ABSTRACT

The hypothesis that there are three Pacific sardine (Sardinops sagax) stocks along the west coasts of Baja California and southern California was evaluated using multivariate discriminant analysis of otolith morphometric variables. Four synoptic data sets were analyzed to test the three-stock hypothesis: warm Magdalena Bay (Warm MB), temperate Magdalena Bay (Temp MB), temperate Ensenada (Temp EN), and cold Ensenada (Cold EN). A gradual increase in the overlap index (Wilks’ Lambda values) reflected the relative degree of similarity among the groups when all comparisons were considered. The Warm MB was the most differentiated group from the others, particularly the Cold EN. The highest degree of similarity was found among the two temperate groups (Temp MB and Temp EN), and they could not be significantly separated in 68% of the repeated tests. Our results support the existence of the three Pacific sardine stocks in the study area and are consistent with previous findings obtained using different methodologies, e.g. temperature-at-catch data.

INTRODUCTION

The Pacific sardine (Sardinops sagax) has a wide geographic distribution, ranging from Southeast Alaska to the southern tip of the Baja California Peninsula and into the Gulf of California (Kramer and Smith 1971; Parrish et al. 1989). It is important to know whether this population consists of one or several stocks and, if multiple stocks do exist, to define their corresponding distributions (Clark 1947). The identification of discrete unit stocks is a basic requirement for fisheries management (Cushing 1968).

It is possible that environmental pressure could segregate the Pacific sardine population into several stocks. The following definition of stock is used in this paper: a unit stock does not necessarily correspond to a genetically distinct group (Saila and Martin 1985). Within each of the large current systems where the sardine occurs, several stocks exist. In the California Current System, the existence of three stocks has been proposed: one off the California coast, the second off the outer Baja California Peninsula, and the third in the Gulf of California (Clark 1947; Vrooman 1964; Mais 1972; Schwartzlose et al. 1999). A fourth stock, the most northern of all, was proposed by Radovich (1982). Identification of these stocks was approached using a variety of methods, including meristics, morphometrics, tags, blood antigens, and population parameters (e.g. differential growth); nevertheless, Hedgecock et al. (1989) found low genetic variability for this species in the Northeast Pacific.

Most recently, Felix-Uraga et al. (2004) provided additional evidence regarding these three groups or stocks of Pacific sardines from Magdalena Bay (México) to San Pedro (USA) based on temperature-at-catch data. Thermal stratification of the sardine population was used to build a conceptual distribution model in space–time for the three sardine groups along the west coast of the Baja California Peninsula and southern California (fig. 1). The conceptual model suggests southward movement of the three groups beginning in winter with the strengthening of the California Current, with all the groups reaching their southern-most distribution in spring. In summer, the northward movement of the three groups begins with the onset of the equatorial countercurrent flow, with all groups reaching their northern-most latitudinal distribution in fall. Figure 2a was taken from Felix-Uraga et al. (2004) and the sea surface temperature (SST) limits (17°C and 22°C) for each putative stock were hand drawn. Figure 2b illustrates the annual
Figure 1. Time-space distribution model of the three-stock hypothesis for Pacific sardines (Sardinops sagax): C = cold stock, T = temperate stock, and W = warm stock.

Figure 2. Pacific sardine (Sardinops sagax) catches aggregated by 1°C intervals of SST for the four landing ports during 1981–2002: (a) whole period; (b) yearly distributed.
catches from Magdalena Bay to San Pedro, 1981 to 2002, grouped by one-degree increments of SST; the annual pattern for the putative stocks is consistent over time.

The development of image analysis systems has facilitated diversification of morphometric methods and expanded the potential of morphometry as a tool for stock identification, providing accurate and efficient measures that traditional methods have not been able to provide (Cadrin and Friedland 1999). Otolith shape analysis has been used for stock determination in several fish species (Campana and Casselman 1993; Bolles and Begg 2000; De Vries et al. 2002). Detection of morphometric differences in fish populations within their geographical range can indicate the presence of stock structure (Thresher 1999; De La Cruz-Agüero and García-Rodríguez 2004). In this paper, we test Felix-Uraga et al.’s (2004) three-stock hypothesis using otolith image analysis in combination with multivariate statistical analysis, in an attempt to distinguish the putative sardine stocks.

MATERIAL AND METHODS

The contour plot of catch by sea surface temperature (SST) and month for Magdalena Bay and Ensenada, originally published in Felix-Uraga et al. (2004; their fig. 4), was redrawn as a three dimensional plot (fig. 3) to further illustrate the hypothesis of three sardine stocks in these locations. Catches to the left of the thin separating curve (freehand traced) correspond to Ensenada, and those to the right of the curve correspond to Magdalena Bay.

To test the three-stock hypothesis, four data groups corresponding to different combinations of locations and SST intervals were identified. The warm stock was present only in Magdalena Bay (Warm MB) at SSTs higher than 22°C and was captured mainly from July to December. The temperate stock, present in Magdalena Bay (Temp MB) at SSTs between 17°C and 22°C, was captured primarily from February to June and, because it shares the same SST range is considered the same stock as that observed from July to November off Ensenada and San Pedro (Temp EN). The cold stock (Cold EN) was present in Ensenada and San Pedro at an SST interval of 13°C–17°C and was caught mainly from December to May.

This a priori stock classification was considered for the application of multivariate discriminant analysis (MDA) to the otolith morphometric variables. The ellipses in Figure 3 indicate that the months considered have a higher probability of representing each one of the three sardine stocks off Baja California and southern California. Synoptic samples of sagittal otoliths from these months and locations were used for the morphometric analysis (August to October for Warm MB and Temp EN; March to May for Temp MB; February to April for Cold EN).

Whole sagittae were imaged, sulcus down at 12x magnification, and digitally measured using Sigma Scan Pro.
4.0 software. Four distances (distance from antirostrum to the posterior edge (AR); distance from posterior edge to rostrum (R); distance of rostrum to antirostrum (RAR); otolith width crossing by focus (W)) were measured on the distal side of the left otolith of each pair extracted from sardines sampled in Magdalena Bay and Ensenada from 1994 to 2002 (fig. 4). The right otolith was measured only when the left was broken. Otolith morphometric samples were restricted to one-year-old sardines to limit variability associated with age and size of fishes and because this was the most abundant age-group in the landings.

MDA was used to compare the otolith morphometric variables from the three putative sardine stocks. These variables were examined for normality using the Kolmogorov–Smirnov (K–S) test (Zar 1996) prior to the Wilks’ Lambda test (Tabachnick and Fidell 1989) for discriminant significance. Statistica 6.1 software was used for these procedures.

The MDA based on otolith morphometric variables from the four synoptic samples (fig. 3) was performed for the six possible paired comparisons. To prevent bias from unbalanced sample sizes, and to obtain a more robust analysis, each synoptic sample was randomly subsampled 50 times, taking a subset of 50 non-repeated otolith morphometric variables each time. Fifty statistical tests for the six paired comparisons were performed, and the frequency distribution of the Wilks’ Lambda values for each comparison was obtained.

To determine whether differences exist within each of the four synoptic groups, MDA was performed based on two balanced, non-replacement, random sub-samples taken from each group, which were then compared. Accuracy of the discriminant classification of otoliths from the three putative stocks was tested using a random sample (n = 100 otoliths) from each group. The temperate stock sample was integrated using 50 otoliths from both temperate locations (Magdalena Bay and Ensenada).

RESULTS

Synoptic samples for otoliths measured from Magdalena Bay and Ensenada, 1994 to 2002, are shown in Table 1. Lack of data at Magdalena Bay from the end of 1997

<table>
<thead>
<tr>
<th>Month</th>
<th>Magdalena Bay</th>
<th>Ensenada</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>April</td>
<td>46</td>
<td>93</td>
</tr>
<tr>
<td>May</td>
<td>38</td>
<td>11</td>
</tr>
<tr>
<td>August</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>September</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>October</td>
<td>48</td>
<td>18</td>
</tr>
</tbody>
</table>

TABLE 1: Monthly number of Pacific sardine (Sardinops sagax) otoliths (age group one) sampled from Magdalena Bay and Ensenada, 1994–2002.
Figure 5. Discriminant scores for each Pacific sardine (Sardinops sagax) otolith depending on morphometric variables for Magdalena Bay warm stock (Warm MB) versus: (a) Ensenada cold stock (Cold EN); (b) Ensenada temperate stock (Temp EN); (c) Magdalena Bay temperate stock (Temp MB).
until 2000 was due mainly to the El Niño phenomenon, which caused a great decrease in the sardine landings in this area and thus affected our sampling program.

All otolith morphometric variables (AR, R, RAR and W) were normally distributed (K-S, \( p > 0.2 \)). MDA results between the Warm MB and the Cold EN, Temp EN and Temp MB groups revealed significant differences for the first three comparisons (fig. 5a, b and c; tab. 2) since the null hypothesis of a common statistical population was rejected. The lowest degree of overlap (\( \Lambda = 0.668 \)) was observed between the Warm MB and Cold EN stocks. Statistically significant differences were also detected for otolith morphometrics between Cold EN and Temp EN and between Cold EN and Temp MB (fig. 6a and b). However, the amount of overlap increased relative to the three previous comparisons (fig. 5).

For the last comparison (Temp EN and Temp MB), we did not expect to detect any difference, but the MDA also showed significant differences despite a high degree of overlap (\( \Lambda = 0.969 \); fig. 7).

### Table 2

Results of comparisons of Pacific sardine (*Sardinops sagax*) otolith morphometric variables with multiple discriminant analysis

<table>
<thead>
<tr>
<th>Stock Comparison</th>
<th>Wilks' Lambda</th>
<th>( F )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold EN and Warm MB</td>
<td>0.6684459</td>
<td>85.189</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Temp EN and Warm MB</td>
<td>0.7462443</td>
<td>59.847</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Temp MB and Warm MB</td>
<td>0.8502735</td>
<td>56.569</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cold EN and Temp EN</td>
<td>0.8638776</td>
<td>21.823</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cold EN and Temp MB</td>
<td>0.9308738</td>
<td>21.238</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Temp MB and Temp EN</td>
<td>0.9694526</td>
<td>9.074</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Figure 6. Discriminant scores for each Pacific sardine (*Sardinops sagax*) otolith depending on morphometric variables for Ensenada cold stock (Cold EN) versus: (a) Ensenada temperate stock (Temp EN); (b) Magdalena Bay temperate stock (Temp MB).
Results of MDA using balanced random samples—presented as frequency distributions of Wilks’ Lambda values for each comparison, average Wilks’ Lambda value, and percentage of non-significant tests—are displayed in Figure 8. A progression from the most dissimilar groups (lower lambdas) to the most similar groups (higher lambdas) was observed. Average lambda values obtained from the 50 tests from the four synoptic samples (subsampled to avoid bias from unbalanced sample sizes) confirmed the results of previous analyses in which all samples were included.

A progression in the percentage of non-significant tests was also observed, from 0% for comparisons between the most dissimilar groups (Cold EN and Warm MB) to 68% for the most similar groups (Temp MB and Temp EN), which were assumed to be the same stock (fig. 8).

The results of the MDA performed for intra-group comparisons were consistent with regard to otolith morphometry because all tests were non-significant ($p > 0.05$), and Wilks’ Lambda values were very close to one (tab. 3).

The MDA test for the three putative stocks was highly significant (Wilks’ Lambda = 0.68, $F_{(8,588)} = 15.42$, $p < 0.05$) and showed that 67% of otoliths from the warm stock (Warm MB), 42% of the temperate stock (Temp MB–EN), and 57% of the cold stock (Cold EN) were well classified; although it was not the objective of this paper to predict group membership from arbitrary otolith samples (tab. 4).

**DISCUSSION**

Variations in size can lead to spurious results when MDA is used to discriminate stocks, since it can be affected by sampling bias and sample design (Dos Reis et al. 1990). Bolles and Begg (2000) found differences in otolith morphometrics of *Merluccius bilinearis* due to age of the organisms. To reduce bias in size and age, we used otoliths of 1-year-old sardines.

Sampling bias is the most common cause of artificial heterogeneity between groups, and results from the uneven collection of specimens over space or time (Reist 1985). We used a random sub-sampling routine to avoid bias originating from unbalanced sample sizes among groups. The MDA results in this paper performed with two types of samples (total number, and equal number of subsamples by group) were consistent with each other (tabs. 2 and 3).

In morphometric studies, it is also essential to consider confounding variation that may be present due to differences among samples in an age-group, year-class, or specific sex, so as not to mistake stock differences for sample differences (Bolles and Begg 2000). Confounding variability produced by sex and year-classes was not detected by MDA performed for intragroup subsamples. These results indicate a consistency between otolith variables of different years and sexes.

Results of the MDA agreed with what we expected *a priori*—that three Pacific sardine stocks exist along the west coast of Baja California and California (Felix-Uraga et al. 2004). The MDA comparison having the greatest difference with respect to temperature and distance (Cold EN vs. Warm MB) revealed the lowest overlap ($\Lambda = 0.668$) among all comparisons.

Comparisons between Temp EN and Warm MB, Temp MB and Warm MB, Temp EN and Cold EN, and Temp MB and Cold EN also revealed significant differences among these putative stocks, with indices of overlap gradually increasing between intermediate values (tab. 2). In the comparison of the Temp EN and Temp
MB groups, which we assumed made up the same stock, the overlap index was the highest among all comparisons ($A = 0.969$); however, the $F$-test still indicated a significant difference.

In these six comparisons, the gradual increase in the overlap index reflected the degree of similarity among the stocks. The warm stock of Magdalena Bay (Warm MB) and the cold stock of Ensenada (Cold EN) were the least similar groups to each other. The temperate stock was more similar to the cold stock than the warm stock. Other authors have also considered sardines from the Gulf of California to be the most distinct group in the Northeastern Pacific (Clark 1947; Vrooman 1964; Mais 1972; Radovich 1982).

The highest degree of similarity was found between the two locations of the temperate stock (Temp MB and Temp EN), a result which was expected based on the conceptual model of time-space distribution proposed by Felix-Uraga et al. (2004). Our results support the hypothesis that the temperate stock present in Magdalena Bay during the first half of the year is the same stock present in Ensenada and San Pedro during the second half of the year.

These results provide new evidence in support of the three-stock hypothesis for the Pacific sardine population previously outlined by Clark (1947 and 1952), Vrooman (1964), Mais (1972), Radovich (1982), and Felix-Uraga et al. (2004), regardless of the low genetic variability evidenced for this species (Hedgecock et al. 1989). The stocks defined by temperature-at-catch data have a clear geographic differentiation, although the high percentage of misclassification from otolith morphometrics does not support the possibility that they are genetically different (Solow 1990).

Otolith development occurs under dual regulation: genetic conditions regulate its form, and environmental conditions (mainly temperature) regulate the quantity of material deposited during formation (Lombarte and Lleonart 1993). On this basis, it is reasonable to believe that otolith morphometric differences found among the stocks are determined by local environmental conditions (Hedgecock et al. 1989; Parrish et al. 1989; Lluch-Belda et al. 1991a).

### Table 3

Statistics resulting for intra-stocks discriminant analysis performed with two random balanced samples from each Pacific sardine (*Sardinops sagax*) group.

<table>
<thead>
<tr>
<th>Stock</th>
<th>Wilks’ Lambda</th>
<th>Statistic $F(m,n)$</th>
<th>Error probability $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm MB</td>
<td>0.9892</td>
<td>$F(4,415) = 1.133$</td>
<td>&lt; 0.3403</td>
</tr>
<tr>
<td>Temp MB</td>
<td>0.9918</td>
<td>$F(4,863) = 1.792$</td>
<td>&lt; 0.1283</td>
</tr>
<tr>
<td>Temp EN</td>
<td>0.9785</td>
<td>$F(4,283) = 1.554$</td>
<td>&lt; 0.1869</td>
</tr>
<tr>
<td>Cold EN</td>
<td>0.9790</td>
<td>$F(4,265) = 1.421$</td>
<td>&lt; 0.2271</td>
</tr>
</tbody>
</table>

### Table 4

Accuracy of discriminant classification of the otoliths from the three putative Pacific sardine (*Sardinops sagax*) stocks. Numbers of well-classified otoliths are on diagonal.

<table>
<thead>
<tr>
<th>Number of otoliths classified by group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Warm MB</td>
</tr>
<tr>
<td>Temp MB-EN</td>
</tr>
<tr>
<td>Cold EN</td>
</tr>
</tbody>
</table>
Based on the working definition for fisheries management, a “stock” is a management unit and not a discrete biological population unit. Consequently, fish stocks having different phenotypic (i.e. morphometric) characteristics or life history (i.e. growth) dynamics should be considered separate units and modeled separately for stock assessment and management purposes, regardless of genetic characteristics (Cadrin and Friedland, 1999).

Environmental temperature is considered the most important factor affecting distribution of marine organisms (Lluch-Belda et al. 1991b). Productivity of sardine populations in the different current systems has been associated with interdecadal regime shifts driven by large-scale changes in ocean climate (Rodriguez-Sanchez et al. 2001). The sardine population extends its geographical distribution during warm periods and contracts it during cold ones (Lluch-Belda et al. 1989). The recent increase and expansion of the Pacific sardine population could have resulted in the current three-stock structure, which may only occur when the fish are very abundant and the population extends to its northern limit.

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LITERATURE CITED


