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**Classification of Otoliths of Fishes Common in the Santa Barbara Basin Based on
2 Morphology and Chemical Composition**

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ABSTRACT

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Morphological and chemical features of fish otoliths are used to distinguish between populations and stocks. We hypothesized that these features can also be used to distinguish between fishes of different taxonomic groups common in and near the Santa Barbara Basin, including mesopelagic, pelagic and demersal fish. Sagittal otoliths obtained from 905 fish representing 6 taxonomic groups were imaged and 12 geometric and 59 elliptic Fourier morphometric features were extracted. A subset of 143 otoliths was also analyzed for Li, Na, Mg, K, Mn, Sr, and Ba. We used chemical composition in addition to morphology because the latter may be altered between otolith formation and analysis. Two sets of classifiers were made, one using only morphometric features and one using both morphometric and element features. Random forest analysis was generally superior to discriminant function analysis. Highest classification success, evaluated using cross-validation and otoliths of masked identity, was achieved with multiple feature types. The ten strongest discriminatory features of all available feature types were used in the final classification models. Our method is applicable to the classification of otoliths recovered from guts, feces, middens and sediments as well to classify other biological objects.

Otolith Classification

34 **KEY WORDS**

36 Otolith – Elliptic Fourier – Discriminant function – Random forest – ICP-MS – Trace
elements – California Current – Shape – Mesopelagic

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Otolith Classification

INTRODUCTION

Otoliths are aragonite and protein structures in the inner ears of teleost fish that function in hearing and balance (Popper et al. 2005). Otoliths are relatively resistant to alteration compared to other hard parts of fish and are often found in the guts and feces of fish and in middens and marine sediments (Pierce et al. 1991; Nolf 1995). Methods are needed to taxonomically classify otoliths from these sources for use in food web analysis and to construct chronologies of past abundances of fishes. Modern fish otoliths have morphological and chemical features that vary between species and with physiology, ontogeny, diet and the environment (Campana and Casselman 1993; Campana 1999; Elsdon and Gillanders 2003). These features can be used to discriminate between otoliths of fish that differ in these characteristics (Campana and Casselman 1993; Campana et al. 2000; Disspain et al. 2015). We develop, test and compare models to classify otoliths from groups of species of modern fish based on otolith shape and elemental composition.

We hypothesized that features from modern otoliths can be used to discriminate between taxa common in and near the Santa Barbara Basin (SBB). We use three types of features: geometric shape (Tuset et al. 2003), elliptic Fourier descriptors (Kuhl and Giardina 1982), and elemental composition (Campana 1999). Elemental composition was included because the morphology of an otolith may be altered between its formation and analysis whereas elemental composition is stable (Degens 1969).

The motivation for our study is to develop a method to identify otoliths found in sediment cores. Fish scales have been most commonly used to create a chronology of past fish abundance in waters overlying anoxic basins (Soutar and Isaacs 1969; Field et al. 2009; Skrivanek and Hendy 2015). However, scale production varies greatly between fish species, whereas all fish have a constant number of otoliths (Field et al. 2009). The present study contributes to our ability to assign otoliths extracted from anoxic sediments to taxonomic groups.

We focus on three objectives that contribute to methods of otolith classification. First, we use multiple types of features to taxonomically classify otoliths. Second, we compare discriminant function analysis and random forest analysis for otolith classification. Third, we use otoliths from mesopelagic as well as pelagic and demersal

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70 fishes to ensure inclusion of all types of fish that might occur in deep water sediments.

72 MATERIALS AND METHODS

74 A flow chart shows the processes from otolith acquisition to classification (Figure
1). All otoliths were from known species and acquired from either live-caught fish or
76 museum collections. Each otolith was imaged and morphometric features were extracted.
A subset of otoliths analyzed for morphometric features was also analyzed for element
78 concentration. Following feature extraction, two classification models were developed:
one used only morphometric features and a second used both morphometric and
80 elemental features.

82 Otolith Sources

Otoliths were obtained from recently captured fish and museum collections of
84 known species. Fish were captured by net and hook-and-line at multiple locations, stored
frozen (-4°C) and thawed immediately before use. Standard length (SL) and wet weight
86 were, for the majority of fish, measured and recorded before otolith removal. Both
sagittal otoliths (sagittae), the largest of three otolith pairs, were extracted with forceps
88 and a dissecting microscope, rinsed in 18 Milli Q water (i.e., quartz-distilled water with
resistivity >18.1 MΩ), excess biological and/or membranous material removed, air dried
90 and stored in vials.

In addition, otoliths from the Fitch Otolith Collection at the Natural History
92 Museum of Los Angeles County (LACM) and the Scripps Institution of Oceanography
Otolith Collection were obtained. Otoliths from these collections were used for
94 morphometric analysis only and returned.

96 Image Acquisition

All otoliths were imaged using Zeiss compound and Wild dissecting light
98 microscopes, each with a trinocular head and Spot 5.0 megapixel camera. An image of
one otolith, either right or left, was randomly selected for each fish, from which
100 morphometric features were extracted. Morphology does not differ between left and right

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otoliths (Zhang et al. 2014). Individual otoliths were imaged when dry and on a glass slide and illuminated by reflected light using a black background. Media Cybernetics Image-Pro Plus 7.0 software was used to acquire and save images as JPEG files. The magnification of each image was recorded. For each magnification, mm/pixel was estimated using a stage micrometer.

Image Processing

MATLAB's Image Processing and Statistics toolboxes were used (MATLAB Release 2014b, The MathWorks, Inc.). A series of pre-processing steps were used on each original JPEG image prior to feature extraction. First, the color JPEG image was converted to gray scale using the 'rgb2gray' function. Second, a threshold was used to create a binary image with the otolith white and the background black. Pixels with gray scale intensity above a threshold of 18 (out of 255) represented the white otolith and those at or below 18 represented the black background. This threshold value worked well for most images but, in some cases, a lower threshold value was used. Next, the 'imfill' function (cf., morphological closing) was used to fill any false black regions within the otolith object and the 'bwareaopen' function (cf., morphological opening) was used to fill any false white regions within the black background.

Geometric Features

The 'regionprops' function in MATLAB was used to extract nine geometric (GEO) features from the otolith in the binarized image: area (A), perimeter (P), major axis length (MAL), minor axis length (mAL), equivalent diameter (EqD), extent (Ext), eccentricity (Ecc), convex area ($CnvA$), and solidity (Sol). Three additional features were derived from the extracted features: roundness (Rnd , $4\pi A/(2P)$), aspect ratio ($AspR$, MAL/mAL), and ellipticity (Ell , $(MAL-mAL)/(MAL+mAL)$). Features are defined at <http://www.mathworks.com/help/images/ref/regionprops.html> and in Table S3. The geometric features were then converted from pixels to mm or square pixels to mm².

Elliptic Fourier Features

An elliptic Fourier series was estimated to mathematically represent the shape of

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the edge of the binary image of each otolith, resulting in a series of normalized elliptic Fourier (EF) features for each otolith (Kuhl and Giardina 1982). EF features have been used to classify a wide variety of biological objects, including grains, shells, plant foliage, and human mandibles (e.g., Innes and Bates 1999; Mebatsion et al. 2012).

First, a series of x and y coordinates were measured along the closed-contour (edge) of the otolith image at predetermined pixel intervals. One pixel in the otolith image was selected as a reference starting point. 100 evenly spaced pixels on the otolith boundary were then selected, moving clockwise around the otolith. The entire perimeter of the otolith was thus described by the 100 coordinates.

Second, this set of 100 coordinates was expressed as a chain code, each link representing one step and indicating the direction moved from one coordinate to the next. Chain coding was completed when the reference point was reached 100 samples later.

Finally, a Fourier series expansion was performed, which decomposes the chain code into series of harmonics (Kuhl and Giardina 1982). Each harmonic is described by four EF descriptors (a_n , b_n , c_n , d_n , where n = harmonic number and a , b , c and d are Fourier coefficients: a (cosine) and b (sine) in the x dimension and c (cosine) and d (sine) in the y dimension). The first harmonic ($n = 1$) is related to the overall size of the otolith and the following harmonics provide increasingly detailed information about the complexity of the otolith's shape. Thirty harmonics were used, capturing greater than 99.99% of the mean cumulated Fourier power (Mérigot et al. 2007; Stransky et al. 2008) and resulting in 120 EF descriptors for each otolith. The EF descriptors were normalized to be invariant with respect to otolith size, orientation, and chain code starting point by fixing $a_1=1$ and $b_1=c_1=0$ (Kuhl and Giardina 1982). The cosine and sine terms of each harmonic for x (a_n , b_n) and y (c_n , d_n) were combined using $EF\ x_n = [a_n+b_n]^2$ and $EF\ y_n = [c_n+d_n]^2$, resulting in 60 total EF features, 59 of which were unique and used in further analyses. Combining the cosine and sine components reduces the number of overall features while retaining information on the relative importance of each harmonic in quantifying an otolith's shape.

Otolith Elemental Composition

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Trace elements in otoliths were measured using a Thermo Finnigan Element2 single collector inductively coupled plasma mass spectrometer (ICP-MS). Solution-based ICP-MS of entire otoliths was used in order to integrate over the entire life history of each fish (Gillanders and Kingsford 2003). The elements ^7Li , ^{23}Na , ^{25}Mg , ^{39}K , ^{55}Mn , ^{88}Sr , and ^{138}Ba were measured and expressed as ratios with respect to measured ^{48}Ca (Bath et al. 2000). Otoliths used in the element analysis were only from live-caught fish; museum specimens could not be destroyed. When possible, otoliths for element analysis came from sampling events with at least six fish of the same species, providing sufficient replicates to test for the effects of year and sample location on elemental composition. One sagittal otolith from a fish was randomly selected. The elemental composition of left and right sagittae has been shown to not differ significantly in fish with bilateral symmetry (Rooker et al. 2001). Otoliths were cleaned in a Class 100 laminar flow hood located within a Class 100 clean room. All polypropylene plasticware that came into contact with the otoliths for element analysis was acid washed for 24 h in heated 10% HNO_3 , rinsed three times in ultra-pure water, air-dried in a Class 100 laminar flow cabinet, and stored in zip-lock bags before use. To remove any remaining organic tissue and/or contaminants, otoliths were transferred to acid-washed 0.6 mL polypropylene vials and rinsed for 5 minutes with 15% ultrapure H_2O_2 buffered with $0.05 \text{ mol L}^{-1} \text{ NaOH}$. Otoliths were then rinsed three times with Milli-Q water and air-dried overnight.

Cleaned otoliths were weighed and dissolved in a known volume of concentrated Seastar ultrapure nitric acid (HNO_3) then diluted with Milli-Q water to make a 5% nitric acid solution. Otolith identity was masked during analysis. Otoliths were analyzed in random order and in groups of approximately ten. Instrument blanks (5% HNO_3) and standards were analyzed at the start of each group. Measured intensity was corrected using a blank value for each sample estimated by linear interpolation between blanks measured before and after a series of samples. Instrument bias was corrected for by measuring values of certified JPR (NIES/WAMRL Red Emperor, *Lutjanus sebae*) and NIST (Standard Reference Material, 1948, fish otoliths) reference material.

Statistical Analyses

Data consisted of 12 GEO, 59 EF and 7 ELM features (Tables S1, S2). Data were

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assessed for normality and homogeneity of variance using Kolmogorov-Smirnov and
194 Levene's tests, respectively (Lilliefors et al. 1967; Brown and Forsythe 1974). All data
were log-transformed prior to further analyses. Bar graphs displaying the mean and
196 standard error for each GEO and ELM feature were used to visualize the differences and
distributions of otolith features across taxonomic groups. The mean and standard error of
198 the EF features were plotted in relation to harmonic number for each taxonomic group.
Single-factor analysis of variance (ANOVA) and the non-parametric Kruskal-Wallis test
200 were used to assess if each otolith feature differed between taxonomic groups. Features
for which there was no significant variation between taxonomic groups were excluded
202 from the classification analysis. The Tukey HSD test was used to perform *post hoc*
comparisons to ascertain which taxonomic groups differed from one another. Sampling
204 location (latitude), otolith size, and date (year) were also examined, using one-way and
multi-factor ANOVA, as potential sources of variation between otolith features. The level
206 of significance for all tests was 0.05, unless otherwise indicated.

We evaluated the ability of two methods to classify otoliths of known taxonomic
208 group whose identity was masked. First, we employed linear discriminant function
analysis (DFA) in which class assignment occurs by sequentially bisecting the data with
210 linear discriminant functions derived from the independent variables (Williams 1983).
Second, we used random forest analysis (RFA), a nonlinear ensemble or forest of many
212 tree classifiers in which class assignment occurs using an average of the forest of trees
(Breiman 2001). We explored DFA and RFA classification models with various
214 combinations of input features and selected the best performing models. We evaluated
model performance using a reduced number of input features using a DFA forward
216 stepwise procedure. Each stepwise procedure was performed 100 times to select a unique
combination of input features for inclusion into the model, typically 5-12 features total.
218 Each feature was ranked according the number of times out of 100 it was included in the
DFA stepwise model. A similar procedure was performed with the RFA, also resulting in
220 5-12 features for each of 100 analyses, which were then ranked by their frequency of
occurrence.

222 To evaluate classification accuracy and to aid in model selection, each model was
evaluated using 10-fold cross-validation (Kohavi 1995). One round of cross-validation

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involved random partitioning of the data into two complementary datasets: a “known” dataset, with 90% of the original data, used to train the model (“training dataset”), and an “unknown” dataset, with the remaining 10% of the original data, used to test the model (“test dataset”). Ten rounds of cross-validation were performed and the results were averaged. The misclassification error is defined as the average percent of errors made in classifying the test datasets in the 10 rounds. The 10-fold cross-validation procedure was performed 100 times and the misclassification error mean and standard deviation were calculated.

Canonical discriminant analysis (CDA) was used to separate the taxonomic groups in multidimensional space associated with the features used in the DFA and RFA classifiers. Features from the best performing DFA and RFA models were used in the CDA and the first two canonical variables used as axes to plot the otolith data by taxonomic group.

RESULTS

Sample Collection

Specimens of fish species common in and near the Santa Barbara Basin were collected in the southern CCS during research cruises. Specimens were also caught by seine and by hook and line from the Scripps Pier (La Jolla, CA) and obtained from bait barges in San Diego and Oxnard, CA (Tables S1, S2). 33 species of fish were collected between 2004 and 2013. The majority (87%) of live fish were collected south of Point Conception (34.51° N, Tables S1, S2). A total of 490 sagittal otoliths from live-caught fish were analyzed, one per fish.

Otoliths were also obtained from the Fitch Otolith Collection at the Natural History Museum of Los Angeles County (LACM) (n = 408) and the Scripps Institution of Oceanography (SIO) Otolith Collection (n = 7). A total of 415 otoliths from 22 species from museum collections were used and returned. Ancillary data, such as SL and collection location, were largely absent for these otoliths.

Otolith Groups

Otolith Classification

Seven otolith groups were defined for this study: entire otoliths of Bathylagidae, Engraulidae, Merlucciidae, Myctophidae, Sebastidae, and ‘Other’ fish, and broken otoliths of Bathylagidae. These groups were created to include the most common taxa found in and near the SBB (Table S9). The group ‘Other’ contained fish species not in the previously defined taxonomic groups but known from CalCOFI ichthyoplankton collections to be present in and near the SBB (Table S9). The group ‘Broken Bathylagidae’ includes Bathylagidae otoliths without the rostrum. This group was included because the rostrum of Bathylagidae otoliths is fragile and easily broken and entire and broken Bathylagidae otoliths differ in shape.

Otolith Data

Morphometric (M) data were obtained for all otoliths (n = 905) (Fig. 1). Element concentration and morphometric (ME) data were obtained for a subset of all otoliths (n = 143) (Fig. 1). M contained GEO and EF features for 905 otoliths from 46 species in seven taxonomic groups (Tables 1, S1). ME contained GEO, EF and ELM features from 143 otoliths from 12 species in six taxonomic groups (Tables 2, S2). In M, the group ‘Other’ contained otoliths from 21 species. In ME, the group ‘Other’ contained otoliths from only one species, *Sardinops sagax*, and therefore this group was referred to as Clupeidae. The group ‘Broken Bathylagidae’ was in M but not ME because we assumed entire and partial otoliths have the same elemental composition.

Feature Transformation

The majority of otolith features had a non-normal distribution (Kolmogorov-Smirnov test) and/or inhomogeneous variance (Levene’s test). Therefore, all features were log-transformed prior to statistical analysis. Even after transformation, the majority of otolith features had a non-normal distribution and/or heteroskedastic variance, as found in other studies (Gillanders et al. 2001; Elsdon and Gillanders 2005). We used transformed data in our analyses because the variances of transformed data departed less from normality than those of untransformed data. The departure from normality led us to use the non-parametric RFA in addition to parametric DFA to classify otoliths.

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Geometric Features

Each GEO feature varied significantly between groups for both M and ME (ANOVA and non-parametric Kruskal-Wallis tests; Tables 3, S4, S5, S10; Figures 2, 3). This was the case when the significance level was set at 0.05 (Table 3) or with Bonferroni corrections for multiple comparisons (Table S10). All GEO features were therefore retained for use in developing classification models. *AspR* and *Ell* were the strongest discriminatory GEO features for M ($F_{6, 483}$ statistics: *AspR* = 363.9, *Ell* = 346.1) and ME (ANOVA, $F_{5, 137}$: *Ell* = 297.3, *AspR* = 247.1) (Tables S4 and S5). Bathylagidae, Engraulidae, and Broken Bathylagidae GEO features differed the most from GEO features of the other groups for M, while Clupeidae and Bathylagidae GEO features differed the most from GEO features of the other groups for ME (Tukey HSD, Tables S6, S7).

Canonical function analysis resulted in two axes (Canonical Variables 1 and 2), which explained variation in the distribution of individual otoliths of the taxonomic groups. The results were similar for M and ME, with the difference due primarily to the larger sample size for M (905 otoliths) than ME (143 otoliths). Canonical Variables 1 and 2 explained 59.5% and 27.6%, respectively, of variation in the GEO features of the otoliths in M (Fig. S1). Canonical Variables 1 and 2 explained 73.4% and 19.6%, respectively, of variation in the GEO features of the otoliths in ME (Fig. S2). The aspect ratio of otoliths (*AspR*) and ellipticity (*Ell*) were most positively associated with Canonical Variable 1, while Roundness (*Rnd*) was most negatively associated with Canonical Variable 1. Extent (*Ext*) was most positively associated with Canonical Variable 2 for M (Fig. S1) and minor Axis length (*mAL*) with Canonical Variable 2 for ME (Fig. S2).

Elliptic Fourier Features

All EF features in M and all EF features except *y29* in ME varied significantly between taxonomic groups (ANOVA, Tables 3, S4, S5; $p < 0.05$). Results of the non-parametric Kruskal-Wallis test ($p < 0.05$) were consistent with those of the ANOVA. Thus, the feature *y29* was the only EF feature excluded from the classification analysis. The results were similar when the significance level reduced using the Bonferroni

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correction for multiple comparisons, although fewer EF features showed significant variation between taxonomic groups (Table S10). The most discriminatory features for M were $y1$ was the most discriminatory feature, followed by $x3$ and $y2$ (ANOVA, $F_{6, 483}$ statistics: $y1 = 51.1$, $x3 = 23.1$, and $y2 = 22.5$; Table S4). Those for ME were $y2$, $x9$ and $y5$ (ANOVA, $F_{5, 137}$ statistics: $y2 = 18.0$, $x9 = 16.8$, and $y5 = 16.6$; Table S5). *Post hoc* analysis of differences between EF feature variation among groups indicated that Merlucciidae and Engraulidae were most different from the other groups in M and Clupeidae and Merlucciidae were most different from the other groups in ME (Tukey HD, Tables S6, S7).

The EF features (29 x and 30 y descriptors) are plotted in relation to harmonic number for each taxonomic group (Fig. 4). For both M and ME, otoliths from Bathylagidae and Clupeidae had higher x and y EF descriptors than other taxa. Merlucciidae showed the greatest variability across the range of harmonics, while Myctophidae showed the least variability. Myctophidae had the lowest x and y EF descriptors. Standard errors for EF descriptors for M were, in general, smaller than those for ME, consistent with the differences in sample size (905 and 143 otoliths for M and ME, respectively).

The relationship between individual EF features and Canonical Variables 1 and 2 are shown in Figures S3 (M) and S4 (ME). Canonical Variable 1 captured 52.0% and 44.5%, for ME and M, respectively. Canonical Variable 2 captured 31.6% and 20.9% of the variability for M and ME, respectively. Variables $x3$ and $x5$ were most positively associated with Canonical Variable 1 for M and most negatively associated with Canonical Variable 1 for ME.

Element Concentration Features

Element concentration ratios for the six taxonomic groups are shown in Figure 5. Sebastidae and Bathylagidae had high values of Mg:Ca, Na:Ca, and Mn:Ca. Merlucciidae had high values of Mn:Ca and K:Ca and Myctophidae low values of these two elemental ratios as well as of Na:Ca and Mg:Ca. Engraulidae had low values of Sr:Ca and high values of Li:Ca. Clupeidae had element concentration ratios in the mid-range of those for all other groups. Significant differences between taxonomic groups were found for Li:Ca,

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Mg:Ca, Mn:Ca, Sr:Ca, Na:Ca, and K:Ca (ANOVA and Kruskal-Wallis test, Table S5; $p < 0.05$). Ba:Ca did not vary significantly between groups using either test and was therefore was not used in classification models. The results were similar using a Bonferroni correction for multiple comparisons, though only Mg:Ca, Na:Ca and K:Ca remained significant using ANOVA (Table S10). Mg:Ca was the most discriminating ELM feature, followed by K:Ca and Na:Ca (ANOVA, $F_{5, 137}$ statistics: Mg:Ca = 14.5, K:Ca = 13.5, and Na:Ca = 9.1). K:Ca values were positively associated with Canonical Variable 1 and negatively associated with the Canonical Variable 2, while Mg:Ca and Na:Ca values were positively associated with both canonical variables (Fig. S5). Canonical Variables 1 and 2 captured 49.6% and 29% of the variability, respectively. Merlucciidae differed the most from the other taxa in elemental composition (Tukey HSD, Table S7).

Factor Analysis

The effect of fish size and collection year and location on otolith features was examined using one-way and multi-factor ANOVA. Otoliths from museum collections that lacked ancillary data were excluded. Three categories of factors were used: five fish size ranges, four collection year ranges, and three collection regions (Table S8). ANOVA was performed for each feature of the three types (GEO, EF, ELM). The three categories (fish size and collection year and location) were not as important as taxonomic group for the large majority of GEO and EF features (Tables 3, S4, S5, S10). For ELM features, variability attributed to taxonomic group was greater than the variability attributed to any of the measured factors for all but two features (multi-factor ANOVA, Table S5; $p < 0.05$). First, Sr:Ca varied more with collection year than taxonomic group. This pattern appeared to be dominated by variability within the group Myctophidae. Myctophidae collected in 2010 had Sr:Ca concentrations that were almost twice as high as those collected in 2012. However, only four Myctophidae were collected in 2010, all *Protomyctophum crockeri*, which was not sampled in 2012. Given the small sample size and our inability to separate the effects of year and species for this feature, Sr:Ca was not excluded from the classification analysis. Second, K:Ca varied significantly with fish size range. This result was also only observed in one group, Merlucciidae. The effect of fish

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size on K:Ca could not be separated from the influence of species on K:Ca. Merlucciidae
380 otoliths were also significantly larger than those of any other group. We decided to not
exclude K:Ca from the classification analysis. Factor analysis results using Bonferroni
382 correction for multiple comparisons yielded similar results (Table S10).

Classification Models

Our goal was to create models with the most success in classifying otoliths of
386 masked origin into taxonomic groups using only morphometric (M) or both
morphometric and elemental composition (ME) data. We evaluated linear discriminant
388 function analysis (DFA) and random forest analysis (RFA) using cross validation.

Five sets of feature combinations were compared for otoliths in M (morphometric
390 data only): (i) GEO, (ii) EF, (iii) GEO, EF (vi) the DFA forward stepwise feature set
(DFA10 SW), and (v) the RFA rank feature set (RFA10 Rank). Eight sets of feature
392 combinations were compared for otoliths in ME (morphometric and element data): (vi)
GEO, (vii) EF, (viii) ELM, (ix) GEO, ELM, (x) GEO, EF, (xi) GEO, EF, ELM, (xii)
394 DFA10 SW, and (xiii) RFA10 Rank. Each set of features was tested using both DFA and
RFA. RFA outperformed DFA in most cases (Table 4). RFA results are reported below
396 unless otherwise noted.

Classification models based on only one type of feature (i, ii, vi, vii, and viii
398 above) were tested first (Table 4). For both M and ME, GEO features outperformed both
EF and ELM features. For M, the classifier using only GEO features had a 10-fold cross-
400 validation misclassification error of 14.6% compared to 25% when only EF features were
used. For ME, the 10-fold cross validation misclassification error was 8% for the GEO-
402 based classifier, 13.2% for EF-based classifier, and 17.1% for the ELM-based classifier.

Classification models based on two or three types of features (iii, ix, x and xi
404 above) were then tested (Table 4). Classification based on both GEO and EF for M had a
10-fold cross-validation misclassification error of 14.1%, compared with 14.6% for a
406 classification model based on only one feature type (GEO). Classification of ME using all
the features and types (GEO, EF, ELM) resulted in the best model, with a
408 misclassification error of 4.6%.

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Knowing that classification success was greatest using all available types of features (GEO and EF for M; GEO, EF and ELM for ME), we next examined if reducing the number of individual features would improve classification by reducing co-linearity among features (Williams 1983). Both DFA stepwise and RFA rank procedures were used to create classification models and rank the discriminatory power of individual features as they were added. Starting with five features, classification success increased as additional features were added until an inflection point was reached at ten features. The classification model with the highest success for M contained ten input features, determined using the RFA rank procedure (RFA10 Rank). This model included 9 GEO features and 1 EF feature and had a misclassification error of 11.8% (Tables 4, 5). The classification model with the highest success for ME included ten features, determined using the DFA stepwise procedure (DFA10 SW). This model had 7 GEO, 1 EF and 2 ELM features and a misclassification error of 3.7% (Tables 4, 5).

Canonical Function Analysis

Canonical function analysis was used to examine patterns in feature and group variation (Fig. 6). Canonical Variable 1 and 2 explained 51.5% and 34.5%, respectively, of feature variation for M and 59.0% and 27.3%, respectively, of feature variation for ME. The aspect ratio of otoliths (*AspR*) and ellipticity (*Ell*) were most positively associated with Canonical Variable 1, while extent (*Ext*) was most positively associated with canonical variable 2 for both M and ME (Fig. 6). In general, Engraulidae and Merlucciidae were most strongly associated with positive values of both Canonical Variables 1 and 2. Myctophidae was most positively associated with negative Canonical Variable 1 and positive Canonical Variable 2. Bathylagidae was also negatively associated with Canonical Variable 2.

DISCUSSION

The present study uses otolith morphology and elemental composition with two classification methods to discriminate between otoliths of groups of fish found in and near the Santa Barbara Basin. We include elemental composition because otolith

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morphology may be altered between otolith formation and analysis. We found consistent differences of otolith features between groups. Classification models with greatest success were based on the ten highest-ranked otolith features.

Otolith Selection

Our focus is on otoliths of fish from in and near the Santa Barbara Basin (SBB). We selected species representative of fish common in this region over the entire water column, assuming that otoliths from those fish accumulate on the bottom of the SBB. The large majority (87%) of otoliths used in this study were from fish caught off the coast of Southern California, while the remaining fish were collected between Point Conception and Southern Oregon or obtained from regional otolith collections. We included mesopelagic fish because they are abundant off Southern California and in the SBB (Moser and Watson 2006; Davison et al. 2015). Mesopelagic fish are also important prey of fish, squid, sea birds, and marine mammals (Fitch and Brownell 1968; Pinkas et al. 1971; Markaida and Sosa-Nishizaki 2003). Recent, revised estimates of abundance of mesopelagic fish worldwide indicate their importance in the global ocean (Irigoien et al. 2014; Koslow et al. 2014; Davison et al. 2015).

The groups Bathylagidae, Engraulidae, Myctophidae, Merlucciidae, Sebastidae, and 'Other' include 17 of the 20 most common species in and around the SSB. The taxonomic group 'Other' contained less common fish species that were not part of the other five taxonomic groups, including sardine (*Sardinops sagax*) and bristlemouths (*Vinciguerrria lucetia*). These groups include 95% of the total number of fish larvae enumerated in CalCOFI 505- μ m-mesh net tows in the southern California Current System since 1951 (Table S9).

Ichthyoplankton collected at stations in the center of the SBB (CalCOFI Lines 81.5-82 and Stations 46-47) are representative of ichthyoplankton collected over most of the CalCOFI grid, with a few exceptions. SBB ichthyoplankton include more Bathylagidae and Sebastidae and fewer Merlucciidae than other areas of the CalCOFI grid (Table S9). We assume that the taxa whose otoliths were used for morphometric analysis (M) in this study were representative of fish common in the SBB. Although only

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a subset of otoliths ($n = 143$) was analyzed for elemental composition, it had representatives from each of the taxonomic groups.

Otolith Features

We are unaware of other studies that have developed classification models for mixtures of otoliths of pelagic, mesopelagic and demersal fishes. Nor are we aware of studies that have developed classification models for otoliths based on both morphology and elemental composition. The three types of features (GEO, EF and ELM) in our study differed in their power to discriminate between otoliths from different taxonomic groups. Bathylagidae and Clupeidae were best separated from other taxonomic groups using GEO features, while Merlucciidae and Engraulidae were best separated from one another using EF and ELM features. Myctophidae and Sebastidae had similar GEO and EF features. While ELM features differed significantly between Bathylagidae and Myctophidae, Clupeidae overlapped with both of these taxa, indicating that there was no strong pattern in otolith elemental composition between pelagic and mesopelagic fish (Table S7).

Twelve geometric features were used to define the geometric (GEO) morphology of each otolith. Of these features, six (*Sol*, *Ecc*, *Ell*, *Rnd*, *Ext*, *AspR*) were dimensionless yet indirectly related to otolith size, as other studies have found (e.g., Zorica et al. 2010). The remaining six GEO features (*A*, *P*, *MAL*, *mAL*, *EqD*, *ConvA*) had either linear or areal dimensions and thus were directly related to otolith size. More variability of the dimensionless GEO features was explained by taxonomic group than by otolith size or any other factor (Tables S4, S5). The most useful GEO features for discriminating between taxa were the dimensionless features *Ell* and *AspR*. Nevertheless, both dimensionless and size-related features ranked sufficiently high to be included in the final classification models (i.e., DFA10 SW, RFA10 Rank). Studies using otolith morphology to discriminate between fish stocks and populations have avoided the use of size-related features in preference of dimensionless features with little or no relation to size (e.g., Tuset et al. 2003; Zorica et al. 2010). Our objective, to classify otoliths into taxonomic groups, warranted our use of size-related GEO features, which have strong discriminatory power between groups that differ in morphology (Reist 1986).

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We normalized the elliptic Fourier (EF) descriptors by otolith size. One hundred
502 evenly spaced boundary pixels comprised the chain-code. Thus, spatial resolution of the
chain code depended on otolith size. However, our classification results indicate that the
504 low harmonic ($n \leq 5$) descriptors, representing the basic shape of an otolith, were most
important (Table 4). We conclude that 100 evenly spaced chain-code pixels were
506 sufficient to describe the contour of each otolith, independent of otolith size. This is also
consistent with the rapid decline in EF descriptor values with increasing harmonic
508 number (Fig. 4). Our use of 30 harmonics captured $> 99.99\%$ of the mean cumulated
Fourier power (Mérigot et al. 2007; Stransky et al. 2008). Each of the final two
510 classification models used only one EF feature (Table 5). EF features have been observed
to vary within species (Campana and Casselman 1993) and between species (Stransky
512 and MacLellan 2005; Kemp et al. 2011). We also observed that variability in EF features
was better explained by taxonomic group than by otolith size, year or location (Table S4,
514 S5). EF features varied most for Merlucciidae and least for Myctophidae (Fig. 4), likely
due to the rugose and high aspect ratio of Merlucciidae otoliths and the smooth and
516 circular nature of Myctophidae otoliths (Jones and Morales 2014).

Otolith shape analysis has been used to taxonomically classify otoliths in gut
518 contents (Casper et al. 2006) and in paleoecological studies (Parisi-Baradad et al. 2005;
Reichenbacher et al. 2007; Disspain et al. 2015). Our results are consistent with these and
520 other studies showing that otolith morphology can be used for taxonomic classification
(Tuset et al. 2008; Zorica et al. 2010) and that otolith shape is principally under genetic
522 control (Lombarte et al. 2010; Reichenbacher and Reichard 2014).

The large differences we observed in the elemental composition of otoliths
524 between taxonomic groups are consistent with studies showing otolith elemental
composition varies between species (Gillanders and Kingsford 2003; Swearer et al. 2003;
526 Hamer and Jenkins 2007). We found Mg to most strongly discriminate between
taxonomic groups, followed by K, Na, Sr and Mn. An extensive analysis of the elemental
528 composition of otoliths, most of which were from European waters, also observed
variability between species within a region, particularly in Sr, Mg, Mn, and Ba (Chang
530 and Geffen 2013). We note that Sr and Mg were strong discriminators between otoliths

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from fish in both the southern California Current System (present study) and European waters (Chang and Geffen 2013).

We observed stronger effects on elemental composition by taxonomic group than year and location, consistent with variation in the species-specific physiology of trace element incorporation in the otolith (Chang and Geffen 2013). However, otolith elemental composition may also vary between taxa due to variation in habitat use and diet (Ruttenberg et al. 2005; Gillanders 2005). The combined variability in elemental composition related to species, date, and location preclude generalizations about the abundances of elements in regard to species, date and location (Campana and Thorrold 2001; Chang and Geffen 2013).

We did not observe a clear pattern of the element concentrations among pelagic, mesopelagic and demersal fishes. Otoliths of benthic fish species have been observed to possess higher concentrations of Sr and lower levels of other trace elements than otoliths of pelagic and demersal species (Swearer et al. 2003, Hamer and Jenkins 2007).

Otolith elemental analysis has been most widely used to infer about stocks (Campana et al. 2000; Tanner et al. 2016), migration pathways (Hamer et al. 2006) and natal origins (Thorrold et al. 2001). Our results and those of other studies support the hypothesis that, with appropriate background data and within appropriate ranges of species, date and location, the concentrations of certain elements can be used in the taxonomic classification of otoliths of unknown origin.

We used elemental composition in addition to morphology to distinguish between taxonomic groups because of the possibility that otolith morphology may be altered between otolith formation and analysis whereas the elemental composition is unlikely to change. Our methods will be useful for the classification fossil otoliths recovered from Santa Barbara Basin sediments. The accuracy of classification relies on the preservation of the features in the archaeological or paleontological otolith samples, which may be altered between otolith formation and analysis (Proctor and Thresher 1998). Alteration of otoliths may result from physical, chemical or biological processes that can occur during ingestion, gut passage, burial and sampling (Nicholson 1996; Zohar et al 2008; Disspain et al. 2015). To reduce potential bias due to otolith shape alteration, we included elemental composition in our analyses. Diagenetic processes have been shown to not

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have significantly altered the elemental composition of fossil otoliths, when compared to modern specimens (Degens 1969; Kern 2012; Cook 2015; Cook 2016).

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We sought to classify otoliths of unknown origin based on their morphology and elemental composition. We used distinguishing features and two types of classification models. We tested our models using masked otoliths and cross-validation. The classifiers we developed are useful for the analysis of otoliths from Santa Barbara Basin sediments, which lie beneath waters containing fish species represented by those in this study. The methods we used may be of general interest for the development of classifiers to analyze otoliths of unknown origin from other sources including feces, gut contents, middens and sediments.

A variety of methods have been used to classify otoliths (e.g., Edmonds et al. 1991; Gillanders and Kingsford 2003; Mercier et al. 2011). Linear discriminant function analysis (DFA) has been used most often. Machine learning, including random forest analysis (RFA) and artificial neural networks (ANN), has been used in medical (Jiang et al. 2007), ecological (Perdiguero-Alonso et al. 2008) and otolith (Mercier et al. 2011) pattern recognition studies. RFA was used with otolith elemental composition to classify stocks of freshwater marine fishes (Paillon et al. 2014, Loewen et al. 2015). These studies focused on the elemental composition of otoliths.

We compared combinations of GEO, EF and ELM features for classifying otoliths of masked taxonomic group (Table 4). We built on prior studies, in which multiple types of features were used to increase discrimination power (Kemp et al. 2011, Longmore 2010), by also using two classification methods. RFA outperformed DFA for M using GEO and EF features singly and in combination (Table 4). RFA also outperformed DFA for ME using all three types of features singly or combined, except for one case (Table 4). These results are consistent with other studies (Jiang et al. 2007; Perdiguero-Alonso et al. 2008; Mercier et al. 2011). Our results are also consistent with the suggestion that RFA is best for complex data (Mercier et al. 2011). RFA may outperform DFA due to inherent differences in their mechanisms. RFA uses ensemble decisions and sequential hyperplanes to partition space, making more complex decision boundaries possible, while

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DFA is limited by the use of a linear hyperplane (Williams 1983). However, DFA was
594 the best model for ME, although the increase in classification success over RFA was
small (1.2%).

596 Morphological (GEO) features outperformed both elliptic Fourier (EF) and
elemental composition (ELM) features when each was tested independently using cross
598 validation (Table 4). This result differs from otolith classification studies that have found
EF or ELM features often superior to GEO features (Kemp et al. 2011; Longmore 2010).
600 This disparity may be due to our focus on distinguishing between taxonomic groups
while other studies focused on distinguishing within species and between populations and
602 stocks, where morphology may be less informative than elemental composition
(Campana and Casselman 1993; Campana et al. 2000; Campana and Thorrold 2001;
604 Geffen et al. 2003; Swan et al. 2006; Chang and Geffen 2013).

Most otolith classification studies have focused on a single type of feature or
606 compare the classification success between types of features (Milton and Chenery 2001;
Lin et al. 2007; Marohn et al. 2009). When different types of features are used together,
608 classification success can increase significantly (Tracey et al. 2006; Burke et al. 2008).
We tested 5 to 12 features of two or three types (GEO, EF, ELM) for our final
610 classification models. Discrimination power approached an asymptote at 10 input
features for both M and ME. Similarly, Mercier et al. (2011) found 8-10 input features to
612 be optimal. Our final classification models for M and ME each contained at least one
feature from all available categories, highlighting the value for discrimination by using a
614 limited but sufficient number of different types of input features.

We show that otolith morphology and elemental composition can be used together
616 to distinguish taxonomic groups of fish found in and near the Santa Barbara Basin.
Morphological and elemental composition features varied significantly among taxonomic
618 groups. Their use in DFA and RFA classifiers provided strong discriminatory power. In
general, RFA was a superior to DFA, especially for ME, which contained both
620 morphometric and elemental composition features. The highest classification success, as
judged by cross validation, was obtained by using the ten features, of all types, that
622 contributed most to discrimination between taxonomic groups and by limiting the total
number of features provided. The methods used here may have a wider application to the

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classification of otoliths from groups of fish from other areas and of other types of objects based on multiple types of features.

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Otolith Classification

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Otolith Classification

Tables and Figures

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Figure 1

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Otolith Classification

Figure 1. Flow chart of otolith analysis and creation of classification models. Feature
types are geometric (GEO), elliptical Fourier (EF) and element (ELM) concentration.
Groups refer to categories of fish species common in and near the Santa Barbara Basin.
M refers to classification models based otoliths (n = 905) with only morphometric feature
types (GEO & EF). ME refers to classification models based on otoliths (n = 143) with
both morphometric and element concentration feature types (GEO, EF & ELM).
Discriminant function analysis (DFA), with stepwise (SW) feature selection, and random
forest analysis (RFA), with rank-based feature selection, were used. The 10-fold cross
validation was performed 100 times on each classifier to estimate classification error. The
ten top-ranked features available in M and ME were used to create the final classifiers.
See text for detail.

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Otolith Classification

Figure 2

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Figure 2. Mean and standard error of log-transformed geometric otolith features for different taxonomic groups of fish from the southern California Current System for M (n = 905). ENG = Engraulidae (n = 82), BTH = Bathylagidae (n = 130), BRB = Broken Bathylagidae (n = 51), OTH = other (n = 231), MRL = Merlucciidae (n = 19), SEB = Sebastidae (n = 24), MYC = Myctophidae (n = 368). Feature abbreviations can be found in Table S3.

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Otolith Classification

Figure 3

Figure 3. Mean and standard error of log-transformed geometric otolith features for different taxonomic groups of fish from the southern California Current System for ME (n = 143). BTH = Bathylagidae (n = 31), CLP = Clupeidae (n = 18), ENG = Engraulidae (n = 11), MRL = Merlucciidae (n = 9), MYC = Myctophidae (n = 59), SEB = Sebastidae (n = 15). Feature abbreviations can be found in Table S2.

Otolith Classification

Figure 4

Figure 4. Log₁₀ of elliptic Fourier descriptors x and y for M (A, B) and ME (C, D) in relation to harmonic number. Mean with standard error bars shown. Abbreviations for taxonomic groups as in Figs. 2 and 3.

Otolith Classification

Figure 5

Figure 5. Mean and standard error element concentrations of six taxonomic groups for ME (n = 143). Taxonomic group abbreviations as in Fig. 3.

Otolith Classification

Figure 6

Figure 6. Canonical function analysis of otolith features for the two best classification models. Symbols represent individual otoliths. Vectors represent relative importance of features in explaining canonical function variability. A) M (morphology only) otoliths from seven taxonomic groups using ten features in the RFA10 Rank model. Canonical Variables 1 and 2 explain 51.4% and 34.5%, respectively, of variability in M otoliths. B) ME (morphology and element concentration) otoliths from six taxonomic groups using 10 features included in the DFA10 SW model. Canonical Variables 1 and 2 explained 59.0% and 27.3%, respectively, of the variability in the ME otoliths. Feature abbreviations as in text and Table S3.

Otolith Classification

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Otolith Classification

Table 1. M otoliths (n = 905) analyzed for geometric (GF) and elliptic Fourier (EF) features. Collection and feature data are in Table S1.

Taxonomic Group	Number of Species in Group	Number of Otoliths
Engraulidae	1	82
Bathylagidae	3	130
Broken		
Bathylagidae	1	51
Merlucciidae	1	19
Myctophidae	15	368
Sebastidae	4	24
Other	21	231

Otolith Classification

Table 2. ME otoliths (n = 143) analyzed for geometric (GEO), elliptic Fourier (EF), and element (ELM) concentration features. Collection and feature data are in Table S2.

Taxonomic Group	Species	Number of Otoliths
Bathylagidae	<i>Leuroglossus stilbius</i>	11
	<i>Lipolagus ochotensis</i>	5
	<i>Bathylagus wesethi</i>	15
Clupeidae	<i>Sardinops sagax</i>	18
Engraulidae	<i>Engraulis mordax</i>	11
Merlucciidae	<i>Merluccius productus</i>	9
Myctophidae	<i>Triphoturus mexicanus</i>	6
	<i>Tarletonbeania crenularis</i>	6
	<i>Stenobrachius leucopsarus</i>	16
	<i>Protomyctophum crockeri</i>	4
	<i>Diaphus theta</i>	11
	<i>Nannobrachium ritteri</i>	16
Sebastidae	<i>Sebastes</i> spp.	15

Otolith Classification

Table 3. ANOVA summary for otolith features in M (morphology) and ME (morphology and elemental composition). Each element in the table body contains the number of tests in which the null hypothesis was rejected (numerator) and accepted (denominator). One-way ANOVA (parametric) and Kruskal-Wallis ANOVA (non-parametric) tested the null hypothesis of no difference between means of a feature among taxonomic groups (seven for M, six for ME). Multi-factor ANOVA (parametric) tested the null hypothesis of no difference between means of a feature among taxonomic groups, fish size (five ranges) and collection location (three regions) and year (four periods). $N_{feature}$ is the number of features in a feature type and hence the number of tests. Test results are in Tables S4 (M) and S5 (ME).

			One-Way ANOVA	Kruskal-Wallis	Multi-Factor ANOVA			
	Feature Type	$N_{feature}$	Taxonomic Group		Taxonomic Group	Fish Size	Location	Year
M	GEO	12	12/0	12/0	12/0	12/0	8/4	12/0
	EF	59	59/0	59/0	58/1	14/45	13/46	21/38
	All	71	71/0	72/0	70/1	26/45	21/50	33/38
ME	GEO	12	12/0	12/0	12/0	9/3	6/6	9/3
	EF	59	59/0	58/1	59/0	39/20	7/52	4/55
	ELM	7	6/1	6/1	5/2	4/3	0/7	2/5
	All	78	78/0	77/1	78/0	52/26	13/65	15/63

Otolith Classification

Table 4. Classification model results using both discriminant function analysis (DFA) and a random forest analysis (RFA) evaluated using a 10-fold cross-validation procedure carried out 100 times.

Feature Set	Group	Number of otoliths	Number of features	Mean cross-val. error (DFA)	Stdev. cross-val. error (DFA)	Mean cross-val. error (RFA)	Stdev. cross-val. error (RFA)
GEO	M	905	12	0.221	0.003	0.146	0.005
EF	M	905	59	0.407	0.008	0.250	0.007
GEO, EF	M	905	71	0.190	0.005	0.141	0.002
DFA10 SW M*	M	905	10	0.220	0.003	0.125	0.001
RFA10 Rank*	M	905	10	0.250	0.003	0.118	0.004
GEO	ME	143	12	0.066	0.012	0.080	0.008
EF	ME	143	59	0.343	0.024	0.132	0.016
ELM	ME	143	6	0.491	0.008	0.171	0.015
GEO, ELM	ME	143	18	0.071	0.008	0.050	0.017
GEO, EF	ME	143	71	0.102	0.012	0.073	0.011
GEO, EF, ELM	ME	143	77	0.108	0.015	0.046	0.008
DFA10 SW ME*	ME	143	10	0.037	0.004	0.049	0.005
RFA10 Rank*	ME	143	10	0.110	0.011	0.056	0.019

*Refer to Table 4 for list features included in these models. GEO = Geometric Features, EF = Elliptic Fourier *x* and *y* components, ELM = Element concentration features.

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Otolith Classification

Table 5. Features included in the ten-feature DFA SW and RFA rank models for M and ME.

Model	Group	GEO*	EF*	ELM*
DFA SW	M	<i>EqD, AspR, P, Sol, CnvA, Ext, A</i>	<i>x3, y3, y2</i>	N/A
RFA10 rank	M	<i>Ext, EqD, CnvA, mAL, Ecc, AspR, Ell, Sol, P</i>	<i>y1</i>	N/A
DFA10 SW	ME	<i>AspR, Ext, Sol, MAL, P, mAL, Ell</i>	<i>x5</i>	Sr, Mg
RFA rank	ME	<i>Ecc, AspR, Ell, Ext, mAL, A, Sol, MAL, P</i>	-	Sr

*Refer to text and Table S3 for feature abbreviations and descriptions.

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Table S7. Tukey HSD test results for ME. Mean and standard error (SE) are displayed. EL DFA10 SW = ME reduced model.

ME Taxa	Number of otoliths	% of total	ELM mean	ELM SE
Bathylagidae	31	21.67832168	0.00076	0.00008
Clupeidae (Other)	18	12.58741259	0.00071	0.00011
Engraulidae	11	7.692307692	0.00062	0.00013
Merlucciidae	9	6.293706294	0.00184	0.00015
Myctophidae	59	41.25874126	0.00060	0.00006
Sebastidae	15	10.48951049	0.00105	0.00012

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M = Elemental features, GEO = Geometric features, EF = Elliptic Fourier Features, ALL = all feature

GEO mean	GEO SE	EF mean	EF SD	ALL mean	ALL SE	DFA10 SW Mean
0.287	0.0007	0.0000008	3.3E-07	-6.06	0.106	-3.20
0.277	0.0010	0.0000025	4.4E-07	-5.67	0.140	-2.64
0.291	0.0012	0.0000011	5.6E-07	-5.80	0.179	-2.95
0.293	0.0014	0.0000030	6.2E-07	-5.39	0.198	-3.27
0.294	0.0005	0.0000006	2.4E-07	-6.25	0.077	-3.44
0.294	0.0011	0.0000004	4.8E-07	-6.19	0.153	-3.00

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is included,

DFA10 SW SE

0.09
0.12
0.15
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0.13

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Table S8. Factor categories for ME and M otoliths used in ANOVA and ANOVAN analyses.

Factor Abbreviation	Factor Description	Number of otoliths (n) M	Number of Bathylagidae in M	Number of Engraulidae in M
S1	MAL < 1.0mm	150	7	0
S2	MAL 1.0 - 1.5mm	129	34	0
S3	MAL 1.5 - 2.5mm	141	18	21
S4	MAL 2.5 - 4.5mm	62	0	61
S5	MAL > 4.5mm	8	0	0
T1	2004	10	0	0
T2	2010	124	18	0
T3	2012	289	41	14
T4	2013	67	0	67
N	North of Point Conception, 39.8 Lat. - 44.3 Lat.	64	9	0
M	Upper Southern California Bite, 33.37 Lat. - 34.5 Lat	165	16	49
S	Lower Southern California Bite, 31.18 Lat. - 33.18 Lat.	261	32	34

Number of Merlucciid ae in M	Number of Myctophidae in M	Number of		Number of Other in M	Number of otoliths (n) ME2	Number of Bathylagida e in ME3
		Number of Sebastidae in M	Broken Bathylagidae in M			
0	103	6	2	32	32	4
0	67	8	5	15	38	21
0	78	9	1	16	32	5
0	0	1	0	0	26	1
7	0	0	0	0	15	0
0	0	0	0	10	6	0
0	51	0	6	25	13	0
0	197	24	2	28	113	31
7	0	0	0	0	11	0
1	54	0	0	10	18	6
0	74	20	0	6	68	15
6	120	4	8	57	37	10

Number of Clupidae in ME4	Number of Engraulida e in ME5	Number of Merlucciida e in ME6	Number of Myctophidae in ME7	Number of Sebastidae in ME8	
0	0	0	27	1	
0	0	0	11	6	
7	1	0	17	2	
11	10	0	4	0	
0	0	9	0	6	
6	0	0	0	0	
0	0	0	4	9	
12	0	9	55	6	
0	11	0	0	0	
6	0	0	6	0	
0	6	0	123	9	
12	5	9	15	6	

Table S9. Percent of total occurrence of ichthyoplankton (larvae only) from 1951 to 2011 during California Oceanic Fisheries Investigations (CalCOFI) cruises. The 20 most common taxa are displayed. **CalCOFI** represents the entire CalCOFI sampling region; **SBB Region** represents line 80 - stations 51-5, lines 80.8-82.7 - stations 83.3 - stations 40.6-55; **SBB Station** represents lines 81.5-82 - stations 46-47. **Taxa category** represents taxonomic categories used in this study.

Species	Family	Taxa category	CalCOFI	SBB Region
<i>Engraulis mordax</i>	Engraulidae	Engraulidae	48.92	47.33
<i>Merluccius productus</i>	Merlucciidae	Merlucciidae	13.77	18.36
<i>Leuroglossus stilbius</i>	Bathylagidae	Bathylagidae	5.95	6.67
<i>Sebastes</i>	Sebastidae	Sebastidae	5.46	10.35
<i>Vinciguerrria lucetia</i>	Phosichthyidae	Other	4.7	0.022
<i>Stenobranchius leucopsarus</i>	Myctophidae	Myctophidae	4.06	4.42
<i>Sardinops sagax</i>	Clupeidae	Clupeidae (Other)	3.48	2.49
<i>Trachurus symmetricus</i>	Carangidae	-	1.39	0.05
<i>Sebastes jordani</i>	Sebastidae	Sebastidae	1.18	2.83
<i>Lipolagus ochotensis</i>	Bathylagidae	Bathylagidae	0.97	0.44
<i>Triphoturus mexicanus</i>	Myctophidae	Myctophidae	0.71	0.13
<i>Bathylagoides wesethi</i>	Bathylagidae	Bathylagidae	0.69	0.01
<i>Protomyctophum crockeri</i>	Myctophidae	Myctophidae	0.62	0.12
<i>Ceratoscopelus townsendi</i>	Myctophidae	Myctophidae	0.56	0
<i>Citharichthys stigmaeus</i>	Paralichthyidae	-	0.51	0.8
<i>Genyonemus lineatus</i>	Sciaenidae	-	0.43	1.08
<i>Nannobranchium ritteri</i>	Myctophidae	Myctophidae	0.4	0.05
<i>Diogenichthys atlanticus</i>	Myctophidae	Myctophidae	0.39	0.02
<i>Symbolophorus californiensis</i>	Myctophidae	Myctophidae	0.37	0.01
<i>Tarletonbeania crenularis</i>	Myctophidae	Myctophidae	0.36	0.17

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SUPPLEMENTARY FIGURES AND TABLES

Figure S1

Figure S1. Canonical function analysis using GEO features for M otoliths. Symbols represent individual otoliths (n = 905). Vectors represent relative importance of features in explaining canonical function variability. Canonical Variables 1 and 2 explained 59.5% and 27.6%, respectively, of otolith features. Feature abbreviations in Table S3.

Otolith Classification

14 Figure S2

16

18 Figure S2. Canonical function analysis using GEO features for ME otoliths. Symbols
 20 represent individual otoliths (n = 143). Vectors represent relative importance of features
 in explaining canonical function variability. Canonical Variables 1 and 2 explained
 73.4% and 19.6%, respectively, of otolith features. Feature abbreviations in Table S3.

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Otolith Classification

26 Figure S3

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30 Figure S3. Canonical function analysis using EF features for M otoliths. Symbols
32 represent individual otoliths (n = 905). Vectors represent relative importance of features
34 in explaining canonical function variability. Only the ten strongest discriminatory
features are displayed as vectors. Canonical Variables 1 and 2 explained 44.5% and
31.6%, respectively, of otolith features. Feature abbreviations in Table S3.

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Otolith Classification

38 Figure S4

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42 Figure S4. Canonical function analysis using EF features for ME otoliths. Symbols
 44 represent individual otoliths ($n = 143$). Vectors represent relative importance of features
 46 in explaining canonical function variability. Only the ten strongest discriminatory
 features are displayed as vectors. Canonical Variables 1 and 2 explained 52.0% and
 20.9%, respectively, of otolith features. Feature abbreviations in Table S3.

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Otolith Classification

Figure S5

Figure S5. Canonical function analysis using ELM features for ME otoliths. Symbols represent individual otoliths (n = 143). Vectors represent relative importance of features in explaining canonical function variability. Canonical Variables 1 and 2 explained 49.6% and 29.0%, respectively, of otolith features. Feature abbreviations in Table S3.

Otolith Classification

Tables S1-S9 are in SI Excel file.

Table S10. ANOVA summary for otolith features in M (morphology) and ME (morphology and elemental composition). Each element in the table body contains the number of tests in which the null hypothesis was rejected (numerator) and accepted (denominator). One-Way ANOVA (parametric) and Kruskal-Wallis ANOVA (non-parametric) tested the null hypothesis of no difference between means of a feature among taxonomic groups (seven for M, six for ME). Multi-factor ANOVA (parametric) tested the null hypothesis of no difference between means of a feature among taxonomic groups, fish size (five ranges) and collection location (three regions) and year (four periods). $N_{feature}$ is the number of features in a feature type and hence the number of tests. Bonferroni correction for multiple comparisons was made for M ($\alpha_{corrected} = 0.05/426$) and ME ($\alpha_{corrected} = 0.05/468$). Test results are in Tables S4 (M) and S5 (ME).

			One-Way ANOVA	Kruskal-Wallis	Multi-Factor ANOVA			
	Feature Type	$N_{feature}$	Taxonomic Group	Taxonomic Group	Fish Size	Location	Year	
M	GEO	12	12/0	12/0	12/0	9/3	4/8	12/0
	EF	59	48/11	50/9	29/30	0/59	0/59	4/55
	All	71	60/11	62/9	41/30	9/62	4/67	16/55
ME	GEO	12	12/0	12/0	12/0	6/6	2/10	4/8
	EF	59	32/27	27/31	8/51	4/55	1/58	0/59
	ELM	7	3/4	6/1	3/4	1/6	0/7	1/6
	All	78	47/31	45/33	23/55	11/67	3/75	5/73