Marine Biology 118, 309–317 (1994)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Population structure of the Spanish sardine *Sardinella aurita*: natural morphological variation in a genetically homogeneous population

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Received: 13 August 1993 / Accepted: 17 August 1993

Abstract. The population structure of the Spanish sardine *Sardinella aurita* in the coastal waters of Florida, USA, was examined using protein electrophoresis (one sample from Charleston, South Carolina, was analyzed as a geographic outlier), morphometrics, and meristics. Electrophoresis of proteins coded by 37 presumed genetic loci revealed low levels of genetic variation, little allele frequency variation among samples, and low genetic distances between samples. Gene flow was high and effectively homogenized genetic variation among sample locations, indicating that a single, panmictic population of Spanish sardines exists at least from South Carolina to the Florida panhandle. Size-corrected principal-components analyses performed on the morphometric data (collected in the form of a truss network) revealed regional allometric patterns. These patterns were most apparent in small fish and less obvious in larger fish, implying that the regional morphological patterns may diminish as the fish grow. Regressions of gill-raker number on fork length demonstrated regional patterns similar to those seen in the morphometric analyses. The absence of genetic evidence for geographic populational structuring and the apparent ontogenetic plasticity of body shape suggests that the morphological variation may be ecophenotypic. Alternatively, the electrophoretic analyses may not have detected substructuring that exists. Both the morphometric and meristic data tended to group samples collected from embayments separately from samples collected from more oceanic environments, implying that proximity to embayments may influence some of the observed morphological variation. It appears that for Spanish sardines, as for other clupeids, protein electrophoresis is useful in determining the evolutionary patterns of population structuring, and morphological analyses are of merit in studying short-term, environmentally induced variation.

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Introduction

Studies of the population structure of commercially important marine fishes are of theoretical interest to evolutionary biologists and of practical value to fishery managers. The interpretation of population structure, however, is largely influenced by the definition of "population" being used by the investigator. In the broadest sense, fish populations can be defined from a biological perspective, which implies some level of reproductive isolation, or from a fishery perspective, which concerns a practical description of a group of fish exploited in a specific area (Smith and Jamieson 1986). In either case, the number and the level of discreteness of the populations are of primary concern.

Morphological characters have traditionally been used to describe the population structure of fishes in the family Clupeidae. However, the relatively recent proliferation of protein electrophoretic studies of clupeid population structure has led to a reevaluation of population concepts based on morphology. Typically, electrophoretic studies have indicated that fewer discrete subpopulations exist than have been suggested by morphological variation (Kornfield et al. 1982, Grant 1984, Grant and Utter 1984, Smith and Jamieson 1986, Hedgecock et al. 1989). While electrophoresis is considered to be a valuable tool for examining, from an evolutionary standpoint, the population structure of fishes (Allendorf et al. 1987), it has been criticized as being insensitive to population differentiation that may occur over relatively short time periods (Kornfield et al. 1982, Grant 1984, Ryman et al. 1984). Conversely, morphological variation in fishes is often environmentally induced and may provide a good record of short-term population structuring, but morphology may be ineffective for studying the evolutionary patterns of population differentiation (Allendorf et al. 1987).

We examined the population structure of the Spanish sardine *Sardinella aurita* in the coastal waters of Florida from a biological perspective, using protein electrophoresis, morphometrics, and meristics. The Spanish sardine is

a small, pelagic herring that inhabits the continental shelves of the western Atlantic from Massachusetts, USA, throughout the Gulf of Mexico and Caribbean Sea to Rio de Janeiro, Brazil (Fisher 1978). In Florida, the Spanish sardine is important as a food source to predatory fishes, including many commercially and recreationally exploited species (Saloman and Naughton 1983, Naughton and Saloman 1984, 1985), and as a commercial fishery resource (Johnson and Vaught 1986). Previous investigations of the population structure of Spanish sardines have typically used only a single analytical technique and either have been geographically restricted or have evaluated samples from only a few locations (Latini and Pettorossi 1977, Johnson and Vaught 1986, Wilson and Alberdi 1991, Tringali and Wilson 1993). Incorporating both protein electrophoresis and morphological analyses into a single study has allowed us to reconcile apparent discrepancies in the structuring of Spanish sardine populations implied by each method. Our results therefore provide a definition of Spanish sardine population structure in Florida that is interpretable from both a theoretical and an applied viewpoint.

Materials and methods

Sampling

From February 1989 through September 1990, 38 collections of *Sardinella aurita* were made at seven locations in Florida coastal waters from Fort Pierce to Panama City; a collection to be used as a geographic outlier was also obtained 20 km offshore of Charleston, South Carolina (Fig. 1). Sardines were captured with cast and gill nets from a boat after being attracted to the surface by flood lights, or they were captured from shore with cast nets. Specimens used in genetic analyses were measured (fork length), labeled, wrapped in aluminum foil, and placed in liquid nitrogen immediately after capture. These sardines were stored in the laboratory at −85 °C until analyzed. Specimens used in morphological analyses were fixed and stored in 10% buffered formalin. Twenty-one of the collections (802 specimens) were included in the genetic analyses, and 25 of the collections (289 specimens) were used for morphological evaluation (Table 1).

Species identification

The Brazilian sardine, *Sardinella brasiliensis*, has been reported to occur sympatrically with *S. aurita* in the Gulf of Mexico (Whitehead 1973, 1985). To ensure that we were examining only *S. aurita*, 40 fish from each sample location in Florida [except CI ($n=34$) and CO ($n=7$)] were identified to species level based on gill-raker shape and number of gill rakers (Whitehead 1973, 1985). In addition, we scrutinized all sample collections used in electrophoresis for the occurrence of fixed alternate alleles at any presumptive gene locus, and we tested for significant differences in allele frequencies between the Gulf of Mexico and Atlantic samples (*S. brasiliensis* has never been reported from the United States Atlantic coast).

Electrophoresis

Analysis of liver and muscle tissue yielded 37 resolvable, presumed genetic loci from 22 enzymatic and non-enzymatic proteins (Table 2). Alleles were coded and scored following Bert (1986). All scores

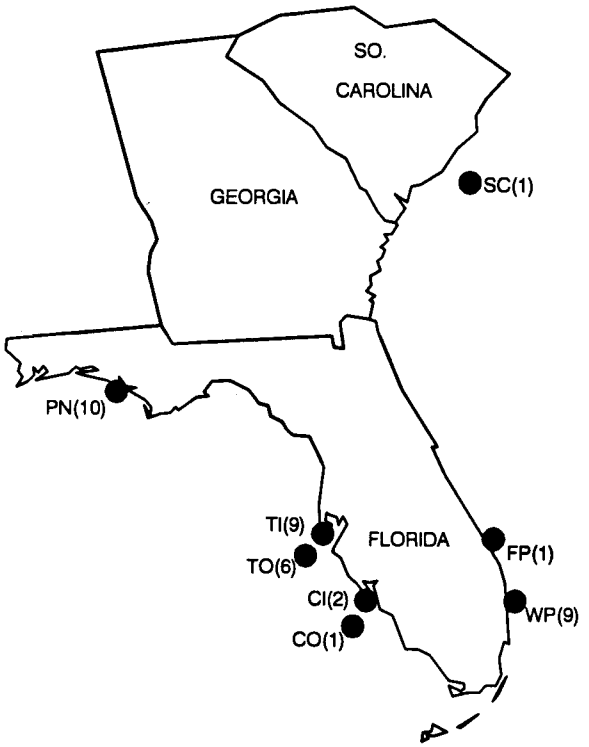

Fig. 1. *Sardinella aurita*. Sampling locations for study of electrophoretic and morphological variation in Florida; location abbreviations are defined in legend to Table 1. Number of samples collected from each location are in parentheses

were agreed upon by two authors (S.T.K. and T.M.B.). The BIOSYS I statistical program (Swofford and Selander 1981) was used to calculate allele frequencies, Wright's fixation index (F_{ST}) genetic distances and distance-based phenograms, and χ^2 statistics for conformation of genotype frequencies to Hardy–Weinberg equilibrium expectations. For the Hardy–Weinberg analysis of each locus, alpha levels were adjusted using the sequential Bonferroni technique (Rice 1989) to avoid Type-I errors resulting from multiple testing of a single hypothesis.

Both Nei's (1978) genetic distance and Rogers' modified genetic distance (Wright 1978) were calculated. Geographic relationships among collection locations were investigated by generating phenograms using the UPGMA on Nei's genetic distances and the distance Wagner procedure on the modified Rogers' distances. Each analysis was performed twice: using all loci and using only loci polymorphic at the P_{95} level (a locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95). We also used the R × C G-test for independence (Sokal and Rohlf 1981) to compare the average heterozygosity per locus ($\bar{H}_0$) and the percentage of polymorphic loci (P_{95}) among collection locations, and to compare allele frequencies among collection locations at loci that demonstrated geographic variation.

Gene flow (N_em: effective number of migrants per generation among populations) was estimated using both F_{ST} values in the island model (Wright 1943) and the "private alleles" method (Slatkin 1985). Mean F_{ST} values were calculated as the arithmetic average of all loci. Slatkin's procedure of systematically omitting collections or groups of collections for the calculation of gene flow was used to examine population structure. To examine temporal variation in geographic patterns, estimations of gene flow were made separately for collections taken during each season for which we had sufficient data, as follows: late winter–spring, around south Florida and between embayment and oceanic collections from the Tampa Bay location; summer, along the Florida east coast; autumn, along the Florida west coast (Table 1). In addition, we used all

Table 1. *Sardinella aurita*. General statistics of collections used for electrophoretic and morphological analyses. FL: fork length; (*n*): number of individuals. SC: Charleston, South Carolina; FP: Fort Pierce, Florida; WP: West Palm Beach, Florida; CI: Charlotte Harbor, Florida (embayment); CO: Charlotte Harbor, Florida (oceanic); TI: Tampa Bay, Florida (embayment); TO: Tampa Bay, Florida (oceanic); PN: Panama City, Florida. Numerical superscripts denote collections used in size-specific morphometric analyses ([1]: fish <100 mm; [2]: fish 100 to 140 mm; [3]: fish >140 mm) and estimates of gene flow during each season ([4]: late winter-spring; [5]: summer; [6]: autumn)

Sample	Allozyme analyses		Morphological analyses				Collection date
			Morphometrics		Gill-raker counts		
	(*n*)	Size range (mm FL)		Size range (mm FL)	(*n*)	Gill-raker count range	
SC1	(44)	95–165					29. VI. 1990
FP1[5]	(37)	147–165					2. VIII. 1989
WP1[4]	(52)						14. III. 1989
WP2[2,5]	(48)	94–118	(3)	94–110	(3)	101–106	13. VII. 1989
WP3[5]	(32)	85–105					19. VII. 1989
WP4[2]			(3)	99–115	(3)	94–112	9. VIII. 1989
WP5[5]	(25)	85–120					25. VIII. 1989
WP6[4]	(23)	152–207					27. III. 1990
WP7[1,4]	(25)	35–96	(9)	82–94	(9)	69–80	3. IV. 1990
WP8[3]			(9)	155–174	(9)	105–126	14. V. 1990
WP10[1,4]	(23)	100–120	(17)	83–103	(16)	75–92	16. V. 1990
CI1[1,4]	(16)		(28)	65–125	(28)	89–135	25. V. 1990
CI2[1]			(6)	82–99	(6)	83–103	24. IX. 1989
CO2[2]			(8)	102–160	(7)	80–120	21. IX. 1990
TI1[4]	(36)						3. II. 1989
TI2[2]			(10)	104–123	(5)	110–143	12. IV. 1989
TI3[2,4]	(54)	94–128	(23)	97–125	(6)	96–127	15. V. 1989
TI4[2,a]			(18)	113–137	(6)	97–112	17. V. 1989
TI5[2]			(10)	97–129	(6)	104–138	30. V. 1989
TI6[2]			(24)	99–135	(6)	115–138	2. VI. 1989
TI7[2]			(24)	104–136	(6)	107–133	15. VI. 1989
TI8[1]			(10)	78–101	(5)	82–108	27. IX. 1989
TI9[6]	(18)	81–96					29. IX. 1989
TO2[4]	(42)						7. II. 1989
TO3[2,4]	(46)		(10)	96–185	(10)	84–125	10. IV. 1989
TO4[5]	(32)	142–189					5. VII. 1989
TO5[3,6]	(50)	144–188	(13)	154–177	(13)	101–131	25. X. 1989
TO6[2]			(9)	120–157	(9)	91–110	6. XI. 1989
TO7[2]			(8)	124–153	(8)	85–99	23. IV. 1990
PN1[3]			(8)	148–163	(8)	97–111	19. V. 1989
PN2[5]	(62)	147–207					30. VI. 1989
PN3[5]	(40)	161–195					16. VIII. 1989
PN4[6]	(50)	147–197					13. IX. 1989
PN5[6]	(47)						29. X. 1989
PN6[3]			(12)	146–187	(12)	96–126	14. XI. 1989
PN7[3]			(10)	145–183	(10)	110–140	24. IV. 1990
PN8[3]			(2)	160, 169	(2)	120, 136	26. VI. 1990
PN9[3]			(2)	165, 169	(2)	120, 121	21. VIII. 1990
PN10[3]			(13)	125–184	(5)	107–129	14. IX. 1990

[a] Collected 1 to 2 km offshore of mouth of Tampa Bay

samples and, separately, used samples collected during each season to calculate correlation coefficients (CORR procedure, SAS Institute Inc. 1985) to test for a relationship between gene-flow values and geographic distance (km).

Morphological analyses

Our measuring protocol incorporated a truss network (Humphries et al. 1981); for each individual, 21 truss measurements, 4 additional cephalic measurements, and fork length were measured by one of us (T.O.) using digital calipers connected to a Polycorder data logger. Truss measurements were labeled according to the morphological landmarks; e.g. Measurement 1–2 was the measure from Landmark 1 to Landmark 2. The landmarks used were: (1) mid-dorsal point between nares, (2) anterior end of dentary median ridge, (3) posterior convergence of fronto-parietal striae, (4) posterior convergence of brachiostegals, (5) anterior base of dorsal fin, (6) mid-ventral point of anterior base of pectoral fin, (7) posterior base of dorsal fin, (8) anterior base of anal fin, (9) dorsal base of caudal fin, and (10) ventral base of caudal fin. Additional measurements were: snout to anterior-most edge of eye (SE), snout to mid-dorsal point

Table 2. *Sardinella aurita*. Enzymes and non-enzymatic proteins resolved and staining procedures used for starch gel electrophoresis. Most proteins were resolved using histochemical staining recipes of Selander et al. (1971); *ADH* and *DIA* were resolved using recipes from Harris and Hopkinson (1976); *GLUDH* and *ODH* were resolved using recipes from Schaal and Anderson (1974). Buffer systems were from Selander et al. (1971). l: liver; s: skeletal muscle. m: monomer (presumed heterozygotes show two bands); d: dimer (presumed heterozygotes show three bands). *SOD* appeared on *G3PDH* stain

Protein	Enzyme No.	(No. of loci)	Buffer system	Tissue used	Enzyme structure
Aspartate aminotransferase (*AAT*)	2.6.1.1	(2)	5	s	d
Alcohol dehydrogenase (*ADH*)	1.1.1.1	(2)	7	l	m
Aldehyde oxidase (*AO*)	1.2.3.1	(1)	3	l	?
Creatine kinase (*CKP*)	2.7.3.2	(1)	3	s	m
Diaphorase (*DIA*)	1.8.1.4	(2)	3	l	m
Esterase (*EST*)	3.1.1.–	(1)	9	l	m
Glutamate dehydrogenase (*GLUDH*)	1.4.1.2	(1)	5	s	?
General protein		(2)	3	s	?
Glycerol-3-phosphate dehydrogenase (*G3PDH*)	1.1.1.8	(3)	5	l, s	d
Glucose-6-phosphate isomerase (*GPIP*)	5.3.1.9	(1)	5	l	d
Isocitrate dehydrogenase (*IDHP*)	1.1.1.42	(2)	5	l, s	d
L-lactate dehydrogenase (*LDH*)	1.1.1.27	(3)	5	l, s	?
Malate dehydrogenase (*MDH*)	1.1.1.37	(2)	5	s	d
Malic enzyme (*MEP*)	1.1.1.40	(2)	9	l, s	m
Mannose-6-phosphate isomerase (*MPIP*)	5.3.1.8	(2)	8		m
Octanol dehydrogenase (*ODHP*)	1.1.1.73	(1)	8		m
Dipeptidase (specific for phenylalanyl-leucine) (*PEP1*)	3.4.–.–	(1)	3		m
Tripeptidase (specific for leucyl-glycyl-glycine) (*PEP2*)	3.4.–.–	(1)	9		m
Phosphogluconate dehydrogenase (*PGDHP*)	1.1.1.44	(1)	3	l	m
Phosphoglucomutase (*PGMP*)	5.4.2.2	(1)	5	s	m
Superoxide dismutase (*SOD*)	1.15.1.1	(2)	5	l, s	m
Xanthine dehydrogenase (*XDH*)	1.1.1.204	(3)	9	l. s	?

between nares (SN), eye width (EW), eye height (EH), and fork length (FL). Measurements taken on several fish collected from one location were followed by measurements taken on several fish collected from one of the other six locations. Gill raker counts were determined for 200 of the 289 specimens used in the morphological analyses (Table 1).

To examine population structure based on variation in shape, we transformed the mensural values ($\log_{10}$) and performed two types of principal-components analyses (PCA) on the covariance matrices. Standard PCA (PRINCOMP procedure, SAS Institute Inc. 1985) was used to analyze all measurement data. Burnaby's (1966) method of size-corrected PCA was performed separately on 20 of the truss measurements (omitting Measurement 1–2, due to software limitations), on only the measurements that loaded heavily on Principal Component (PC) 2 or PC3 (Strauss 1985), and on each of three subsets of the total data set, as defined by size classes (Table 1). The Burnaby method adjusts for residual size effects in standard PC2 and PC3, allowing comparisons of variation in shape of the fish among samples that differ in their size distributions. We designated characters significantly correlated with PC2 or PC3 as heavily loaded shape characters (Strauss 1985). Gill-raker numbers were regressed against fork length separately for each collecting location. Slopes and *Y*-intercepts were compared among all pairs of locations (except CO2, due to low sample size) and among collections pooled by habitat type using analysis of covariance [general linear model (GLM) procedure, SAS Institute Inc. 1985]; significance levels were adjusted using the sequential Bonferroni technique (Rice 1989).

Results

Species identification

We characterized all specimens examined for gill-raker number and shape as *Sardinella aurita*, in accordance with Whitehead's (1973, 1985) description. In addition, our protein electrophoretic results concurred with those of Wilson and Alberdi (1991) in that we also found no differences in allele frequencies among the Gulf of Mexico and Atlantic samples that suggested the occurrence of two species in the Gulf. Furthermore, Montero and Perez (1981) conducted a protein electrophoretic study of the Spanish and Brazilian sardine from the Venezuelan coast, where both species reportedly occur (Fisher 1978), and concluded that *S. aurita* and *S. brasiliensis* were a single species.

Electrophoresis

Of the 37 loci we examined, 22 were polymorphic but only two, *DIA-2* and *EST-1*, were polymorphic at the P_{95} level. Significant departures of genotype frequencies from Hardy–Weinberg equilibrium expectations occurred at only three loci in single samples. Deviations from expected frequencies were caused by one rare-allele homozygote in Sample SC1 at *PEP2-1* and in Sample PN4 at *MEP-2*, and by two homozygotes of the uncommon allele (possibly misscored) in Sample TO5 at *PEP1-1*. The close agreement to Hardy–Weinberg expectations suggests a genetic basis for all loci observed; however, the expected frequencies of many rare alleles were <0.01, below the level of confident interpretation of the χ^2 goodness of fit test (Zar 1974). Allele frequencies of all polymorphic loci are available from the authors upon request.

Values for P_{95} and $\bar{H}_0$ were low, ranging from 5.4 to 10.8 ($\bar{x}=6.8$) and from 0.02 to 0.06 ($\bar{x}=0.04$), respective-

ly. No pattern of geographic structuring of genetic variability was found in P_{95} or $\bar{H}_0$ values among locations. Geographic patterns in allele frequencies among locations also were not apparent, and phenograms derived from genetic distances demonstrated no geographic or temporal pattern. Nei's (1978) genetic distances calculated using all loci were very low (≤ 0.007), and the UPGMA phenetic tree had no branching patterns. The corresponding modified Rogers' distances (Wright 1978) ranged from 0.017 to 0.097, and the resultant distance Wagner tree suggested no structuring of populations attributable to geographic variation or to inferred migratory patterns (Fig. 2A). Using only loci polymorphic at the P_{95} level, the UPGMA phenogram generated from Nei's (1978) genetic distances also indicated no discernable geographic patterns (Fig. 2B), and the corresponding Distance Wagner phenogram generated from the Rogers' distances (Wright 1978) was similar to the phenogram depicted in Fig. 2A.

Significant variation in allele frequencies occurred at a few polymorphic loci, but the variation in allele frequencies did not reveal a pattern that could be attributed to geographical population structuring. Using both F_{ST} and the "private alleles" method, both overall and seasonal estimates of gene flow were high ($N_e m \geq 1$), indicating that allele frequencies were similar in all samples. $N_e m$ among all samples was estimated to be 10.9 (private alleles method) and 6.2 (F_{ST} method). Gene flow was lowest among southwest Florida samples during late winter–spring (CI1; TI1, 3; TO2, 3), and $N_e m$ estimates were 1.9 (private alleles method) and 4.7 (F_{ST} method). The highest gene flow occurred among northwest Florida samples during autumn (PN4, 5), and $N_e m$ estimates were 24.2 (private alleles method) and 41.4 (F_{ST} method). $N_e m$ values generated using either method of calculation were not correlated with geographic distance.

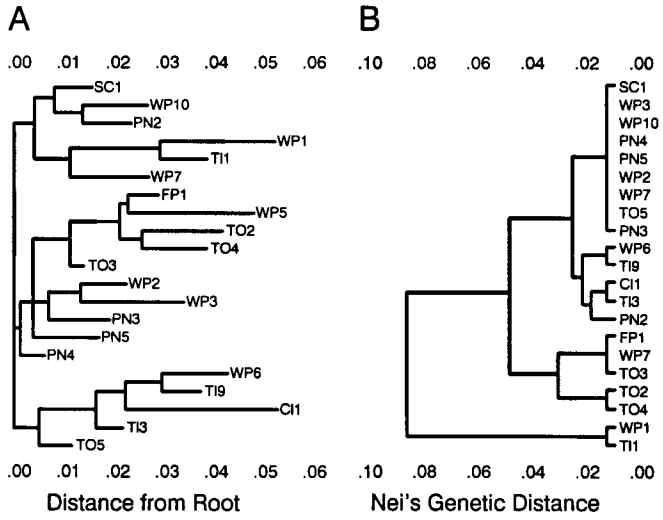

Fig. 2. *Sardinella aurita.* Phenograms generated from genetic distances; sample abbreviations as in Table 1. (A) Distance Wagner phenogram based on Rogers' modified genetic distances (Wright 1978) calculated using all loci; (B) UPGMA phenogram based on Nei's (1978) genetic distances calculated using only loci polymorphic at P_{95}

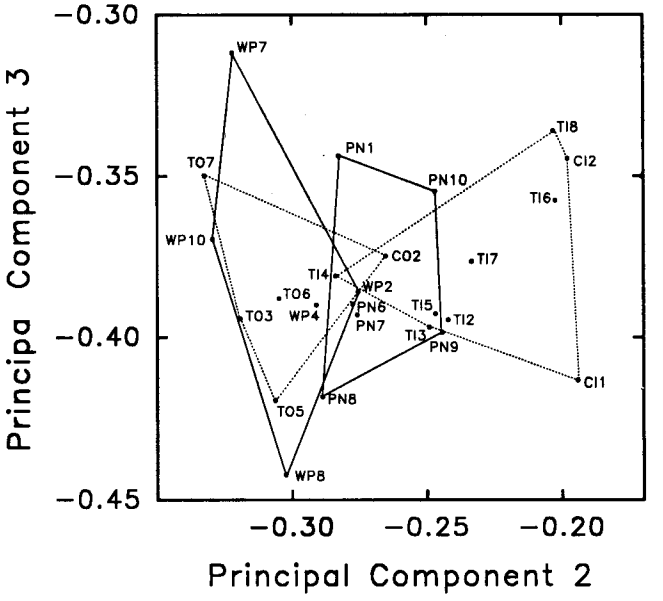

Fig. 3. *Sardinella aurita.* Bivariate plot of Principal Component 2 and 3 scores generated using Burnaby's (1966) size-corrected principal-components analysis. Within samples, individuals clustered relatively tightly; therefore, group centroids (calculated as mean scores on PC axes) are shown. Continuous polygons encompass either WP or PN samples, dashed polygons either embayment (CI, TI) or oceanic (CO, TO) samples collected from west-central Florida. Sample abbreviations as in Table 1

Morphological analyses

Most of the morphometric variation among our samples was size-related. The standard PCA using all measurements yielded a first principal component that accounted for $\simeq 96\%$ of the total variation; PC2 and PC3 each accounted for $\simeq 1\%$ of the total variation. All characters were highly correlated with PC1 ($P < 0.0001$), demonstrating that PC1 is a general "size" axis.

All PCAs demonstrated that fish at different locations tended do be characterized by different morphological forms; this was most clearly illustrated by PC2 in the Burnaby size-corrected PCA on the 20 truss measurements (Fig. 3). Scores on PC2 were low for WP and TO collections, intermediate for PN and CO, and high for CI and TI. Similar results (not shown) were obtained using the ten measurements that loaded heavily on PC2 or PC3 (Measurements 1–2, 2–4, 3–4, 5–6, 6–7, 7–9, 7–10, 9–10, eye width, and eye height). The distribution along the PC2 axis of samples collected from the west-central Florida coast (TI, TO, CI, CO) indicated a difference in morphology between inshore collections (those from embayments) and offshore collections (those from oceanic locations). Samples CI1 and 2, and TI2, 3, and 5–8, which all had comparatively high PC2 scores, were collected from within Charlotte Harbor or Tampa Bay. The CO2 sample and all TO samples, which tended to have low PC2 scores, were collected from 20 km or more offshore. The TI4 sample was collected in coastal waters (1

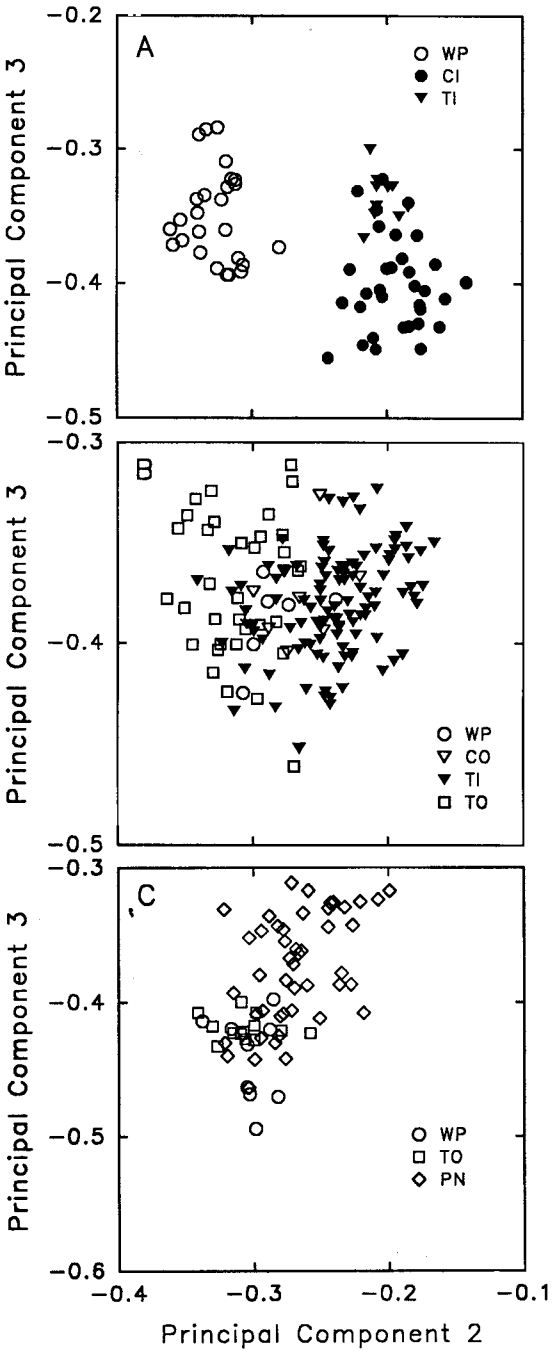

Fig. 4. *Sardinella aurita*. Size-class-specific bivariate scatterplots of Principal Component 2 and 3 scores generated using Burnaby's (1966) size-corrected principal-components analysis; samples included in each size class are shown in Table 1. (A) Mean fork length <100 mm; (B) mean fork length 100–140 mm; (C) mean fork length >140 mm

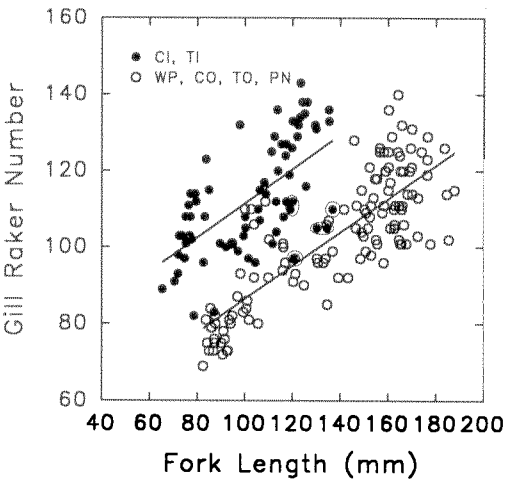

Fig. 5. *Sardinella aurita*. Linear regressions of gill-raker number on fork length for all samples pooled into two groups based on collection location habitat. Embayment samples (TI, CI): $Y=0.45X+66.57$; $r^2=0.42$; oceanic samples (WP, CO, TO, PN): $Y=0.44+42.78$; $r^2=0.67$. Individuals from TI4 sample (circled) were taken 1 to 2 km offshore from Tampa Bay. Sample abbreviations as in Table 1

to 2 km from the mouth of Tampa Bay), but had an average PC2 score that was similar to the offshore collections. Sample means from the PN collections had PC2 values that were intermediate relative to the inshore and offshore extremes, and the WP samples all clustered toward the region of the PC2 continuum occupied by the oceanic TO samples. The WP and PN samples were collected from beaches or piers in areas with a very narrow continental shelf and nearshore oceanic water. Size-class-specific PCAs demonstrated that in the smallest size class, the oceanic WP samples were distinct from Gulf of Mexico samples collected from embayments (CI and TI; Fig. 4A). In the middle size class, however, oceanic (WP, CO, TO) and embayment (TI) samples exhibited a greater degree of morphological homogeneity (Fig. 4B); in the largest size class, no morphological distinctions among locations were found (Fig. 4C), but all samples were from oceanic collections.

In the regressions of gill-raker number on fork length for each collection location, the slopes did not differ between any pair of locations. However, pairwise comparisons of Y-intercepts revealed differences among locations that were related to habitat. Samples from embayment locations (CI, TI) were not significantly different from one another, but each was significantly different ($P<0.001$ in most cases) from every sample collected from an oceanic location (WP, CO, TO, PN). Samples from oceanic locations were not different from one another except for the TO–PN pair ($P=0.0002$). The regression generated using the pooled embayment samples had a different Y-intercept than did the regression using the pooled oceanic samples ($P<0.0001$, Fig. 5). Fish from the TI4 collection, taken in coastal waters 1 to 2 km offshore, had gill raker numbers that were generally intermediate to those of fish from the embayment and oceanic samples (Fig. 5).

Discussion

Electrophoretic variation

Allozyme variability in *Sardinella aurita* was low, but within the range found in other marine teleosts (Smith and Fujio 1982, Waples 1987, Mitton and Lewis 1989)

and other members of the Clupeiformes (Hedgecock et al. 1989). The low level of variability is difficult to explain based on historical events. The occurrence of a genetic bottleneck in the recent or distant past is possible, perhaps as a result of natural fluctuations in population size due to the inherent instability of clupeid populations (Cushing 1971), but such a bottleneck is unlikely, given the extensive geographical range of *S. aurita*. Also, the Spanish sardine in Florida waters may have originated from a small founder population. However, levels of heterozygosity and polymorphism in the Spanish sardine from South Carolina through Florida are similar to those reported for the Spanish sardine from Santos, Brazil (Wilson and Alberdi 1991).

The overall levels of genetic variation ($\bar{H}_0$, P_{95}), variation at specific loci (allele frequencies), and genetic distance values each gave no indication of population structuring of Spanish sardines. The genetic homogeneity is reflected in the high estimates of gene flow we calculated for all combinations of samples. Values of $N_em > 1$ typically indicate that migration rates of individuals between geographic locations are too high for genetic subdivision of populations to occur (Slatkin 1985). The general absence of significant differences among locations in the level of allozyme polymorphism and in allele frequencies among samples, and the high N_em estimates suggest that either gene flow effectively neutralizes population differentiation due to genetic drift or that balancing selection maintains genetic homogeneity over large geographic areas (Karl and Avise 1992). For the Spanish sardine, it seems likely that genetic homogeneity is due principally to gene flow. The Spanish sardine is a highly mobile, pelagic species that reportedly migrates north and south along the east Florida continental shelf (Hildebrand 1963), which probably facilitates gene exchange among geographic locations. In addition, Spanish sardines spawn offshore, and in the eastern Gulf of Mexico their eggs and larvae occur throughout the year and over a larger geographic area than those of any other clupeid species (Houde 1976). Utter et al. (1992) point out that studies using protein-coding loci may fail to identify genetic differentiation that exists if an insufficient number of loci are examined. Our study examined 37 loci from two tissues and Wilson and Alberdi (1991) used 41 loci from three tissues to examine the population structure of the Spanish sardine in Florida. Both studies found similar levels of polymorphism and little variation in allele frequencies, making it unlikely that substructuring exists. The absence of evidence for population structuring of Spanish sardines along the west coast of Florida using mtDNA restriction-site analysis (Tringali and Wilson 1993) further supports this idea.

Morphological variation

The mensural and meristic data show that different morphological forms of Spanish sardines exist in Florida. Fish collected from embayments had a different overall body shape and a relatively higher number of gill rakers than did fish collected from oceanic locations. Fish from the coastal TI4 sample had body shapes and gill-raker numbers intermediate to the fish collected from within embayments and from offshore. Along west-central Florida, our CI and TI collection locations were as little as 20 km away from our CO and TO locations, but the water masses from these two regions have different biological characteristics. Zooplankton is the principal food of Spanish sardines, and zooplankton species composition differs considerably between the mouth of Tampa Bay and 20 km offshore of the bay (Hopkins 1977, Hopkins et al. 1981). Also, in the mouth of Tampa Bay, chlorophyll *a* can be an order of magnitude higher and particulate organic carbon two to ten times higher than from locations 20 km offshore from Tampa Bay and Charlotte Harbor (Paul et al. 1985). These and other water-mass characteristics, or ecological differences associated with the different water masses, may influence Spanish sardine morphology. The habitat-specific nature of the morphological variation and the lack of corresponding genetic variation among fish from the different locations suggests that the observed differences in morphology are environmentally induced.

In general, fishes demonstrate greater variance in morphological traits both within and among populations than do other vertebrates, and they are more susceptible to environmentally-induced morphological variation (Allendorf et al. 1987, Wimberger 1992). Hedgecock et al. (1989) found little genetic variation among sample locations for the Pacific sardine, but variation in morphology was detected and was attributed to environmental differences among sample locations. The greatest differences in the shape of *Sardinella aurita* from the different locations occurred in the smallest size class; in larger fish, these differences were less obvious. This pattern may reflect the local environmental conditions experienced by a cohort at a specific location during early ontogeny. However, as the fish from a specific location grow and presumably become more mobile, and thereby more dispersed, the environmental conditions they experience would become more diverse and allometric changes might be homogenized throughout the region. If individuals do, in fact, become more dispersed as they grow, larger individuals may simply migrate between locations so that our samples contain fish from a variety of locations, effectively reducing the location-specific morphological patterns seen in the small fish.

Counts of meristic characters in fishes are partly determined during a critical period of development, and an increase in the length of the critical period may lead to an increase in meristic counts (Leary et al. 1985). The Spanish sardine may spend its early life in an embayment, in continental shelf water, or as far offshore as the shelf break. Fish collected from embayments had higher gill-raker counts at a given size than did fish collected from oceanic samples. The significantly different *Y*-intercepts (and the homogeneity of the slopes) of the regressions calculated for inshore and offshore samples may indicate that sardines collected from embayments have a longer critical period (perhaps due to slower overall growth) than do sardines collected from oceanic regions.

Population structure

From an evolutionary standpoint, we found no evidence for population substructuring of the Spanish sardine in Florida coastal waters in either the electrophoretic or the morphological data. Our data indicate that a single, panmictic population of the Spanish sardine exists at least from Charleston, South Carolina, to Panama City, Florida.

Grant and Utter (1984) stated that morphological data may be more useful than electrophoretic data in detecting short-term, environmentally induced variation in clupeids. Our data for the Spanish sardine support Grant and Utter's assertion. If the observed morphological variation is caused by habitat effects and not by geographic isolation, then the patterns in morphology demonstrate natural levels of variation within a homogeneous population. However, Smith and Jamieson (1986) proposed that herrings may form "transient assemblages" that do not necessarily diverge genetically and have no taxonomic or evolutionary status but that are important from a fishery management perspective. It is unclear whether the observed morphological variation in *Sardinella aurita* is indicative of "transient assemblages" that occur over time scales relevant to fisheries management.

Acknowledgements. This research was supported by Florida saltwater recreational fishing license revenue. We thank F. Sutter III, R. Trapani, L. Foushee, D. Pierce, S. Branstetter, and F. McIntyre for providing field collections. We also thank D. Camp, J. McKenna, T. Perkins, and M. Tringali for critically reviewing the manuscript and L. French and J. Leiby for editorial assistance.

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Communicated by N. H. Marcus, Tallahassee