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Low genetic variability in a widely distributed and abundant clupeid species, *Sardinella aurita*. New empirical results and interpretations

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Considering the wide geographic distribution and the catches of *Sardinella aurita*, the observed allozyme diversity (H=0·011) is strikingly small. Potential explanations for this low gene diversity, which include restrictions on effective population size owing to variance in reproductive success, demographic instability, historical bottlenecks in population size, selection, and technical artifacts are examined and quantified. This quantification, though rough, shows that no single factor can account for the lack of diversity. However, demographic instability of *S. aurita*, especially if this instability has persisted over evolutionary time scales, together with much greater than Poisson variance in individual reproductive success, could account reasonably for the results. Pleistocene reduction of population size seems also a necessary co-factor. Technical artifacts, mostly scoring difficulties linked to liver autolysis, are considered also and analysed in different clupeid species.

Key words: allozymes; population size; demographic instability; reproductive success; bottlenecks.

INTRODUCTION

The round sardinella *Sardinella aurita* Valenciennes is an abundant and widely distributed clupeid present in the Mediterranean Sea and off the West African coast from the Strait of Gibraltar to Southern Angola (Ben-Tuvia, 1960). Its range extends in the western Atlantic Ocean from Cape Cod to southern Brazil (Ben-Tuvia, 1960; Whitehead, 1985). The most important concentrations of *S. aurita* on the west side of the Atlantic are encountered off the Venezuelan and southern Brazilian coast (Longhurst & Pauly, 1987; Lowe-McConnell, 1987) where up to 200 000 t of fish are caught every year (FAO, 1992). Off West Africa, annual catches are even larger (up to 650 000 t: Marchal, 1991) and are concentrated in three areas, on the so-called Senegalo-Mauritanian, Ivoro-Ghanaian and Congo-Angolese stocks (Fréon, 1986; Do Chi, 1994).

With an average weight of 100 g, tens of billions of individuals are caught every year off Africa, as compared to hundreds of millions off the American coasts. Because of both its economic importance and population size fluctuations, *S. aurita* has attracted much interest (Cury & Roy, 1991; Bard &

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Koranteng, 1994) though few genetic studies have been undertaken (Baron, 1973; Wilson & Alberdi, 1991; Tringali & Wilson, 1993; Chikhi *et al.*, 1994, 1997; Kinsey *et al.*, 1994).

Variable genetic markers are a prerequisite for stock and population structure studies (Ryman *et al.*, 1984; Allendorf *et al.*, 1987; Carvalho, 1993). The present study estimates the amount of allozymic variation and geographical structuring in *S. aurita*. However, an unusually low level of genetic diversity was found. This makes the description of population structure difficult but is of fundamental interest because of the crucial role of genetic diversity as the basis of evolutionary change. It is still a matter of debate why some species may exhibit low levels of genetic variability (Nei & Graur, 1984).

Results already obtained for other clupeids are reviewed looking for a plausible explanation of the low levels of genetic variability observed even in some abundant and widely distributed species. History, demography, variance in reproductive success, selection and technical artifacts are considered and quantified as potential explanations of the low genetic diversity of *S. aurita*.

MATERIALS AND METHODS

SAMPLING AND ELECTROPHORESIS

S. aurita specimens were caught during 1992 and analysed at the Centre de Recherche Océanographique d'Abidjan (Côte d'Ivoire). The sample from Isla Margherita (Venezuela) was sent on dry ice and dissected in Abidjan. The West African samples were collected directly from fishermen and kept in ice until arrival at the laboratory of the country of sampling. Samples from Pointe Noire (Congo) were then kept in liquid nitrogen until arrival in Côte d'Ivoire while those from Accra (Ghana) were frozen at $-20°$ C (see Fig. 1).

Eyes, muscle and liver were taken from every individual and stored at $-20°$ C. Enzyme products of 25 presumptive loci (Table I) were resolved on 12·5% starch gels using staining recipes described by Pasteur *et al.* (1988).

Alleles were designated alphabetically following Wilson & Alberdi (1991), with allele a being the most anodal, b the next, and so on.

STATISTICAL METHODS

Unbiased expected average multilocus heterozygosity, H, hereafter called heterozygosity or gene diversity, was estimated as an average of Nei's (1978) single locus estimates, h. Weir & Cockerham's (1984) method was used to estimate Wright's F-statistics (Wright, 1951). Wright's F_{st} is a measure of population differentiation based on the variance of allelic frequencies among populations. In order to test for a departure from zero (i.e. no genetic differentiation), random permutations of genotypes among samples were performed, and F_{st} was recomputed for the new, permuted, data set. By permuting the data set many times (usually 1000) an empirical distribution of the F_{st} statistic was generated under the null hypothesis of no structure (see for instance Noreen (1989) for a more general description of computer-intensive methods). Randomization of genotypes rather than of alleles circumvents the effect of correlation of alleles within individuals (measured by Wright's F_{is}). The real (unknown) significance level P, was estimated as $P=(n+1)/(N+1)$ where n is the number of permuted data sets that produced a value of F_{st} as great or greater than the real value, and N is the number of permutations performed. Sometimes, $P'=n/N$ is used as an estimate of P (Roff & Bentzen, 1989; García de León *et al.*, 1997) but P is more conservative and statistically more valid than P' (Noreen, 1989). Unless N is small, however, the difference between P and P' is minor.

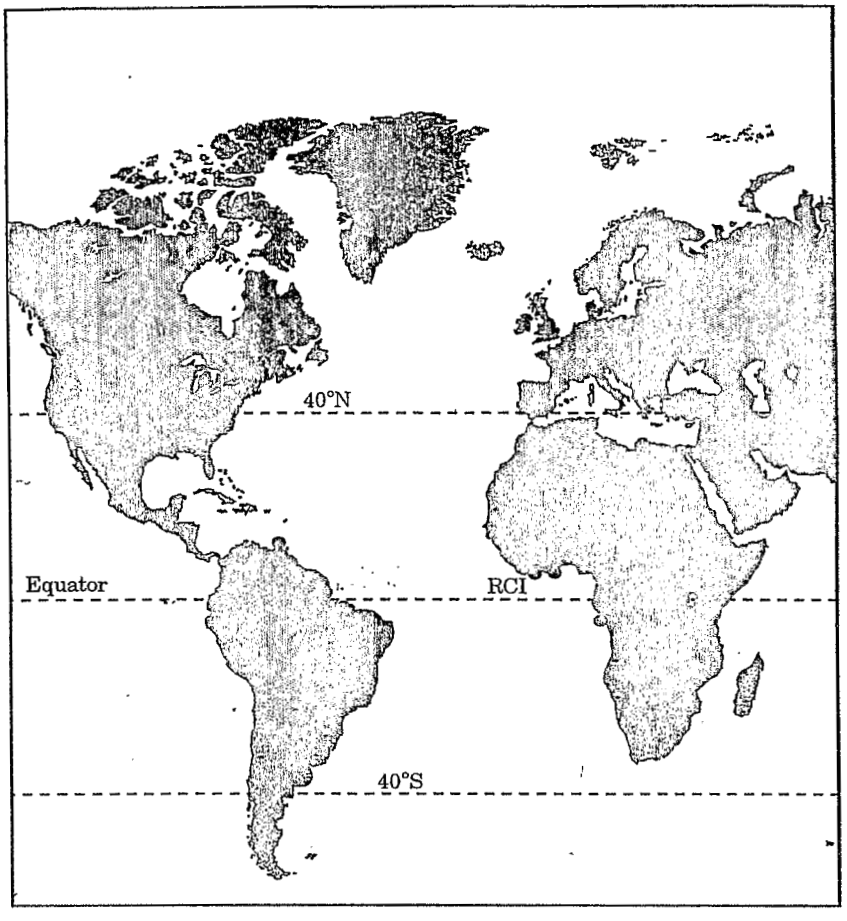

FIG. 1. Sampling locations of *Sardinella aurita*.

Genetic homogeneity (departure from Hardy–Weinberg proportions) within samples was assessed using Wright's F_{is}. The null distribution of F_{is} was approximated by permutations of alleles within samples and the significance level was assessed as described for F_{st}.

All computations and permutation tests were performed using the Genetix 3.07 software (Belkhir *et al.*, 1997) freely available at http://www.univ-montp2.fr/genome-pop/genetix.htm.

RESULTS

NUMBER OF POLYMORPHIC LOCI AND HETEROZYGOSITIES

Among the 25 loci analysed (Table I) three were polymorphic by the 0·99 criterion (P_{99}=3/25=0·12; Tables II and III) while only G3PDH-1 was polymorphic by the 0·95 criterion (P_{95}=0·04). The overall mean heterozygosity was ~0·01 (Table II), with all sample heterozygosities ranging from 0·003 to 0·011, indicating a very low level of genetic variation. Besides, when the most variable locus (G3PDH-1, h=0·156, Table III) was excluded from the analysis, the

TABLE I. Enzymes investigated in *Sardinella aurita*; the buffer was TC 8·0 in all cases

Enzyme	Locus abbreviation	E.C. no	Tissue
Aspartate aminotransferase	AAT-1	2.6.1.1	L
	AAT-2		L
	AAT-3		L
Alcohol dehydrogenase	ADH	1.1.1.1	L
Adelynate kinase	AK	2.7.4.3	L
Creatine kinase	CK-1	2.7.3.2	L
	CK-2		L
Esterase	EST	3.1.1.1	L
Fructose biphosphatase	FBP	3.1.3.11	L
Fumarate hydratase	FUM	4.2.1.2	L
Glycerol-3-phosphate dehydrogenase	G3PDH-1	1.1.1.8	L
	G3PDH-2		L
Glucose-6-phosphate isomerase	GPI	5.3.1.9	M, E
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	L
Isocitrate dehydrogenase	IDH-1	1.1.1.42	M, E
	IDH-2		M, E
1-iditol dehydrogenase	IDDH	1.1.1.14	L
L-lactate dehydrogenase	LDH-1	1.1.1.27	M, E
	LDH-2		M, E
Malate dehydrogenase	MDH-1	1.1.1.37	M, E
	MDH-2		M, E
Phosphoglucomutase	PGM	5.4.2.2	M, E
6-phosphogluconate dehydrogenase	6-PGDH	1.1.1.44	L
Superoxyde dismutase	SOD	1.15.1.1	M, E
Xanthine dehydrogenase	XDH	1.1.1.204	L

heterozygosity was halved (Table III, case A). Moreover, the lack of gene diversity was nearly unaffected by variation in the number of loci and the sample size considered (Table III). When samples were taken individually, the levels of gene diversity were still very low (range 0·003–0·011; Tables II and III), as was the mean number of alleles in each sample (1·14–1·45 and Table II).

No departure from Hardy–Weinberg (HW) expectations was observed in any sample as demonstrated by the non-significant and extremely small F_{is} values (Table IV). Neither did the pooled sample exhibit any HW departure ($F_{is} = -0{\cdot}001$, NS).

POPULATION STRUCTURE

The Venezuelan sample had the smallest number of alleles per locus because of its smaller size but its heterozygosity was not smaller than that of the Congolese sample. The overall heterozygosity of African samples was not significantly greater than that of the Venezuelan sample indicating no differentiation ($F_{st} = -0{\cdot}0019$, i.e. $F_{st}=0$). Thus, no strong heterogeneity existed across the Atlantic: analysis of the population structure yielded an average F_{st} estimate of -0.0009 (i.e. $F_{st}=0$). When all pairwise comparisons were computed (Table IV), distant populations were no more significantly differentiated than closer

TABLE II. Allele frequencies, sample sizes, and heterozygosity of polymorphic loci

	Congo	Ghana	CI	Venezuela	Pooled	Heterozygosity
IDH-1	89	95	99	30	313	
a	1·000	1·000	0·995	1·000	0·998	
b	0·000	0·000	0·005	0·000	0·002	0·003
IDH-2	85	97	96	30	308	
a	0·994	1·000	1·000	1·000	0·997	
b	0·006	0·000	0·000	0·000	0·003	0·003
LDH-1	81	97	99	28	305	
a	0·000	0·000	0·005	0·000	0·002	
b	1·000	1·000	0·995	1·000	0·998	0·003
MDH-2	94	85	75	30	284	
a	1·000	1·000	1·000	0·983	0·996	
b	0·000	0·000	0·000	0·017	0·004	0·004
GPI	75	97	75	29	276	
a	0·007	0·007	0·007	0·000	0·005	
b	0·993	0·986	0·993	1·000	0·992	
c	0·000	0·007	0·000	0·000	0·002	0·014
PGM	95	97	81	26	299	
a	0·000	0·000	0·012	0·020	0·005	
b	1·000	1·000	0·988	0·980	0·995	0·001
AAT-2	—	30	90	30	150	
a		0·017	0·000	0·000	0·003	
b		0·983	1·000	1·000	0·997	0·007
SOD	—	37	93	30	160	
a		0·986	1·000	1·000	0·997	
b		0·014	0·000	0·000	0·003	0·006
AAT-3	—	56	—	30	86	
a		0·992		1·000	0·994	
b		0·009		0·000	0·006	0·012
6-PGDH	—	—	45	21	66	
a			0·011	0·000	0·008	
b			0·989	1·000	0·992	0·015
EST	58	—	—	—	58	
a	0·017				0·017	
b	0·983				0·983	0·034
G3PDH-1	25	—	—	—	25	
a	0·080				0·080	
b	0·920				0·920	0·156
n_a	1·25	1·45	1·38	1·14	1·64	—
H	0·003	0·009	0·006	0·005	0·006	0·011

Single locus heterozygosities are calculated also for the pooled data (last column) and mean heterozygosity is calculated for each sample (last row).
n_a, Mean number of alleles per locus; CI, Côte d'Ivoire.

ones. Though the highest pairwise F_{st} value was between the Congo and Venezuela samples (F_{st}=0·0094; Table IV) it was not significant (P=0·117, 1000 permutations); strangely, the only significant value was that between the Côte d'Ivoire and Ghana samples which are the geographically closest ones (F_{st}=0·0055, P<0·01; Table IV and Fig. 1).

TABLE III. Variation of heterozygosity according to the size of samples and the sampling of loci in *S. aurita*

	Mean sample size	Mean heterozygosity
A	145	0·0055
B	175	0·0065
C	238	0·0069
D	271	0·0073
Whole data set*	140	0·0113

*The whole data set comprises all samples and all loci. A, The most variable locus is excluded (G3PDH-1, see Table II); B, samples with less than 50 individuals are excluded; C, samples with only one location are excluded; D, samples with less than 100 individuals are excluded.

TABLE IV. Matrix of pairwise F_{st} and N_m values

	Congo	Ghana	CI	Venezuela
Congo	− 0·0000 NS	− 0·0034 NS	− 0·0001 NS	0·0094 NS
Ghana	∞	− 0·0005 NS	0·055 *	0·0032 NS
CI	∞	45	− 0·0019 NS	− 0·0061 NS
Venezuela	27	78	∞	0·0000 NS

F_{st} values are given above the diagonal. The number of migrants, N_m, exchanged under the island model hypothesis, $N_m=(1-F_{st})/4F_{st}$ (Wright, 1951), is given below the diagonal. Negative F_{st} estimates may arise between genetically very similar samples. In such cases, F_{st} values and computation of N_m, are meaningless and the infinity symbol has been used for the latter. F_{is} values are given on the diagonal. NS, Not significant; *$P<0·01$.

DISCUSSION

NUMBER OF POLYMORPHIC LOCI AND HETEROZYGOSITIES

The low level of variability observed in *S. aurita* cannot be attributed to small sample sizes since the mean number of individuals analysed for the 25 loci was quite large ($\tilde{n}=140$, Table III), and confirmed the results of Wilson & Alberdi (1991) for specimens sampled in the Gulf of Mexico and off Brazil ($P_{95}=0·06$, $0·02<H<0·03$) and those of Kinsey *et al.* (1994) from the Gulf of Mexico to the coast of South Carolina ($P_{95}=0·07$, $0·02<H<0·06$). In both studies the number of loci surveyed was large (41 and 37, respectively) though the area covered was smaller than in the present one (Table V). Earlier, Baron (1973) studied one polymorphic serum esterase locus in a Senegalese sample. His frequency data

TABLE V. Genetic variability among clupeid species

	Locations*	Loci	Distance†	Abundance‡	*H*§	Reference
Alosa alosa (L.)	8	22	1000	+	0·000	Boisneau *et al.* (1992)
Alosa fallax (Lacépède)	8	22	1000	+	0·000	Boisneau *et al.* (1992)
Alosa fallax (Lacépède)	2	27	400	+	0·004	O'Maoleidigh *et al.* (1988)
Sardinella longiceps Valenciennes	3	19	400	+++	0·006–0·009	Menezes (1994)
Sardinella aurita Valenciennes	4	25	2200–5000	+++	0·003–0·011	This study
Sardinella aurita Valenciennes	5	41	500–5000	+	0·020–0·027	Wilson & Alberdi (1991)
Sardinella aurita Valenciennes	8 (38)	37	500	+	0·020–0·060	Kinsey *et al.* (1994)
Sardinella maderensis (Lowe)	4	18	5000	+	0·105	Chikhi *et al.* (unpublished)
Sardinops sagax caerulea (Jenyns)	5	27	2000	++++	0·005–0·016	Hedgecock *et al.* (1989)
Sardinops melanosticta (Temminck & Schlegel)	1	22	—	+++++	0·064¶	Fujio & Kato (1979)
Ethmalosa fimbriata (Bowdich)	6	24	2200	++	0·004–0·025	Gourene *et al.* (1993)
Alosa pseudoharengus (Wilson)	12	27	2000–5000	++	0·029–0·044	Ihssen *et al.* (1992)
Clupea harengus L.	7 (14)	44	1000	+++++	0·040–0·059	Kornfield *et al.* (1982)
Clupea harengus L.	6	40	1000–10 000	++++	0·062–0·068	Grant (1986)
Clupea harengus L.	3	25	1000	+++	0·068–0·073	Andersson *et al.* (1981)
Clupea pallasi Valenciennes	21 (24)	40	6000–12 000	++++	0·067–0·106	Grant & Utter (1984)
Opisthonema bulleri Regan‖	1 (2)	29	—	+/++	0·051–0·054	Hedgecock *et al.* (1989)
Opisthonema libertate Günther‖	3 (5)	29	1200	+/++	0·055–0·072	Hedgecock *et al.* (1989)
Opisthonema medirastre Berry & Barrett‖	2	29	800	+/++	0·076–0·108	Hedgecock *et al.* (1989)

*Locations (samples).

†Approximate distance (following the coast line) between the two most distant locations. When samples are not homogeneously distributed the greatest distances both within and between a group of populations are given. For instance, in Wilson and Alberdi's study the samples were located in the Gulf of Mexico, except one Brazilian sample.

‡Catches in metric tons (t) of the respective sea area as reported in the FAO statistics and averaged over 1983–1992.
+: 0–50 000 t; ++: 50 000–200 000 t; +++: 200 000–500 000 t; ++++: 500 000–1 000 000 t; +++++: >1 000 000 t.

§Gene diversity.

¶Observed heterozygosity.

‖FAO statistics group *Opisthonema* species under the name *O. libertate* in the Pacific making difficult the estimation of each species' contribution.

yielded the very high value of $h=0{\cdot}71$. Baron's data are difficult to integrate with ours since even for a polymorphic species exhibiting $H=0{\cdot}1$, the probability to observe a locus with $h>0{\cdot}7$ is *c.* 7×10^{-4} for neutral genes (Fuerst *et al.*, 1977). If $H<0{\cdot}05$ the probability is $\ll 10^{-4}$. Thus, the probability that we missed a neutral highly polymorphic locus is $\ll 10^{-4}$ and this cannot explain the small H value which is much smaller than that observed for fishes in general ($H=0{\cdot}064$, 113 species: Ward *et al.*, 1994).

Among other clupeids, the levels of gene diversity observed vary by more than one order of magnitude (Table V) but this does not seem to reflect any obvious difference in the current census sizes (as estimated by annual catches, see Table V, Spearman rank correlation coefficient $r_S=0{\cdot}294$, $P=0{\cdot}217$). Indeed, *S. aurita*'s H is comparable to that of the Californian sardine *Sardinops sagax caerulea* (Jenyns) (Table V) which was one of the most abundant clupeids of the world.

POPULATION STRUCTURE

Sardinella aurita showed no significant differentiation across the Atlantic but significant differentiation between Ghana and Côte d'Ivoire.

Since permutations were used to test these results, the statistical problem that arises with a classical χ^2 goodness-of-fit test (which is only asymptotically correct: Roff & Bentzen, 1989) was avoided. This significant result cannot therefore be caused by the small number of heterozygotes observed. Even correcting levels of significance for the multiple pairwise comparisons by dividing the significance level by the number of tests (Bonferroni method), the F_{st} value is still significantly different from zero. Thus, the only explanations left are that (i) there is differentiation between the two closest samples but not between samples separated by the Atlantic Ocean, or (ii) this differentiation is artifactual and due to sampling error, or (iii) some other sampling phenomenon is responsible for a microgeographic differentiation (e.g. Campton *et al.*, 1992). Since the overall F_{st} is virtually zero and since *S. aurita* is a migratory fish, the last two explanations seem most likely. In particular, when there is a high variance in reproductive success, microgeographic differentiation can arise that would not be stable over time (García de León *et al.*, 1997). Interestingly, this result is in agreement with Wilson & Alberdi's (1991) observation that Gulf of Mexico samples of *S. aurita* exhibited significant differences of allele frequencies that were absent between Florida and Brazilian samples.

Clearly, low F_{st} values concur with low levels of genetic differentiation among pelagic fishes (Gyllensten, 1985; Hedgecock, 1994*a*; Ward *et al.*, 1994). This does not mean that the species acts as a single panmictic unit: it simply underscores the problem of seeking population structure when variation is scarce. Until more polymorphic loci are studied the question of stock structure for *S. aurita* remains open.

EFFECTIVE SIZE

With billions of individuals ($N>10^9$) and average mutation rates usually reported for allozymes ($\mu=10^{-6}$–10^{-7}, Nei & Graur, 1984; Nei, 1987) *S. aurita* should exhibit for $N_e=N=10^9$ a mean heterozygosity $H\sim0{\cdot}99$, since:

$$H=4N_e\mu/(4N_e\mu+1) \quad (1)$$

at equilibrium (Kimura & Crow, 1964).

Since there is a large discrepancy between the expected and observed levels of genetic diversity in *S. aurita* and since, even in other clupeid species, the census population size does not seem to be simply related to H (r_S=0·294, P=0·217; Table V), we shall attempt to quantify roughly the effects of various factors on N_e.

The so-called *equilibrium* equations are based not only on the mutation-drift equilibrium but on other underlying hypotheses (Poisson variance in reproductive success of the adults, constant population sizes, etc.) that represent features of the ideal population. A departure from *equilibrium* equations may be caused by departures from any of these hypotheses. Three of these were explored in quantitative terms: variance in reproductive success, demographic instability, and long-term population size reductions.

When one factor is studied (for instance demographic instability) the other factors are considered to be those of an ideal population (i.e. Poisson variance in reproductive success and long-term mutation-drift equilibrium).

Variance in reproductive success

Variance in the reproductive success can greatly reduce the effective population size (Crow & Kimura, 1970; Ewens, 1979) and thus genetic variation. The pelagic environment is highly unpredictable, causing mass mortalities in the first stages of clupeids (Murphy, 1973; Cury, 1988). Strong upwellings can drive eggs away from coastal retention areas (Cury & Roy, 1989) while predation can reduce population densities of *S. aurita* eggs and larvae by two orders of magnitude in a few days (Conand, 1977). Since *S. aurita* can hatch tens of thousands of eggs in short periods, those that do survive may be derived from relatively few females that hatched in the favourable environmental window (Cury & Roy, 1989). This variance, suggested in numerous marine species (Avise, 1989; Bowen & Avise, 1990; Hedgecock & Sly, 1990) has been quantified rarely (Hedgecock, 1994*b*).

In a stable population of N individuals where the number k of alleles that an individual contributes to the next generation has a given variance V_k, the effective size can be expressed as:

$$N_e=(4N-4)/(V_k+2), \quad \text{Crow \& Kimura (1970).} \quad (2)$$

From equations (1) and (2) one has:

$$V_k=16(N-1)\mu[(1-H)/H]-2\approx 16N\mu[(1-H)/H] \quad (3)$$

Thus for a census size of $N=10^9$, with H=0·01 to 0·1 and $\mu=10^{-8}$–10^{-6}, V_k is *c.* 10^3–10^7 (or a standard deviation of *c.* 30–1000 alleles).

This range is extremely large but is in agreement with V_k around 10^2–10^5 by which Hedgecock (1994*b*) accounted for the small effective sizes in the Pacific oyster, *Crassostrea gigas* (Thunberg). Though the exact distribution of k cannot be inferred, its expectation is 2 in a stable population. Thus, with a variance which is orders of magnitude greater than the mean, it is clearly far from a Poisson distribution, indicating that perhaps a minority of the population is

taking part effectively in the reproduction. A high V_k is expected to create genetic heterogeneity at a microgeographic scale, as found here between Ghana and Côte d'Ivoire (Table IV; Wilson & Alberdi, 1991).

Demographic instability

Demographic instability of many clupeid species is well documented (Murphy, 1973; Longhurst & Pauly, 1987). For example, for the last 2000 years, the Californian sardine populations have shown crashes comparable in intensity and frequency to those observed since exploitation (Baumgartner *et al.*, 1992), as have the sardine and anchovy populations of Namibia over periods of hundreds of years (Shackelton, 1986), hence indicating that demographic instability may be a typical feature of clupeids.

When population size fluctuates, the long-term effective size is given by

$$N_e \simeq t/[\Sigma(1/N_i)] \quad (4)$$

where t is the time in generations and N_i the effective population size at generation i (Crow & Kimura, 1970). It is clear that N_e will be particularly affected by the smallest size reached (i.e. during a population crash) but also by the frequency and duration of these crashes.

Given the observed level of genetic diversity ($H \approx 0{\cdot}01$, Table IV), and $\mu = 10^{-7}$ the long-term effective size will be $N_e \approx 25\,000$ assuming $V_k = 2$ and that the populations are in equilibrium. If the population size is constant (with $N_e = 10^9$) except during the crash generation, then in order to generate a value of $H = 0{\cdot}01$ (or long-term $N_e \approx 25\,000$), for crashes taking place every 10 or 100 years, the crashed population will have $N_e = 2500$ or $N_e = 250$, respectively. In other words, a reduction by a factor of 400 000 every 10 years (or 4 000 000 every 100 years) is necessary to account for the observed gene diversity. These figures imply that if the low genetic diversity were caused by demographic instability only, this instability should have been orders of magnitude higher than usually believed.

Current data do not support such a frequency and intensity for *S. aurita* or probably for any clupeid species (Cury, 1988). Even the dramatic collapse of the Japanese sardine stock during the 1950s did not reduce its genetic variability substantially (Fujio & Kato, 1979 *in* Hedgecock *et al.*, 1989). Thus, though demographic instability can substantially reduce genetic variability it can be rejected as a unique causal factor.

Evolutionary population size reductions

Evolutionary population size reductions (e.g. during glaciations) may also cause loss of genetic diversity even when the species is currently extremely abundant (Nei & Graur, 1984). In principle, the near-absence of variation of *S. aurita* may have been caused by one of the dozen or more glacial-interglacial cycles of the last 2 million years (Roberts, 1984), that affected the distributions of many species including tropical ones (Van Zinderen Bakker, 1982; Maley, 1991). For instance, the decrease of the sea level, up to 175 m during the last 25 000 years (Roberts, 1984), reduced the continental margins (currently 100–200 m deep) whose width affects the duration and intensity of upwellings and their associated planktonic blooms on which *S. aurita* trophically depends.

However, a long-term $N_e \approx 25\,000$ individuals over the species range is still necessary to explain the observed low heterozygosity. If a glaciation event occurred every 10 000 years and if the reduction was maintained for 100 years, then to produce a heterozygosity of $H=0{\cdot}01$ (or $H=0{\cdot}1$), the bottleneck population N_e must have been *c.* 250 (or 2500). Since there have been only *c.* 12 glaciations in the last two million years (Roberts, 1984) the corresponding bottlenecks must have been either longer or with smaller populations.

Since the time required for heterozygosity to reach a new equilibrium approximates the reciprocal of the mutation rate (Nei *et al.*, 1975), millions of years would be necessary to reach a new mutation-drift equilibrium (with one generation per year and $\mu=10^{-7}$). But Nei *et al.* (1975) showed that even with a bottleneck population of $N_0=100$ lasting 100 years, as much as 60% of the pre-bottleneck heterozygosity may be preserved. Since the population sizes must have been $\geqslant 100$, this means that the pre-bottleneck population must have had low heterozygosity and so one single bottleneck cannot account for the data.

Although one bottleneck scarcely explains our data, allelic diversity may indicate it as a potential co-factor. Allelic diversity should be lost more quickly than gene diversity (Nei *et al.*, 1975; Carvalho & Hauser, 1995) leading to the absence of medium frequency alleles. Moreover after a severe ($N_0=100$ for 100 years) or less severe bottleneck (e.g. $N_0=100$ for 10 years) all allelic diversity should be lost. A population recovering from such bottlenecks should present only rare alleles, which is what we observe.

Thus, these calculations indicate that (i) it is unlikely that any single event explains the overall low diversity of *S. aurita*, and (ii) the species may not have had the opportunity to build up a large population over long periods of time. At the same time the distribution of allelic diversity in rare alleles suggests a bottleneck, but cannot identify how severe. These points argue for the joint intervention of V_k and demographic instability.

It is concluded that the discrepancy between total and effective population size as estimated by the *equilibrium* equations must result from at least two of these factors acting together. The exact combination of these factors cannot be determined but some reasonable combinations may be considered. A population of size $N=10^9$ might be reduced by a factor 10 000 every 100 years, for 10 years. Then N_e would be 10^6. Considering ancient bottlenecks every 10 000 years that leave 10 000 individuals for 100 years, then N_e will only be halved $N_e \simeq 10^6/2$. The variance in reproductive success necessary finally to produce $H=0{\cdot}01$, would be $V_k \approx 77{\cdot}2$.

Other combinations may work as well but this simple calculation suggests that regular demographic variations and variance in reproductive success, that is factors that are primarily affected by every day life in upwelling regions, may be the two main causes for persistent low heterozygosities. Pleistocene events (Roberts, 1984) may have influenced *S. aurita*'s distribution more than its overall genetic diversity.

OTHER EXPLANATIONS

Selection

Purifying selection might explain the lack of genetic variation in *S. aurita*, as invoked for green turtles *Chelonia mydas* L., (Bonhomme *et al.*, 1987) to

interpret the near-absence of allozymic variation among Atlantic, Indian and Pacific ocean samples. However, evidence for selection in our results is insufficient to reject the simpler hypothesis of neutrality (Fuerst *et al.*, 1977).

Artifacts and biases

Statistical bias: more than 1000 species have shown diverse levels of genetic variation when the same allozyme loci were compared by electrophoresis (Nevo, 1988). So, for some species, the apparent lack of genetic variation may be a type-I error because only poorly polymorphic loci have been studied due to the huge stochasticity among loci (Stewart, 1976). For a given value of H, the probability that the polymorphic loci have been missed can be estimated using the theoretical distributions of single locus heterozygosity computed by Fuerst *et al.* (1977).

For $H=0{\cdot}05$ they showed that 83% of all loci should exhibit values of $h \leq 0{\cdot}05$. A simple (and conservative) binomial test can be used to estimate the probability that only the less variable loci were sampled by chance. With 25 loci sampled $P=0{\cdot}06$. The hypothesis of statistical bias cannot be rejected on this basis but this result may be due to having only 25 loci. It was shown earlier that the probability of missing highly polymorphic loci was extremely low.

When applied to the data of Wilson & Alberdi (1991) and Kinsey *et al.* (1994), who analysed 41 and 36 loci respectively, P-values become significant. This bias hypothesis should be rejected, at least in the area covered by their studies. Since our Venezuelan sample was not less variable than our African samples (Table II) our inability to reject this hypothesis might be due simply to the analysis of 25 loci.

Technical bias: allozymes may degrade, affecting their electrophoretic mobility, and misleading the assessment of polymorphisms. Since such loci are difficult to read they are usually excluded from the analysis but then genetic variation can be underestimated, especially if variable loci are read in a fragile part of the fish's body. In *S. aurita*, the polymorphic loci are all read using the liver, which autolyses quickly.

The hypothesis was tested that most polymorphic loci in clupeids were read from the liver (Fig. 2), and a two-tailed sign test on the data plotted was statistically significant ($P \approx 0{\cdot}011$) indicating that loci read in the liver contributed most of the variation. Though data from Spanakis *et al.* (1989) and from Alexandrino *et al.* (1993) cannot be plotted (number of loci unknown) they can be used for a sign test (all polymorphic loci were read in the liver) which yields a value of $P \approx 0{\cdot}006$.

Scoring problems have been observed by some of the authors cited but degradation has been considered just a small technical problem likely to be artifactual. Only one study showed a significant frequency difference (Table VI).

Although there may be a tissue-specific sampling bias it is not clear if degradation would lead to an underestimation of genetic variation for all species. For *S. aurita* 16 out of 25 loci were studied in liver extracts and thus should lead to an overestimation of H.

To conclude, the degradation effect is not a major cause of the results presented here. If the level of genetic variation of *S. aurita* was underestimated because of technical problems, the lack of variation would still be significant.

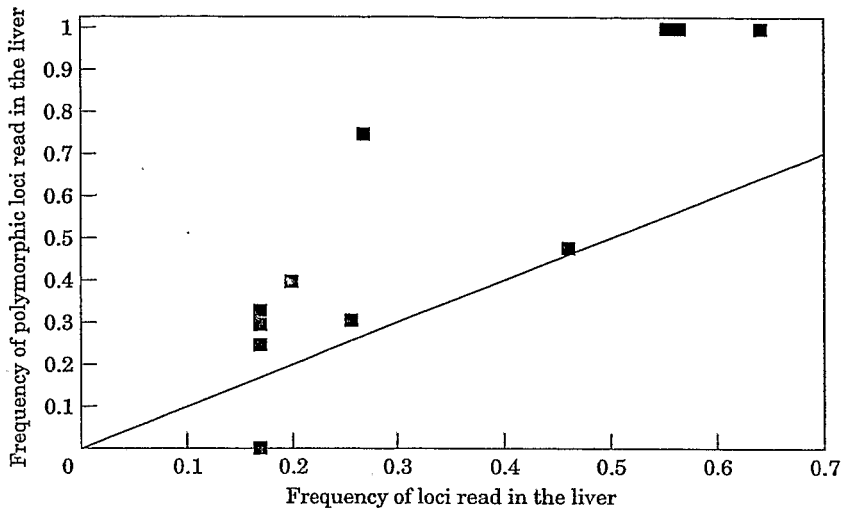

FIG. 2. Importance of the liver in revealing allozyme variation in clupeid species. This figure represents the proportion of loci read in the liver among all loci analysed plotted against the proportion of loci read in the liver considering only polymorphic loci. If loci read in the liver were in no way different from other loci the dots should be randomly distributed around the straight line $y=x$.

Heterozygosities of $\sim 0{\cdot}1$, which are the highest observed in clupeids (Table V), still correspond to effective sizes of $\sim 250\,000$ (for $\mu = 10^{-7}$). Demographic instability over evolutionary periods of time and variance in reproductive success must be invoked to understand the polymorphism shown by this species and other clupeids.

CONCLUSION

The use of allozymes on a large geographical scale confirmed the apparent absence of genetic variation in *S. aurita* which was not a consequence of technical artifacts.

The wide distribution and abundance of this species made this result surprising and a combination of demographic instability, variance in the reproductive success and ancient mass extinctions seem necessary to explain it. However, ancient events are unlikely to have played a major role in reducing the species' gene diversity overall.

Regular population crashes by three orders of magnitude every 10 years would reduce N_e by a factor of 100. Evolutionary population reduction by three more orders of magnitude every 1000 years would reduce it further only by a factor of 10. Finally, a variance $V_k \approx 100$ would reduce it further by a factor ~ 25. This particular combination assumes quite large effects for the three factors and would lead to a final N_e value of *c.* 40 000 (with $N = 10^9$ and $\mu = 10^{-7}$) or an expected heterozygosity of $H \approx 0{\cdot}016$. Any other combination that would minimize one of the three parameters, say V_k, would necessarily increase the effect of the other ones, i.e. demographic instability or the importance of bottlenecks.

TABLE VI. Influence of liver on gene diversity

	Loci	Polym. loci	Liver/Total*	Liver/polym.†	Z‡		
Clupea harengus L.	25	13	0·26	0·31	−0·312	NS	Andersson *et al.* (1981)
Clupea harengus L.	44	5	0·20	0·40	−0·993	NS	Kornfield *et al.* (1982)
Opisthonema bulleri Regan	29	4	0·17	0·00	0·902	NS	Hedgecock *et al.* (1988)
Opisthonema medirastre Berry & Barrett	29	5	0·17	0·30	−0·670	NS	Hedgecock *et al.* (1988)
Opisthonema libertate Günther	29	6	0·17	0·25	−0·445	NS	Hedgecock *et al.* (1988)
Sardinops sagax caerulea (Jenyns)	32	4	0·27	0·75	−1·959	*	Hedgecock *et al.* (1989)
Sardinella aurita Valenciennes	36	3	0·17	0·33	−0·723	NS	Wilson & Alberdi (1991)
Sardinella aurita Valenciennes	37	2	0·55	1·00	−1·243	NS	Kinsey *et al.* (1994)
Sardinella aurita Valenciennes	25	1	0·64	1·00	−0·742	NS	This study
Sardinella maderensis (Lowe)	18	5	0·56	1·00	−1·846	NS	Chikhi *et al.* (unpublished)
Ethmalosa fimbriata (Bowdich)	25	8	0·46	0·48	−0·094	NS	Gourene *et al.* (1993)
Sardina pilchardus Walbaum	X	3	X	1·00	<0	X	Spanakis *et al.* (1989)
Alosa fallax (Lacépède)	X	1	X	1·00	<0	X	Alexandrino *et al.* (1993)
All species	329	56	0·31	0·44	−1·964	*	

*Proportion of loci scored in the liver.
†Proportion of loci scored in the liver among polymorphic loci (by the 0·95 criterion).
‡Z the statistic used for the large-sample test when comparing two proportions, p_1 and p_2 (H_0: $p_1=p_2$) (Montgomery & Runger, 1994).
X, Only polymorphic enzymes were used. Though Z could not be estimated, a sign test could be used (see text) since p_2 is one and Z is thus necessarily negative.
NS, Not significant; *$0{\cdot}01<P<0{\cdot}05$.

These over-simplified calculations suggest that the marine environment (notably upwelling regions) might be orders of magnitude more unstable than believed.

Since genetic variation generally is correlated positively with the abundance and geographic distribution of a species (Nevo, 1988), *S. aurita*'s low *H* remains surprising. Although the relations between heterozygosity and extinction risks cannot be addressed properly, it seems relatively clear that low variability is unlikely to be the best solution to increase survival on evolutionary time scales. Indeed, low levels of genetic variation may favour the spread of infectious diseases or parasites which in turn will reduce genetic variation by mass mortality and selection (O'Brien & Evermann, 1988). This may be true particularly for clupeids which live in large high density shoals. Genetic variation may also play a major role in unstable environments, so as to favour quick responses to newly occurring conditions (Nevo, 1988; O'Brien & Evermann, 1988).

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References

Alexandrino, P. J., Sousa, C., Pereira, A. & Ferrand, N. (1993). Genetic polymorphism of adenosine deaminase (ADA, E.C. 3.5.4.4) in allis shad, *Alosa alosa* and twaite shad, *Alosa fallax*. *Journal of Fish Biology* **43,** 951–953.

Allendorf, F. W., Ryman, N. & Utter, F. (1987). Genetics and fishery management: past present and future. In *Population Genetics and Fishery management* (Ryman, N. & Utter, F., eds), pp. 1–20. Seattle, WA, University of Washington Press.

Andersson, L., Ryman, N., Rosenberg, R. & Ståhl, G. (1981). Genetic variability in Atlantic herring (*Clupea harengus harengus*): description of protein loci and population data. *Hereditas* **95,** 69–78.

Avise, J. C. (1989). Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution* **43,** 1192–1208.

Bard, F.-X. & Koranteng, K. A. (eds) (1994). *Dynamics and Use of Sardinella Resources from Upwelling off Ghana and Côte d'Ivoire*. Paris: Editions de l'ORSTOM.

Baron, J.-C. (1971). Les transferrines de deux espèces de sardinelles: *Sardinella aurita* (C.V.) et *S. eba* (C.V.). *Cahiers ORSTOM Série Océanographie* **IX,** 85–96.

Baron, J.-C. (1973). Les estérases du serum de *Sardinella aurita* (Valenciennes, 1847, in C.V.). Application à l'étude des populations. *Cahiers ORSTOM Série Océanographie* **XI,** 389–418.

Baumgartner, T. R., Soutar, A. & Ferreira-Bartrina, V. (1992). Reconstruction of the history of Pacific sardine and northern anchovy populations over the past two millenia from sediments of the Santa Barbara basin, California. *CalCOFI Report* **33,** 24–40.

Belkhir, K., Borsa, P., Chikhi, L., Goudet, J. & Bonhomme, F. (1997). *GENETIX 3.07, Windows® Software for Population Genetics*. Montpellier, France: University of Montpellier II, Laboratoire Génome et Populations.

Ben-Tuvia, A. (1960). Synopsis of biological data on *Sardinella aurita* of the Mediterranean sea and other waters. *FAO Fisheries Biology Synopsis* **14,** 287–312.

Boisneau, P., Mennesson-Boisneau, C. & Guyomard, R. (1992). Electrophoretic identity between allis shad, *Alosa alosa* (L.) and twaite shad, *A. fallax* (Lacépède). *Journal of Fish Biology* **40,** 731–738.

Bonhomme, F., Salvidio, S., LeBeau, A. & Pasteur, G. (1987). Comparaison génétique des tortues vertes (*Chelonia mydas*) des Océans Atlantique, Indien et Pacifique: une illustration apparente de la théorie mullerienne classique de la structure génétique des populations? *Genetica* **74,** 89–94.

Bowen, R. W. & Avise, J. C. (1990). Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: influence of zoogeographic factors and life-history patterns. *Marine Biology* **107,** 371–381.

Campton, D. E., Berg, C. J., Roblson, L. M. & Glazer, G. L. (1992). Genetic patchiness among populations of the queen conch *Strombus gigas* in the Florida Keys and Bimini. *Fisheries Bulletin* **90,** 250–259.

Carvalho, G. R. (1993). Evolutionary aspects of fish distribution: genetic variability and adaptation. *Journal of Fish Biology* **43** (Suppl. A), 53–73.

Carvalho, G. R. & Hauser, L. (1995). Genetic impacts of fish introductions: a perspective on African lakes. In *The Impact of Species Changes in African Lakes* (Pitcher, T. J. & Hart, P. J. B., eds), pp. 457–494. London: Chapman & Hall.

Chikhi, L., Bonhomme, F. & Agnèse, J.-F. (1994). The search for genetic differentiation of two sardine species (*Sardinella aurita* and *S. maderensis*). In *Dynamics and Use of Sardinella Resources from Upwelling of Ghana and Côte d'Ivoire)* (Bard, F.-X. & Koranteng, K. A., eds), pp. 152–168. Paris: Editions de l'ORSTOM.

Chikhi, L., Agnèse, J.-F. & Bonhomme, F. (1997). Fortes différences des ADN mitochondriaux de populations de *Sardinella aurita* de la mer Méditerranée et de l'Atlantique Est. *Comptes Rendus de l'Académie des Sciences, Paris, Série III, Sciences de la vie* **320,** 289–297.

Conand, F. (1977). Oeufs et larves de la sardinelle ronde (Sardinella aurita) au Sénégal: distribution, croissance, mortalité, variations d'abondance de 1971 à 1976. *Cahiers ORSTOM Série Océanographie* **XV,** 201–214.

Crow, J. & Kimura, M. (1970). *An Introduction to Population Genetics Theory.* New York: Harper and Row.

Cury, P. (1988). Pressions sélectives et nouveautés évolutives: une hypothèse pour comprendre certains aspects des fluctuations à long terme des poissons pélagiques côtiers. *Canadian Journal of Fisheries and Aquatic Sciences* **45,** 1099–1107.

Cury, P. & Roy, C. (1989). Optimal environmental window and pelagic fish recruitment success in upwelling areas. *Canadian Journal of Fisheries and Aquatic Sciences* **46,** 670–680.

Cury, P. & Roy, C. (eds) (1991). *Pêcheries Ouest-africaines: Variabilité, Instabilité et Changement.* Paris: Editions de l'ORSTOM.

Do Chi, T. (ed.) (1994). *Groupe de travail* ad hoc *sur les sardinelles et autres espèces de petits pélagiques côtiers de la zone Nord du COPACE.* Rome: FAO.

Ewens, W. J. (1979). *Mathematical Population Genetics.* Berlin: Springer-Verlag.

FAO (1992). *Fishery Statistics, Catches and Landings.* Rome: FAO yearbooks **74.**

Fréon, P. (1986). Réponses et adaptations des stocks de clupéidés d'Afrique de l'Ouest à la variabilité du milieu et de l'exploitation: analyse et réflexion à partir de l'exemple du Sénégal, Tome I. Ph.D. thesis, Université de Marseille II. Paris: Editions de l'ORSTOM.

Fuerst, P. A., Chakraborty, R. & Nei, M. (1977). Statistical studies on protein polymorphism in natural populations I. Distribution of single locus heterozygosity. *Genetics* **86,** 455–483.

Fujio, Y. & Kato, Y. (1979). Genetic variation in fish populations. *Bulletin of the Japanese Society for Scientific Fisheries* **45,** 1169–1178.

García de León, F. J., Chikhi, L. & Bonhomme, F. (1997). Microsatellite polymorphism and population subdivision in natural populations of sea bass *Dicentrarchus labrax* (Linnaeus, 1759), Perciformes: serranidae. *Molecular Ecology* **6,** 51–62.

Gourene, A. B., Pouyaud, L. & Agnèse, J.-F. (1993). Importance de certaines caractéristiques biologiques dans la structuration génétique des espèces de populations: le cas de *Ethmalosa fimbriata* et *Sarotherodon melanotheron*. *Journal Ivoirien d'Océanologie et Limnologie d'Abidjan* **2**, 55–69.

Grant, W. S. (1986). Biochemical genetic divergence between Atlantic, *Clupea harengus*, and Pacific, *Clupea pallasi*, herring. *Copeia* **1986**, 714–719.

Grant, W. S. & Utter, F. M. (1984). Biochemical population genetics of Pacific herring (*Clupea pallasi*). *Canadian Journal of Fisheries and Aquatic Sciences* **41**, 856–864.

Gyllensten, U. (1985). The genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous and freshwater species. *Journal of Fish Biology* **26**, 691–699.

Hedgecock, D. (1994*a*). Population genetics of marine organisms. *U.S. GLOBEC News* **6**, 1–11.

Hedgecock, D. (1994*b*). Does variance in reproductive success limit the effective population sizes of marine organisms? In *Genetics and Evolution of Aquatic Organisms* (Beaumont, A. R., ed.), pp. 122–134. London: Chapman & Hall.

Hedgecock, D., Nelson, K. & Lopez-Lemus, L. G. (1988). Biochemical genetic and morphological divergence among three species of thread herring (*Opisthonema*) in Northwest Mexico. *CalCOFI Report* **29**, 110–121.

Hedgecock, D., Hutchinson, E. S., Li, G., Sly, F. L. & Nelson, K. (1989). Genetic and morphometric variation in the Pacific sardine, *Sardinops sagax caerulea*: comparisons and contrasts with historical data and with variability in the northern anchovy, *Engraulis mordax*. *Fisheries Bulletin* **87**, 653–671.

Hedgecock, D. & Sly, F. L. (1990). Genetic drift and effective population size of hatchery-propagated stocks of the Pacific Oyster, *Crassostrea Gigas*. *Aquaculture* **88**, 21–38.

Ihssen, P. E., Martin, G. W. & Rodgers, D. W. (1992). Allozyme variation of Great Lake alewife, *Alosa pseudoharengus*: genetic differentiation and affinities of a recent invader. *Canadian Journal of Fisheries and Aquatic Sciences* **49**, 1770–1777.

Kimura, M. & Crow, J. (1964). The number of alleles that can be maintained in a finite population. *Genetics* **49**, 725–738.

Kinsey, S. T., Orsoy, T., Bert, T. M. & Mahmoudi, B. (1994). Population structure of the Spanish sardine *Sardinella aurita*: natural morphological variation in a genetically homogeneous population. *Marine Biology* **118**, 309–317.

Kornfield, I., Sidell, B. D. & Gagnon, P. S. (1982). Stock definition in Atlantic herring (*Clupea harengus harengus*): genetic evidence for discrete fall and spring spawning populations. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 1610–1621.

Longhurst, A. R. & Pauly, D. (1987). *Ecology of Tropical Oceans*. San Diego: Academic Press, Inc.

Lowe-McConnell, R. H. (1987). *Ecological Studies in Tropical Fish Communities*. Cambridge: Cambridge University Press.

Maley, J. (1991). The African rain forest vegetation and palaeoenvironments during late quaternary. *Climatic Change* **19**, 79–98.

Marchal, E. (1991). Location of the main West African pelagic stocks. In *Pêcheries Ouest-africaines Variabilité, Instabilité et Changement* (Cury, P. & Roy, C., eds), pp. 187–191. Paris: Editions de l'ORSTOM.

Menezes, M. R. (1994). Little genetic variation in the oil sardine, *Sardinella longiceps* Val., from the western coast of India. *Australian Journal of Marine and Freshwater Research* **45**, 257–264.

Montgomery, D. C. & Runger, G. C. (1994). *Applied Statistics and Probability for Engineers*. Chichester: John Wiley.

Murphy, G. I. (1973). Clupeoids. In *Fish Population Dynamics* (Gulland, J. A., ed.), pp. 283–308. Chichester: John Wiley.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.

Nei, M., Maruyama, T. & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution* **29**, 1–10.

Nei, M. & Graur, D. (1984). Extent of polymorphism and the neutral mutation theory. *Evolutionary Biology* **17,** 73–118.

Nei, M. (1987). *Molecular Evolutionary Genetics*. New York: Columbia University Press.

Nevo, E. (1988). Genetic diversity in nature, patterns and theory. *Evolutionary Biology* **23,** 217–245.

Noreen, E. (1989). *Computer-intensive Methods for Testing Hypotheses: An Introduction*. Chichester: John Wiley.

O'Brien, S. J. & Evermann, J. F. (1988). Interactive influence of infectious disease and genetic diversity in natural populations. *TREE* **3,** 254–259.

O'Maoileidigh, N., Cawdery, S., Bracken, C. C. & Ferguson, A. (1988). Morphometric, meristic character and electrophoretic analysis of two Irish populations of twaite shad, *Alosa fallax* (Lacépède). *Journal of Fish Biology* **32,** 355–366.

Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J. & Britton-Davidian, J. (1988). *Practical Isoenzyme Genetics*. Paris: Ellis Horwood Limited.

Roberts, N. (1984). Pleistocene environments in time and space. In *Hominid Evolution and Community Ecology, Prehistoric Human Adaptation in Biological Perspective* (Foley, R., ed.), pp. 25–53. London: Academic Press.

Roff, D. & Bentzen, P. (1989). The statistical analysis of of mitochondrial DNA polymorphism: χ^2 and the problem of small samples. *Molecular Biology and Evolution* **6,** 539–545.

Ryman, N., Lagercrantz, U., Andersson, L., Chakraborty, R. & Rosenberg, R. (1984). Lack of correspondence between genetic and morphologic variability patterns in Atlantic herring (*Clupea harengus*). *Heredity* **53,** 687–704.

Shackelton, L. Y. (1986). Fossil pilchard and anchovy scales—indicators of past fish populations off Namibia. In *International Symposium on Long Term Changes in Marine Fish Populations* (Wyatt, T. & Larraneta. M. G., eds), pp. 55–68.

Spanakis, E., Tsimenides, N. & Zouros, E. (1989). Genetic differences between populations of sardine, *Sardina pilchardus*, and anchovy, *Engraulis encrasicolus*, in the Aegean and Ionian seas. *Journal of Fish Biology,* **35,** 417–437.

Stewart, F. M. (1976). Variability in the amount of heterozygosity maintained by neutral mutations. *Theoretical Population Biology* **9,** 188–201.

Tringali, M. D. & Wilson, R. R. (1993). Differences in haplotype frequencies of mt-DNA of the Spanish sardine *Sardinella aurita* between specimens from the eastern Gulf of Mexico and southern Brazil. *Fishery Bulletin U.S.* **91,** 363–374.

Van Zinderen Bakker, Sr, E. M. (1982). African palaeoenvironments 18 000 yrs BP. In *Palaeoecology of Africa and the Surrounding Islands* (Coetze, J. A. & Van Zinderen Bakker, Sr, E. M., eds), Vol. 15, pp. 77–99. Pretoria: Southern African Society for Quaternary research.

Ward, R. D., Woodwark, M. & Skibinski, D. O. F. (1994). A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. *Journal of Fish Biology* **44,** 213–232.

Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38,** 1358–1370.

Wilson, R. R. & Alberdi, P. D. (1991). An electrophoretic study of Spanish sardine suggests a single predominant species in the eastern Gulf of Mexico, *Sardinella aurita*. *Canadian Journal of Fisheries and Aquatic Sciences* **48,** 792–798.

Whitehead, P. J. (1985). Clupeoid fishes of the world (suborder Clupeoidei). *FAO Fishery Synopsis* **125,** 1–303.

Wright, S. (1951). The genetical structure of populations. *Annals of Eugenics* **15,** 323–354.