen stable isotopes in the otoliths were colder than the sea surface temperatures recorded in CalCOFI surveys of the SCB (Dorval et al. 2011). We did not include the temperature comparison with the 1991–92 Ensenada samples because we did not have data collected and averaged by the same methods for northern Baja California waters where currents and water masses are influenced by the Ensenada Front (McClatchie 2014).

Otolith Preparation

Otoliths were removed primarily from fresh fish with the remainder extracted from frozen fish.

After initially cleaning in deionized water (MilliQ™, MQ-H$_2$O), the otoliths were dried, weighed on a Cahn C-33 microbalance (0.005 mg accuracy), and stored in plastic microfuge tubes. They were further prepared in a Class 100 clean room using acid-cleaned implements. Following the procedure of Javor and Dorval (2016), the fragile otoliths were soaked in 2% sodium dodecyl sulfate for 30 min, washed at least 4 times with MQ-H$_2$O, soaked 3–5 min in 30% H$_2$O$_2$, and washed again with at least 4 final MQ-H$_2$O changes. Primarily left sagittal otoliths were analyzed. Preliminary measurements showed no difference in trace element composition between left and right otoliths (Javor and Dorval 2016).

There are significant linear and nonlinear relationships between otolith weight and trace element ratios in juvenile sardine, indicating strong ontogenetic effects on elements such as Mg and P (Javor and Dorval 2016). That study showed these effects were not significant when otolith weight was greater than 0.6 mg in age-0 sardine. To minimize size and age effects while comparing trace element ratios among groups of fish, we only used age-1 otoliths. The average weight of otoliths in this study was 1.0 mg (range = 0.8–1.4 mg) except for the 2004 collection off San Diego that had an average weight of 0.7 mg.

Inductively Coupled Plasma Mass Spectrometry (ICPMS)

Otoliths were dissolved in 2% HNO$_3$ with 2 ppb In as an internal standard for trace element analysis by solution-based ICPMS on a Finnegan MAT Element 2 instrument at Old Dominion University (Norfolk, Virginia). Samples were randomized in sets of 8 between a blank (to determine minimum detection limits and baseline) and a repeating standard (to determine drift). Average detection limits were: ^{24}Mg, 0.01 ppb; ^{31}P, 0.22 ppb; ^{48}Ca, 0.01 ppm; ^{55}Mn, <0.01 ppb; ^{88}Sr, 0.01 ppb; and ^{137}Ba, <0.01 ppb. Mean RSD(%) were: Mg, 6.16%; Ca, 5.27%; Sr, 2.85%; and Ba, 3.68%. RSD values for P and Mn were not provided by the analytical laboratory. Trace element ratios are reported as mmol mol^{-1} for Mg/Ca, P/Ca, and Sr/Ca; and μmol mol^{-1} for Mn/Ca and Ba/Ca.

Standards, reagents, and their sources were reported by Javor and Dorval (2016) and are briefly summarized here. Standards for solution-based ICPMS were diluted from a master solution to bracket similar element ratios and concentrations as sardine otoliths: 200 ppm Ca, 30 ppb Mg, 300 ppb P, 2 ppb Mn, 300 ppb Sr, 8 ppb Ba, and 2 ppb In as internal standard. Element ratios in the standard mixture were: Mg/Ca, 0.247 mmol mol^{-1}; P/Ca, 1.935 mmol mol^{-1}; Sr/Ca, 0.685 mmol mol^{-1}; Mn/Ca, 7.280 μmol mol^{-1}; and Ba/Ca, 11.660 μmol mol^{-1}.

Data Analysis

Several statistical approaches were used to evaluate the data sets collected from different sites and sampling years. To compare otolith composition of sardine captured across the SCB, we used a nonparametric discriminant analysis, the k-nearest neighbor method with $k = 3$. This method evaluated the accuracy of classifying individual otolith samples to their site and year of collection in the SCB based on five trace element ratios (Hand 1981; Souza et al. 2003; Dorval et al. 2005). Classification rates of otolith samples within site-year were predicted for fish collected in the SCB off southern California from 1995 to 2004 and Ensenada in 1991 and 1992. Contrary to many other methods, and particularly for explanatory analyses, the k-NN approach also provides classification rates for an "Other group" that is assigned to individual samples that have a low probability of belonging to the groups explicitly specified in the analysis. The k-NN method is suitable for the kind of survey we conducted. A jackknife (leave one out) method was used to estimate final classification accuracy within site-year. Because the years of capture of the Ensenada samples did not overlap with the capture dates of the California collections, our main objective was to determine whether trace element profiles from these southernmost samples (with the highest likelihood of influence by temperate stock) had characteristics that could discriminate fish caught in Mexican waters from those collected over multiple years from US waters. We present the results as the percent of otoliths from each annual collection that self-identified.

We also evaluated how temperature may have influenced the temporal variability of otolith trace element composition within the US waters of the SCB over multiple years (1995–2003) for spring-caught sardine. We used a nonmetric, weighted, multidimensional scaling analysis based on Euclidian distance (Krustal and Wish 1978; Schiffman et al. 1981). We used six variables (Mg/Ca, P/Ca, Mn/Ca, Sr/Ca, and Ba/Ca ratios, plus sea surface temperature) to compute Euclidian distances. Both the nearest neighbor and the multidimensional scaling analyses were performed using SAS software (version 9.4). In addition to the multidimensional analysis, we compared temporal trends of otolith element ratios and temperature with Spearman correlation coefficients. We considered correlation coefficients of 0.5 or less to be insignificant.

We also used 2 X 2 ANOVA tests to compare trace element ratios for the 1996 and 1997 cohorts that were born during the same annual spring spawning events off California and sampled as age-1 fish from central and southern California waters. Based on Hartley's (1950) F_{max}-test described by Sokal and Rohlf (1995), these ratios showed homogeneity of variance within year and site of collection after log transformation. In the log
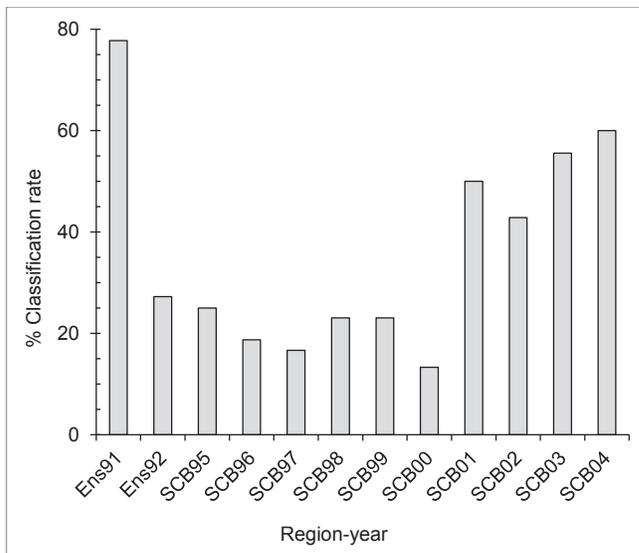

Figure 2.   Classification rates using nearest neighbor analysis to compare otolith trace element profiles of *S. sagax*. Abbreviations: Ens = Ensenada 1991, 1992; SC = southern California 1995–2004.

scale, ratios were normally distributed within year and site based on the Kolmogorov-Smirnov test, except for Ba/Ca and Mn/Ca measured in 1996 from southern California otoliths. Mg/Ca ratios did not meet either the normality or the homogeneity of variance assumption. We performed simple 2-way ANOVA on P/Ca and Sr/Ca ratios using the Type III sum of squares method, and robust 2-way ANOVA on Mg/Ca, Mn/Ca, and Ba/Ca ratios. The R function *raov* from the *Rfit package* (*R version 3.2.5*) was used to conduct the robust test for main effects and interaction based on the algorithm developed by Hocking (1985).

RESULTS

Predictability of Trace Element Profiles Within and Between Sites

We compared otolith composition of samples collected off southern California and Ensenada using nearest neighbor classification methods to evaluate site-specific homogeneity calculated as rates of self-classification. Greater self-identity percentages indicated uniqueness of those sampled fish from trace element profiles of sardine otoliths in the SCB over the course of years surveyed. Self-classification rates were high (nearly 80%) for sardine collected off Ensenada in the spring of 1991, but they were less than 30% for sardine captured off Ensenada in 1992 and Los Angeles in 1995–2000 (fig. 2). The SCB samples were relatively distinctive in the 2001–04 collections (40%–60% rates). Among the sardine analyzed by the k-NN method, less than 4% could not be assigned to any region–year group. Overall, trace element composition of sardine otoliths gener-
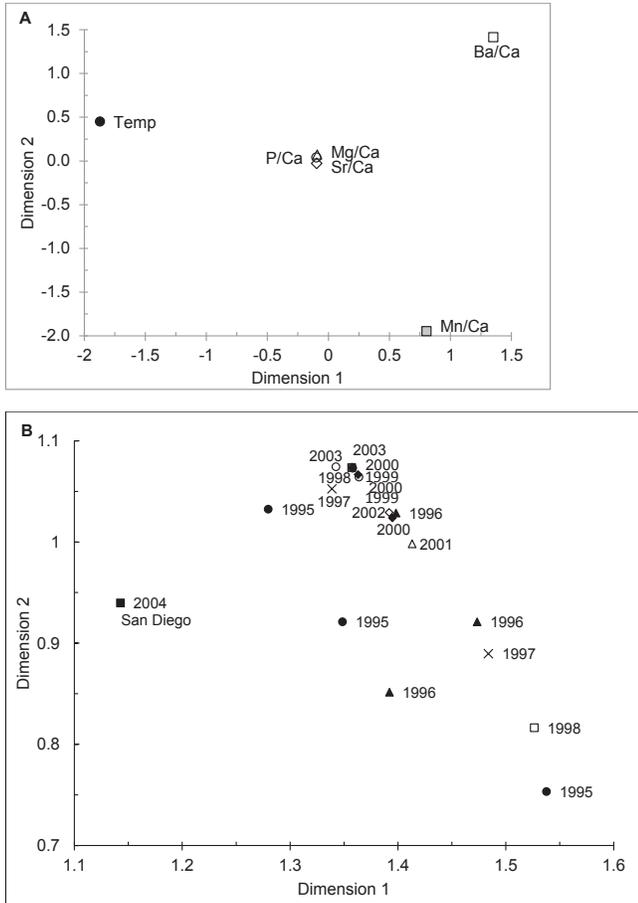
Figure 3. Multidimensional discriminant analysis of *S. sagax* otoliths from the SCB separated by trace element ratios (A) and by year (B).

ally followed regional patterns in some years in the SCB, but in other years they had relatively distinctive profiles.

We also evaluated the distinguishing factors of the spring, 1991 sardine from Ensenada by comparing Mn/Ca and Ba/Ca ratios in otoliths (table 1). All the otoliths from that collection had low Mn/Ca ratios (average 1.587 μmol mol^{-1}) together with very low Ba/Ca ratios (average 0.876 μmol mol^{-1}). Although some individual otoliths in this study had similarly low Mn/Ca ratios, otoliths having both low Mn/Ca and Ba/Ca ratios together were rare except in the Ensenada samples from spring 1991. This feature did not persist in subsequent samples from Ensenada. By the end of 1991 and through 1992, Mn/Ca ratios in Ensenada sardine resembled those measured in the SCB in the 1995–2003 samples while Ba/Ca ratios equaled and surpassed the averages measured in the 9-year survey. These observations on Mn/Ca and Ba/Ca trends were not further evaluated because the sample sizes were small and only two years were compared.

The predictability of assigning sardine to collection area was also analyzed with a multidimensional scale model that evaluated the annual seawater temperature and trace element ratios of otoliths sampled in the SCB from 1995 to 2004. If otolith composition profiles had been similar over the collection years, the cluster patterns would have been simple. However, the results were complex. The configuration of temperature and trace elements based on Dimension 2 against Dimension 1 showed a tripod shape with Mg/Ca, Sr/Ca and P/Ca ratios forming a cluster at the center (fig. 3A). Dimension 1 separated this cluster from temperature, Ba/Ca, and Sr/Ca. In contrast, Dimension 2 showed the cluster was more related to temperature than to Ba/Ca (at the top, positive) and Mn/Ca (at the bottom, negative).

The plot of coefficients for annual samples in the SCB exhibited three distinct groups when depicted as Dimension 2 versus Dimension 1 (fig. 3B). Dimension 2 mainly separated years with low annual mean temperature (2001–03, with coefficients between 0 and 1.1) and low concentration in Mg/Ca and P/Ca from samples that were collected in years of warmer water conditions. Some fish collected in 1995–98 grouped with the 2001–03 collections, but others formed a wide range grouping along Dimension 2 (from 0.75 to 0.95). Fish collected in 2004 off San Diego in the SCB formed a unique cluster along Dimension 1, and thus they were not similar to any groups of fish collected off Los Angeles in 1995–2003. Multidimensional scale analysis of trace element ratios in the multiyear survey of cohorts from the SCB did not clearly depict sardine otoliths as having distinct trace element profiles that clustered as a single stock nor did they exhibit characteristics that varied every year that would suggest stock mixing.

Temporal and Spatial Trends in Samples from the SCB

The data for the 1995–2003 collections (corresponding to spawning years 1994–2002) from off Los Angeles were evaluated to assess trends over time and to compare them with sardine from other collection sites in the SCB. Otoliths from the 1995–2003 collections had average Mg/Ca, P/Ca, and Sr/Ca ratios of less than 1 mmol mol^{-1}, and average Mn/Ca and Ba/Ca ratios of 2–4 μmol mol^{-1} (table 2A). Over this 9-year period, average Mg/Ca ratios generally decreased as did mean temperature (fig. 4) although overall there was poor correlation between the two variables when all the years were considered (Spearman ρ = 0.43, p = 0.25). P/Ca and Mg/Ca ratios covaried over the 9-year period (ρ = 0.990; table 2B). There was no significant correlation between mean temperature and the other element ratios over the 9 years ($\rho \leq 0.4$; data not shown).

Sardine collected off San Diego in 2004 had some distinct characteristics from sardine captured about 150 km to the north off Los Angeles in 1995–2003. Mg/Ca ratios

TABLE 2
Trace element ratios in age-1 *S. sagax* juveniles from the SCB off Los Angeles, 1995–2003, *n* = 122.
A: Molar trace element ratios, average ± S.E. B: Spearman correlations between trace element ratios.

A

Mg/Ca	P/Ca	Sr/Ca	Mn/Ca	Ba/Ca
mmol mol^{-1}	mmol mol^{-1}	mmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}
0.106 ± 0.005	0.824 ± 0.048	0.511 ± 0.007	2.481 ± 0.058	3.770 ± 0.135

B

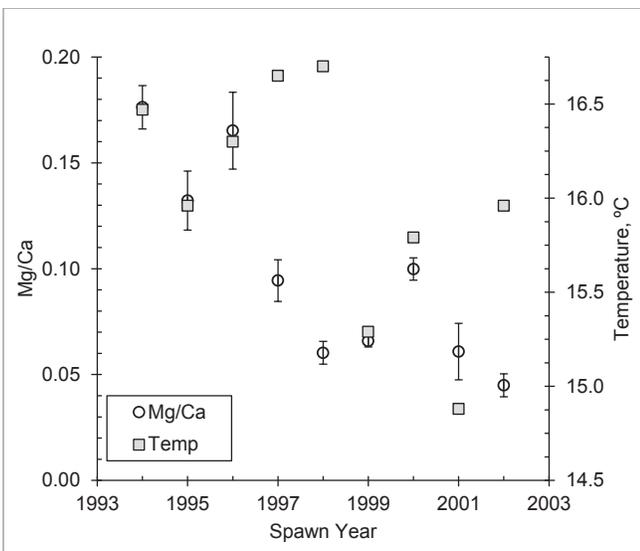|	Mg/Ca	P/Ca	Mn/Ca	Sr/Ca
P/Ca	0.990			
Mn/Ca	0.702	0.674		
Sr/Ca	−0.006	−0.047	−0.096	
Ba/Ca	0.488	0.424	0.469	−0.001

Figure 4. Temporal trends in average sea surface temperatures in the SCB, otolith Mg/Ca ratios (mmol mol^{-1}), and ranges of ratios (± S.E.) in age–1 *S. sagax* collected between 1995 and 2003 in the SCB off Los Angeles. Because the sardine spawned in the spring and spent the first ~8–9 months of their lives in the calendar year prior to their collection, the data are plotted according to the year of spawn.

averaged 0.276 ± 0.036 (± S.E.) mmol mol^{-1}, which were higher than most of the samples collected off Los Angeles (fig. 4). The 2004 otoliths were generally smaller (0.693 ± 0.018 mg) than the average size of age-1 otoliths which might have influenced the results for correlations and for the multidimensional analyses described above.

Because P/Ca and Mg/Ca ratios covaried in sardine otoliths, we compared P/Mg ratios in the collections to evaluate possible trends for both trace elements together. P/Mg ratios of sardine otoliths collected in 1991 and 1992 off Ensenada (9.071 ± 0.498 [S.E.]) differed little from ratios in otoliths of similar sized sardine caught off Monterey in 1996–97 (8.167 ± 0.401) and Los Angeles in 1995–2003 (7.816 ± 0.147). Lower P/Mg ratios were found in otoliths from sardine captured near San Diego in 2004 (4.498 ± 0.445).

Comparing Cohorts from Southern and Central California

We further assessed whether the Mg/Ca and P/Ca relationships in otoliths might be useful for differentiating and identifying sardine in neighboring regions and over time by comparing cohorts from 1996 and 1997 collected from Monterey Bay and off Los Angeles. The interaction between sites and years was significant for both P/Ca and Mg/Ca ratios (table 3). Although there were no significant differences between years, the Monterey sardine had significantly lower Mg/Ca ratios (fig. 5) and P/Ca ratios than southern California sardine. Comparisons of 1996 and 1997 results at the two sampling sites showed no significant site or year differences between other element ratios except for Ba/Ca ratios in Monterey that differed between the two years.

DISCUSSION

The overall picture of trace element profiles of age–1 otoliths of Pacific sardine captured in the SCB and Monterey is one of complex interactions between collection sites, year of capture, and seawater temperature. Of the five trace elements monitored, Mg, P, Mn, and Ba provided useful data for assessing profile similarities and differences. Sr/Ca ratios were largely similar in all the samples and were not useful for differentiating collections.

Predictability of Trace Element Profiles Within and Between Sites and Over Time

Trace element ratios in otoliths in our study were similar to those reported in a number of fish (Campana 1999), including Pacific sardine from the SCB grown in the laboratory (Javor and Dorval 2016) and *S. sagax* from Australia (Edmonds et al. 1995). By limiting the samples to age-1 and >0.6 mg, our study minimized ontogenetic effects on trace element composition (Javor and Dorval 2016). This stringency should have improved the predictability if otolith composition followed regu-

TABLE 3
Results of two-way ANOVA performed on trace element ratios in sardine otoliths collected in 1996 and 1997
from central (Monterey Bay) and southern (Los Angeles) California waters. $n = 84$ with $df = 1$ (see Methods).
A: Parametric model. B: Nonparametric model.

A. Parametric model

Element ratio	Source	MS	F	𝑃
P/Ca	Site	19.51	137.85	0.0000
	Year	0.30	2.12	0.1490
	Site★Year	1.08	7.83	0.0071
	Error	0.14		
Sr/Ca	Site	0.00	0.03	0.8720
	Year	0.00	0.03	0.8564
	Site★Year	0.02	1.40	0.2397
	Error	0.02		

B. Nonparametric model

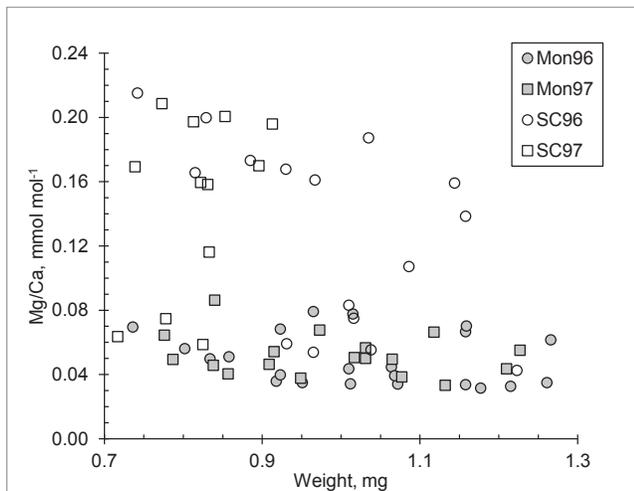| Element ratio	Source	RD	F	𝑃
Mg/Ca	Site	1.54	117.51	0.0000
	Year	0.08	5.99	0.1648
	Site★Year	0.07	5.25	0.0244
	Error	0.01		
Mn/Ca	Site	1.12	3.98	0.0493
	Year	0.35	1.26	0.2643
	Site★Year	0.16	0.58	0.4499
	Error	0.28		
Ba/Ca	Site	0.59	1.25	0.2665
	Year	15.97	33.96	0.0000
	Site★Year	1.01	2.39	0.1494
	Error	0.42		

Figure 5.   Relationships between molar ratios of Mg/Ca in age-1 *S. sagax* otoliths from cohorts collected near Monterey and in the SCB off Los Angeles, 1996 and 1997.

lar geographic and annual patterns. Our results showed similarities with studies of otolith trace element profiles in other fish species that compared sites and times of capture in that consistent profile patterns were often not detected (Gillanders 2002; Standish et al. 2011).

The annual self-classification rates in sardine were generally lower and more homogeneous in 1995–2000

than in 2001–04. Spring samples from Ensenada in 1992 resembled the 1995–2000 SCB samples, but the spring 1991 sardine from Ensenada had a largely unique profile. Mn/Ca and Ba/Ca ratios were both low only in the spring 1991 collection. To address whether those Ensenada sardine might have resembled a different stock, we compared the spring 1991 Ensenada samples with four monthly samples taken in 2004 from sardine captured in Bahía Magdalena farther south in Baja California. Those otoliths from presumably temperate and/or warm stocks did not share the low Mn/Ca–Ba/Ca feature (unpublished data). With limited sampling that lacked an overlap of dates, it is not possible to conjecture if and how low Mn–Ba trace element composition in sardine otoliths might be interpreted as an indicative population or stock feature.

The annual trends in self-classification in the SCB could have resulted from temporal changes in Pacific sardine stock size and composition. After the US stock collapsed in the 1940s and 1950s, the sardine population in US waters began to recover in the 1980s presumably by expansion of the population that had contracted into Mexican waters where the southern, temperate stock dominated. The US population biomass (northern or cold stock) was about 0.73 mmt in 1995, reached a peak biomass of about 0.91 mmt in

1999, and declined to 0.35 mmt by 2003 (Hill et al. 2014). During the period of the US sardine recovery in the 1980s and 1990s, the population in the SCB was likely influenced by the influx of temperate stock from Mexico and increasing local recruitment from the growing biomass of cold stock spawned in US waters. Shifts in sardine population sizes in the SCB, northern and southern sources of migrating stock, and environmental factors during this period could have altered trace element profiles recorded in age-1 sardine otoliths in 2001–04 following a period of relatively homogeneous trace element profiles in 1992–2000 when the US population was generally increasing.

Temporal Trends in Samples from the SCB

Although Mg/Ca ratios showed only modest overall correlations with temperature in the 9-year survey of sardine captured off Los Angeles, in 6 of the 9 years Mg/Ca ratios and temperature did correlate well (fig. 4, 1994–96 and 1999–2001, $\rho = 0.9$). These results indicate temperature could have played a significant role in otolith Mg incorporation during the first year of growth. Other variables that may have influenced trace element composition include overall stock composition (discussed above), local and regional stock mixing during seasonal migrations, variations in birth dates (and hence ontogeny), and physiological state (Sturrock et al. 2014, 2015).

Variations in temperatures actually experienced by the fish sampled in the collections as a result of depth preferences may have played a role as well. Otolith $\delta^{18}O$ composition indicates sardine in the SCB recorded temperatures colder than sea surface temperature which likely reflected their time spent in deeper water during daylight hours (Dorval et al. 2011; Javor and Dorval 2014). Sardine can avoid warm temperatures during El Niño events by remaining deeper for longer periods, but they cannot escape cold water during La Niña periods.

We did not assess seasonal trends in regional populations in this study. However, our preliminary unpublished data from a limited sample of sardine otoliths collected in summer and autumn months of 2004 from Bahía Magdalena in southern Baja California indicated trace element profiles in the November collection differed from summer collections, coinciding with expected seasonal migrations of temperate and warm stocks (Félix-Uraga 2004, 2005). Expanded annual surveys in different seasons from the SCB, Bahía Magdalena, and the Gulf of California might improve the resolution of regional identification of stocks of age-1 sardine. Used in combination with temperature and relevant morphometric criteria that might indicate stock identity (Javor et al. 2011; Javor 2013), otolith trace element profiles could

prove to be a useful tool to monitor stocks of subadult sardine throughout the California Current Ecosystem before they are large enough to undertake long-distance migrations.

Comparing Cohorts from Central and Southern California

The investigation comparing age-1 cohorts from central and southern California detected significant differences in Mg/Ca and P/Ca ratios between the two sites, but not the other trace elements measured. Salinity was similar at the two locations (Javor and Dorval 2014), hence differences in the composition of these trace elements might be attributable to temperatures during the first year of life. Based on oxygen stable isotopes in otoliths, sardine experienced an estimated average of 12°–14°C near Monterey (Javor and Dorval 2014), and 14°–17°C off Los Angeles in the SCB (Dorval et al. 2011).

Temperatures between 13° and 21°C affected the incorporation of Mg, P, Mn, Sr, and Ba in otoliths of juvenile sardine maintained in aquaria (Javor and Dorval 2016). That study showed negligible variation in Mg/Ca ratios of age-1 sardine grown at 13° and 17°C, two temperatures that juveniles likely experienced between central and southern California, respectively, where significant differences in Mg/Ca ratios were determined in this study. To reconcile these contradictory findings on temperature-Mg/Ca relationships in experimental and wild-caught sardine, we hypothesize incorporation and retention of Mg in sardine otoliths might respond to thermal influences differently during early life stages which were not assayed in this survey that analyzed dissolved whole otoliths of age-1 fish. Based on mass calculations, experiments showed Mg and P incorporated into juvenile sardine otoliths were metastable and partially lost during subsequent growth at 16°–19°C (Javor and Dorval 2016). We suggest the low Mg/Ca and P/Ca ratios in age-1 sardine captured in Monterey Bay may have resulted from greater losses of Mg and P from otoliths at cooler temperatures early in juvenile life relative to the sardine that presumably spent their first year in the SCB. Establishing geographic variations in the loss of entrapped or poorly bound Mg and P in juvenile sardine otoliths would be a novel application of addressing trace element ratios in biogenic carbonates. It might provide a key to differentiating populations in regions (i.e., between central California and the SCB) and in local sardine habitats (i.e., between Ensenada and San Diego where seasonal currents affect temperatures) (McClatchie 2014). This theory could be tested by time-course sampling of age-0 cohorts in growth series (e.g., monthly) from different sites.

CONCLUSIONS

Trace element composition in age-1 Pacific sardine otoliths off central California and in the SCB showed predictable profiles in some years and relatively distinctive profiles in others. Mg/Ca and P/Ca ratios were important for defining trace element profiles in cohorts between central and southern California, and were consistent with the possible loss of these trace elements from juvenile otoliths as the fish grew. Seawater temperature was a factor in Mg and P composition in most years. Interannual variations in trace element composition within collection sites of the SCB made identification of cold stock characteristics challenging. Our analyses showed that more frequent sampling within and between sites would be required for assessing population characteristics from trace element profiles in sardine otoliths.

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