

Long-term changes in maturation of sardine, *Sardina pilchardus*, in Portuguese waters

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SUMMARY: Long-term changes in sardine maturation were described using samples collected from landings off the western Portuguese coast since 1947. Estimates of the length at 50% maturity (L_{50}) were calculated in 44 years of the study period and proved to be good proxies of the maturation length of first-year spawners (Lp_{50} of age 0-1 fish). Sardine probability of maturing at a given length declined from the early 1950s to the late 1960s, corresponding to an increase of ca. 2 cm in both L_{50} and Lp_{50} . This trend reversed in the early 1970s and halted in the early to mid-1990s. The tendency for sardine to mature at a lower length was positively correlated with improved body condition in the growing season preceding maturation. Long-term trends in sardine maturation and body condition were parallel to trends in sea surface temperature reported in the literature. The results suggest that maturation at a lower size is directly influenced by increased temperature, and that higher temperatures improve body condition through increased feeding efficiency or a combination of both. We found no evidence that fishing intensity has contributed to long-term changes in sardine maturation.

Keywords: maturation, condition, sardine, Portuguese waters.

RESUMEN: CAMBIOS A LARGO PLAZO DE LA MADUREZ DE LA SARDINA, *SARDINA PILCHARDUS*, EN AGUAS PORTUGUESAS. – Se describen los cambios a largo plazo de la madurez de la sardina utilizando muestras recogidas desde 1947 en los desembarcos en la costa oeste de Portugal. Las estimas de la talla de primera madurez al 50% (L_{50}) fueron calculadas en 44 años del periodo de estudio y se mostraron como una buena aproximación de la talla de madurez de los individuos desovantes de primer año de vida (Lp_{50} peces de edad 0-1). La probabilidad de madurar de la sardina a una talla determinada disminuye desde principios de los años 50 hasta finales de los años 60, correspondiendo a un aumento de 2 cm en ambas tallas, L_{50} y Lp_{50} . Esta tendencia cambió a principio de los años 70 y se detuvieron a mediados de los años 90. La tendencia de la sardina a madurar a tallas inferiores se correlacionó positivamente con la mejora de la condición corporal en la estación de crecimiento que precedía a la madurez. Las tendencias a largo plazo de la madurez y la condición corporal eran paralelas a las tendencias en la temperatura superficial del mar reportadas en la literatura. Los resultados sugieren que la madurez a tallas pequeñas está influida directamente por el aumento de la temperatura, que temperaturas más elevadas mejoran la condición mediante un aumento de la eficiencia en la alimentación o a una combinación de ambas. Por otra parte, no encontramos evidencias de que la presión de la pesca haya contribuido a los cambios a largo plazo en la madurez de la sardina.

Palabras clave: madurez, condición, sardina, aguas portuguesas.

INTRODUCTION

Fish maturation or puberty is the process by which an immature individual acquires for the first time the capacity to reproduce (Okuzawa 2002). Several internal factors such as sex steroid hormones, insulin-like growth factor-I and leptin, a hormone produced by adipocytes, are involved in the activation of the brain-

pituitary-gonad axis at the onset of maturation (Okuzawa 2002, Carrillo *et al.* 2009). External factors such as photoperiod, temperature and food availability have been shown to influence internal factors and trigger maturation. Like other life-history traits, maturation is determined by the individual genetic pool, which reflects selective pressures to which the population has been subject, and by environmental conditions that the

individual experiences during maturation or at an earlier period when the “decision” to mature takes place (Wright 2007).

Trends towards maturation at lower size and age have been reported for a large number of commercial fish species (Jørgensen *et al.* 2007, Kuparinen and Merilä 2007). Such trends may be caused by environmental variation, fishing intensity or a combination of both (Marshall and McAdam 2007). Increased water temperature may induce maturation at a younger age by increasing the growth rate (Sinclair *et al.* 2002) or even independently of growth (Dhillon and Fox 2004, Tobin and Wright 2011). Increased food availability may have a similar effect since it usually leads to higher growth and improved body condition (Rochet *et al.* 2000; Kuparinen and Merilä 2007). Higher food availability per capita may be a direct response to the physical environment (e.g. upwelling intensity) or to reduced intra-specific competition for food resources at low population abundance resulting, for example, from high fishing pressure.

Besides reducing abundance, fishing shifts the demographic structure of exploited populations towards younger and smaller individuals (juvenescence) (Hilborn and Walters 2001). If mostly small and young fish contribute to reproduction, maturation age and size are expected to decline and other life-history traits with strong size/age-dependency, such as fecundity and egg and larval survival may decrease, magnifying abundance fluctuations and susceptibility to over-fishing (Rochet *et al.* 2000, Kuparinen and Merilä 2007, Anderson *et al.* 2008). The persistence of fishing effects may lead to evolutionary changes in life-history traits at decadal time scales, with important consequences for the dynamics of exploited populations (Law 2000, Jørgensen *et al.* 2007, Kuparinen and Merilä 2007, Allendorf *et al.* 2008). Since life-history traits determine population dynamics, impacts on stock biomass, productivity and yield are also expected from fisheries-induced changes in maturation (Jørgensen *et al.* 2007, Kuparinen and Merilä 2007, Allendorf *et al.* 2008). In particular, the reproductive potential of populations may decline if adult size declines because of shorter spawning periods, lower fecundity and lower probability of survival of eggs and larvae (Law 2000).

Disentangling genetic and plastic influences on life-history traits is important to evaluate the persistence and likelihood of reversal in trends; plastic changes are temporary because they are not transferred to subsequent generations while evolutionary changes alter the genotype and can be slow or even impossible to reverse (Jørgensen *et al.* 2007, Kuparinen and Merilä 2007, Dunlop *et al.* 2009). Probabilistic maturation reaction norms have been widely applied to investigate fisheries-induced evolution on maturation (reviews by Dieckmann and Heino 2008, Heino and Dieckmann 2008). Probabilistic maturation reaction norms describe the probability of fish maturation as a function of age and size and possibly other life-history traits

such as condition. Their application to disentangle environmental from evolutionary effects on maturation assumes that (i) variation in growth and survival is mainly of environmental origin and (ii) environmental variability is reflected mainly in growth, recorded as the size at age achieved by each individual at maturation time. Thus, trends in maturation after discounting growth are likely to be of genetic origin.

The sardine, *Sardina pilchardus* (Walb.), is a small pelagic clupeoid distributed in the northeast Atlantic, from the North Sea to Senegal, and in the Mediterranean Sea (Parrish *et al.* 1989). It is a batch spawner with indeterminate fecundity, fast growth, early maturation, moderate natural mortality and moderate longevity (Silva *et al.* 2006, 2008, Ganius 2009, Nunes *et al.* 2011a). In Portuguese waters, sardine spawns from October to March (Stratoudakis *et al.* 2007, Nunes *et al.* 2011a). Maturation takes place during the first two years of life (individuals aged 0 to 2 years) but most individuals from a cohort mature within their first spawning season, i.e. as old age 0 or young age 1 (ICES 2010).

Silva *et al.* (2006) showed that the length of sardine at first maturity (L_{50}) decreased from the early 1980s to the early 2000s and associated this trend with an increase in fish condition. However, they did not examine possible alternative causes of the maturity trend such as density-dependent and evolutionary effects caused by fishing. Sardine has a long fishing history in the Iberian Peninsula, being the target species of the purse seine fishery since the early 20th century (Mendes and Borges 2006). The annual landings of sardine in Portugal have fluctuated between 55000 t and 160000 t and the number of vessels licensed for purse seining have ranged between 100 and 450 since 1940 (Fig. 1A). Over this period, there were phases of intense exploitation, such as those in the late 1990s and possibly also in the mid-1960s (Jorge 1972). The assessment of the stock shows extensive changes in the abundance of the population and a downward trend in recruitment since 1978 (ICES 2010).

In this study we extend the analysis of sardine maturity-at-length back to the 1940s and examine changes in the maturation probability of sardine first-year spawners following the probabilistic maturation reaction norm approach. We relate trends in maturation and fish body condition over the study period and discuss concurrent changes in sea surface temperature, population abundance and fishing intensity. Finally, we discuss the potential impact of long-term maturation changes on sardine reproductive potential and productivity.

MATERIALS AND METHODS

Sampling

Biological samples collected from commercial landings on the western Portuguese coast in the period

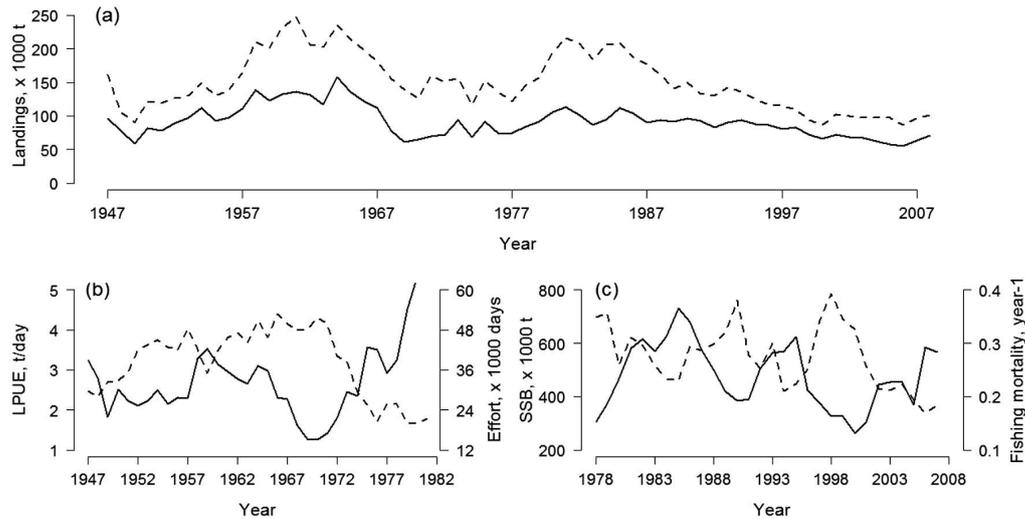


FIG. 1. – (a) Historical series of Portuguese (solid line) and total stock (dashed line) landings of sardine 1947-2008 (b) LPUE (solid line) and fishing effort (dashed line) for the Portuguese purse seine fishery in 1947-1981; (c) spawning biomass, SSB (solid line), and fishing mortality, F (dashed line) for the Atlanto-Iberian stock in 1978-2008. Plot (b) constructed from data in Mendes and Borges (2006), plots (a) and (c) constructed from stock assessment in ICES (2010).

1947-2008 were used to describe long-term changes in sardine maturation and body condition (Fig. 2). Samples obtained within the spawning season (October to March) were used to estimate maturity and maturation ogives (see below). Samples obtained within the growing season (June to October) were used to examine fish body condition. Sampling intensity and regularity varied substantially during the study period, providing better seasonal and annual coverage during a short period in the 1950s and since the early 1980s (Fig. 3). In general, one or two random samples of 100-150 fish (until 2001) or length-stratified samples of 10-15 fish per half-centimetre length class (since 2002) were collected per month per port. Age was determined in length-stratified samples of 5 to 15 individuals per length class.

Fish length (cm) was recorded for all individuals during the period; fork length (FL) was measured until 1978, after which total length (TL) became the standard measurement. FL was converted to TL using data collected in the transition period (1978 and 1979), when both measurements were recorded on the same individuals. The linear regression used in the conversion is the following: $TL = 1.12 FL + 0.046$ ($r^2 = 0.99$, $p < 0.001$, $n = 1451$). Macroscopic maturity and visceral fat were recorded since 1947 while gutted weight (g) started to be recorded in 1986. In the period 1951-1957 data were available only in aggregated format, namely as numbers of individuals in each maturity and fat stage by half-centimetre length class. Age data were only available since 1986.

The macroscopic maturity stage of each fish was determined by visual examination of the gonads. Until 1957, an eight-stage maturity key was used (Furnestin 1943); thereafter, a six-stage key was used: 1, immature; 2, developing; 3, pre-spawning; 4, spawning; 5, spawning/recovering; 6, post-spawning (Pinto 1957,

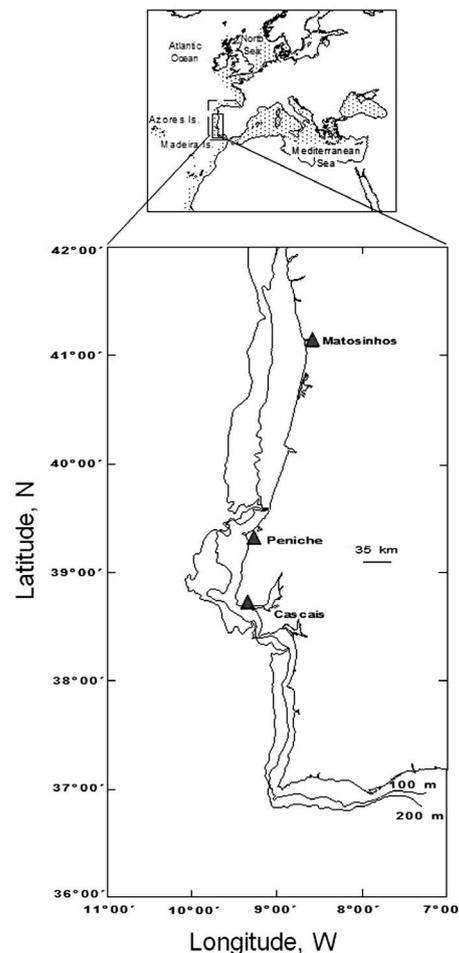


FIG. 2. – Map of the study area with the location of sampling ports (triangles). The map on the top shows the area of distribution of the species and the delimitation of the Atlanto-Iberian sardine stock (dashed line).

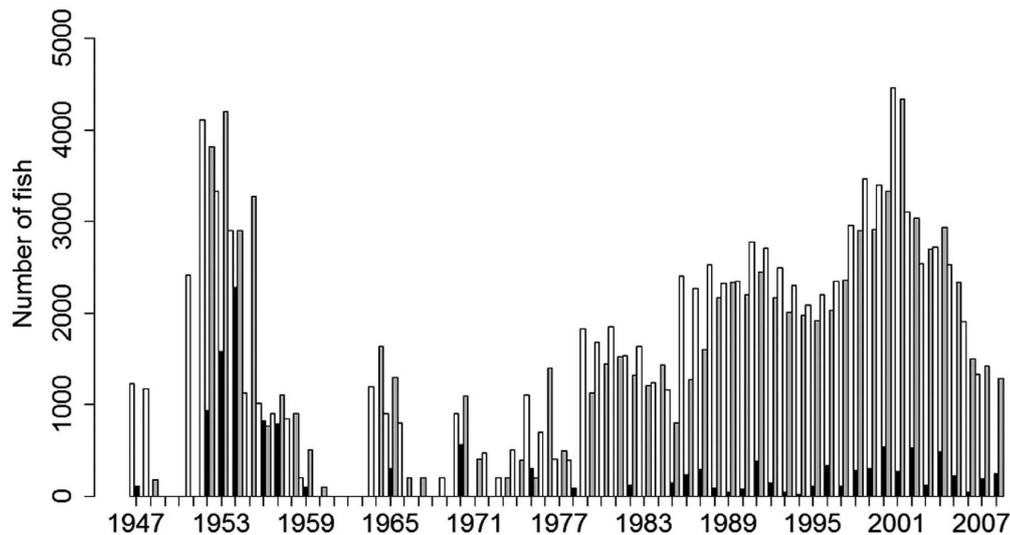


Fig. 3. – Total number of individuals (white bars) and number of first-year spawners (black bars) sampled per spawning season (October to March) and total number of individuals sampled per growing season (grey bars) in the period 1945-2007.

Pinto and Andreu 1957). Stages 2-3, 5-6 and 7-8 of the earlier maturity key were converted to stages 2, 4 and 5 of the current key, respectively. Recently, maturity stages 3 and 6 were merged as stage 3 due to difficulties in their separation (Afonso-Dias *et al.* 2008). Fish at maturity stage 2 and above were considered to be maturing or mature and to spawn within the current spawning season. Therefore, the proportion of stage 2+ individuals was used to fit maturity ogives and maturation ogives.

The classification of the fat stage was based on the key proposed by Furnestin (1943): 1, no fat visible; 2, thin thread of fat surrounding part of the gut; 3, thicker layer of fat partially surrounding the gut; 4, thick layer of fat surrounding the gut. For the purpose of this analysis, stages 1-2 and 3-4 were pooled into two groups, “lean” and “fat”, respectively. There are indications that the fat stage is a reliable indicator of sardine condition. For example, Bandarra *et al.* (1997) found a good correspondence between fat stages and the amount of lipid in the muscle of sardine and Nunes *et al.* (2011a) report a good correspondence between the seasonality in the proportion of fat individuals and their relative condition factor.

Maturity and maturation models

Maturity ogives describe the probability of an individual fish being mature at a given length in a given spawning season (or year). L_{50} is the length at which individuals have a 50% probability of being mature.

Maturation ogives describe the probability of an individual becoming mature at a given length within a given age and spawning season (or year), i.e., conditional to being immature in the previous age and year. Sardines maturing in their first spawning season are termed here first-year spawners (FYS). The length at

which FYS from each cohort have a 50% probability of becoming mature, L_{p50} , corresponds to the first midpoint of the probabilistic maturation reaction norm for the species. In sardine, FYS are not distinguished from second- or third-year spawners by visual examination of the gonads or histological analysis (Afonso-Dias *et al.* 2008). Therefore, they can only be separated in samples by combining information on maturity status and age. Here, we examine variation in sardine maturity ogives per spawning season and variation in the maturation ogives of FYS per cohort.

Generalized linear models (GLM) assuming binomial errors and a logit link were used to estimate maturity ogives and FYS maturation ogives. The model has the general form,

$$\text{logit}(m) = a + bX \quad (1)$$

where a and b are coefficients relating m , the probability of being mature (maturity ogives) or becoming mature (maturation ogives), to predictor variables X (see below). To simplify model interpretation, only two-way interactions were considered. The response variable was binary in maturation ogives for the recent period and binomial in the remaining cases. Goodness of fit was measured by the percentage of correctly classified observations in the binary case and by the percentage of explained deviance in the binomial case (Crawley 2007). Models were selected by backward selection of variables based on the Akaike Information criterion (AIC).

To estimate maturity ogives, model (1) was fitted to the proportion of mature fish by length class (LC, continuous variable) and spawning season (Y, factor variable). Monthly samples collected between October and March in each spawning season were pooled and length was aggregated in one-centimetre length classes

TABLE 1. – Properties of the models fitted to sardine maturity and maturation of FYS in 1947-2007. LC, length class; C, cohort; M, month. The first line for each period describes the final model. Subsequent lines correspond to simplified models by deleting terms involving a given predictor (e.g. LC terms deleted indicates both LC and LC×C terms have been deleted). The change in AIC is relative to the final model.

Model	Period	Terms deleted from full model	df	Residual deviance	AIC	Change in AIC (%)	P
Maturity	1947-2007	None	441	29	333	-	<0.001
Maturation	1947-1985	None	38	113	372	-	-
		LC	50	1372	1607	332	<0.001
		C	60	1105	1320	255	<0.001
		LC×C	49	432	668	80	<0.001
Maturation	1986-2007	None	4779	2154	2252	-	-
		L	4801	3495	3549	58	<0.001
		C	4821	2668	2682	19	<0.001
		M	4784	3609	3697	64	<0.001
		L×C	4827	6192	6194	175	<0.001

to increase sample size. Ogives could not be estimated for 17 out of 61 spawning seasons due to insufficient numbers of immature fish. Spawning seasons are here referred to by the starting year, i.e. the spawning season October 1999-March 2000 is termed the 1999 spawning season; because FYS of the 1999 cohort are the major contributors to the ascending limb of the 1999 maturity ogive, a broad correspondence can be assumed between spawning season and cohort.

Maturation ogives of FYS from landing samples were estimated separately for the periods 1947-1985 and 1986-2007 due to differences in the abundance and level of aggregation of the data. Moreover, ogives could not be estimated for 30 out of 61 cohorts present in the period 1947-2008 due to sparseness of data mainly in the earlier years. For the recent period, model (1) was fitted to maturation stage data by individual (binary variable), using total length (L, continuous variable), cohort (C, factor variable) and month (M, factor variable) as predictors. December was used as the reference month to calculate model coefficients and estimate L_p points. For the earlier period, age data were not available and, in some years, maturity data were aggregated by length class. Therefore, the proportion of immature and mature FYS per length class (LC) and cohort was derived from the combination of length frequency data and maturity ogives as follows: (i) each annual length distribution (samples pooled during the spawning season) was assumed to be a mixture of normal probability density functions corresponding to year-spawner groups with the first normal component assumed to represent FYS; (ii) densities of fish per one centimetre length class were estimated by fitting mixture distribution models to length distributions by maximum likelihood using the R package *mixdist* (Macdonald 2008); (iii) the proportion of mature FYS per length class was calculated by multiplying the maturity ogive by the number of fish in each length class (only classes containing more than 80% of FYS were included); and (iv) a GLM was fitted to the proportion of mature FYS by length class using cohort (factor) as a predictor variable. Maturation ogives were estimated for 12 cohorts during the period; in the remaining

cases, either mixture models did not fit significantly to length distributions or the first normal component of the mixture was not satisfactorily separated from the remaining components.

Point estimates of the length at 50% maturity or maturation were derived from model parameters as:

$$L_{50}/L_{p50} = -a/b$$

Standard errors were estimated using the formula for the approximate variance of a ratio of two random variables (Mood *et al.* 1974):

$$\text{s.e. } L_{50}/L_{p50} = (a/b)^2 \times [\text{var}(a)/a^2 + \text{var}(b)/b^2 - 2 \text{covar}(a,b)/(a \times b)]$$

Condition models

Long-term variation in condition within the growing season (June to October) was modeled using generalized additive models (GAMs; Wood 2006). GAMs are appropriate to model the seasonal nonlinear variation in condition and also have the advantage of allowing robust interpolation, a relevant property in the present case, in which sampling size per length class and month is unbalanced with periods of sparse data.

The models had the general form:

$$C = f_1(Y) + f_2(LC, M) \quad (2)$$

where C is the condition variable, f_1 is a univariate smooth function and f_2 is an anisotropic bi-variate function. Model (2), assuming binomial errors and logit link, was fitted to the proportion of fat fish (i.e. fish in fat stage 3 and 4) in the period 1947-2007, using length class (1 cm), month and year as continuous predictors. Individual gutted weight was modelled as a function of fish length (logged), month and year in the period 1986-2007, assuming gamma errors and a log link. Model selection was carried out by backward selection of variables based on generalized likelihood ratio tests (Wood 2006).

All calculations were carried out in R 2.10.1 (R Development Core Team 2009).

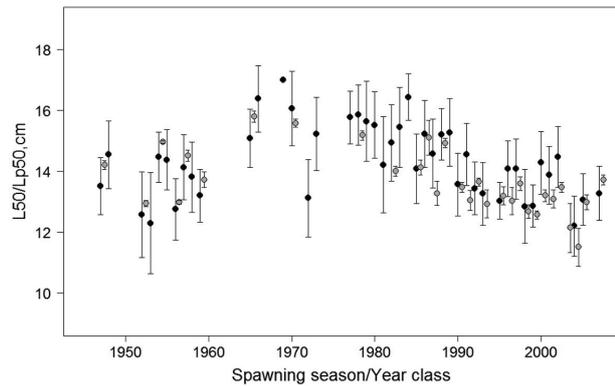


FIG. 4. – Variation in L_{50} (black symbols) and Lp_{50} (grey symbols) of first-time spawners during the period 1948-2007. Bars represent ± 2 standard errors of estimates.

RESULTS

Maturity and maturation

Sardine maturity, including interaction between fish length and spawning season (94.2% of the deviance explained, Table 1), was well explained by the model. L_{50} ranged from 12.2 ± 0.99 cm to 16.4 ± 0.74 cm (mean = 14.3 cm, CV=8.4%) over the last 60 years (1947-2007) (Fig. 4). Short term variability is also great, with L_{50} shifting up to 3 cm between consecutive years. In the long-term, there is an indication of an increase in L_{50} from ca. 13 cm in the 1950s to ca. 16 cm in the 1970s and of a declining trend thereafter (Fig. 4). The latter trend is strongly supported by abundant data collected since the late 1970s, which also point to a weakening of the downward trend in L_{50} since the mid-1990s. From the late 1970s to the early 2000s, L_{50} decreased by 2.5 cm, corresponding to a pronounced increase in the probability of being mature at a given length; for example, a 14-cm sardine showed a 25% probability of being mature in the late 1970s and a 81% probability in the mid-2000s. Changes in the maturity have apparently affected fish of different sizes similarly, as indicated by the slopes of annual maturity ogives over time (not shown).

The final models fitted to the maturation of sardine FYS in 1947-2007 included the interaction between length and cohort and, for the recent period, month as a factor (Table 1). In both cases, the models fitted the data well, as indicated by the high explained deviance in the earlier period (89.5%) and the high per-

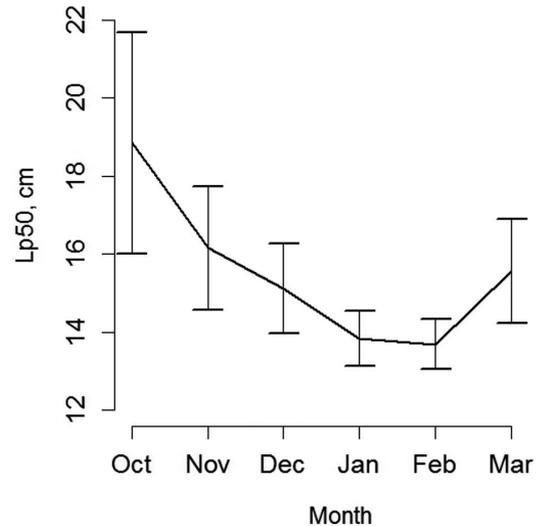


FIG. 5. – Variation in Lp_{50} estimates by month during the spawning season for the 1986 cohort. Bars show ± 2 standard errors of estimates.

centage of correctly classified individuals from each cohort in the recent period (70%-100% immature and 78%-100% mature). Not unexpectedly, maturation is mainly explained by fish length, the relationship being variable among cohorts, as indicated by significant interactions between length and cohort in both periods. In the model for the recent period, the sampling month has an influence on maturation comparable to that of fish length; as the spawning season progresses, the proportion of maturing individuals varies in a dome-shaped pattern with maximum values in December/January. On the logit scale, the odds of a FYS sardine becoming mature in October or March are 0.8% and 56% of those of it becoming mature in December. Thus, for each cohort, Lp_{50} varies with the spawning month in an inverted dome-shaped pattern: for example, point estimates of Lp_{50} for the 1986 cohort are 18.8, 13.8 and 15.6 cm in October, January and March, respectively (Fig. 5). Month-corrected estimates of Lp_{50} based on model predicted maturation probabilities at length in December are lower and more uncertain than uncorrected estimates (not shown) based on pooled samples during the spawning season. However, the two types of estimate are significantly correlated ($r=0.71$, $p<0.001$, $n=18$).

Lp_{50} varied in parallel to L_{50} (Fig. 4): in the period 1947-2007, Lp_{50} varied from 11.5 ± 0.62 cm to 15.8 ± 0.19 cm (mean = 13.7 ± 1.03 cm, CV=7.5%),

TABLE 2. – Properties of the models fitted to the percentage of fat individuals and to the individual gutted weight of sardine in the growing season (June-October).

Model	Period	Explained deviance (%)	N	Smooth terms	Degrees of freedom	χ^2 or F	p
Fat	1948-2008	44	1869	L	3,4	137,1	<0.001
				M	2	262,3	<0.001
				Y	8,5	161	<0.001
Gutted weight	1987-2008	96	51339	L×M	23,9	63190,2	<0.001
				Y	8,9	502,8	<0.001

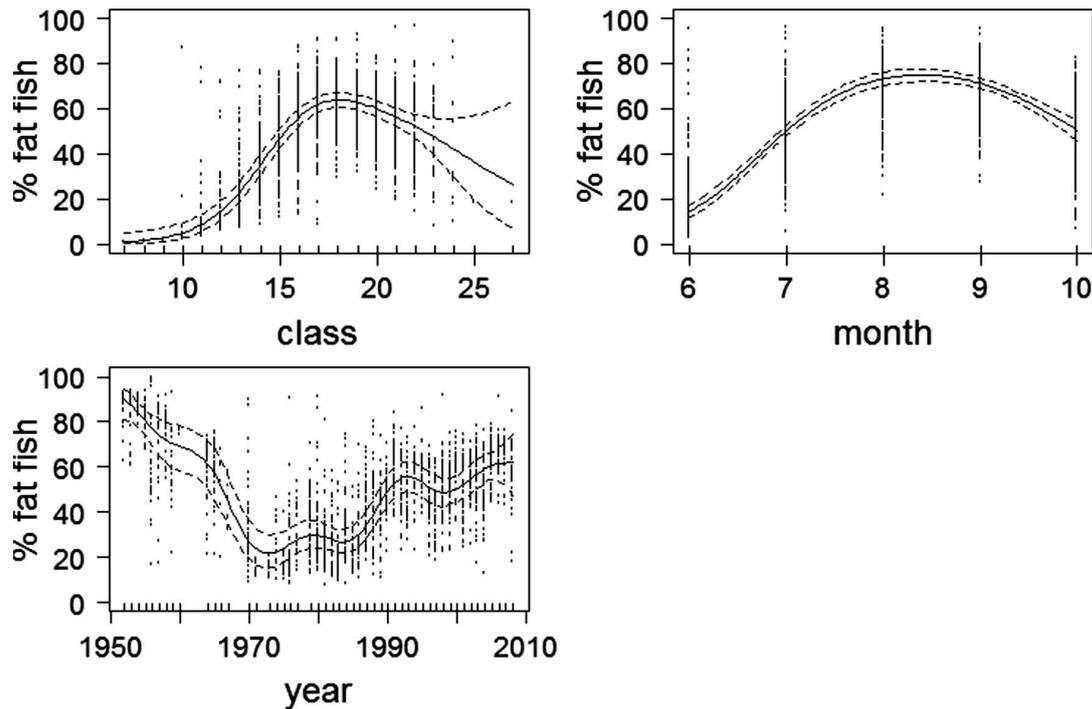


FIG. 6. – Plots of the smooth functions of the GAM fit to the percentage of fat individuals in relation to length class, month and year (see Table 2). Fat fish correspond to individuals in visceral fat stage >2 in the growing season, June–October. Continuous lines show model predicted values; dashed lines show ± 2 standard errors. Symbols correspond to partial residuals.

increasing by about 3 cm from the early 1950s to the early 1970s and decreasing by *ca.* 2.5 cm until the mid-1990s. Thereafter, L_{p50} showed interannual fluctuations but no obvious trend. The probability of a 14-cm sardine becoming mature declined from 66.5% to 24.4% between 1952 and 1970 and increased to 89.0% in 1995.

The historical series of L_{p50} and L_{50} are significantly correlated ($r=0.87$, $p<0.001$, $n=29$), supporting the use of L_{50} as a proxy of sardine FYS L_{p50} . L_{p50} is generally lower than L_{50} in corresponding spawning seasons. A linear regression of L_{p50} on L_{50} has an intercept of 11.3 ± 0.36 cm (estimated at $L_{50}=11$ cm) and a slope of 0.81 ± 0.12 , indicating that differences between L_{p50} and L_{50} increase towards higher values.

Condition

The GAMs fitted to condition data in the growing season are shown in Table 2. In the model describing the proportion of fat fish as a function of fish length, month and year, the interaction terms were not significant, indicating that temporal variation is comparable among fish of different sizes. As expected from the seasonal cycle of sardine condition (Nunes *et al.* 2011a), the proportion of fat fish follows a dome-shaped curve during the growing season with a maximum in August–September (Fig. 6). The increase in the percentage of fat fish with increasing fish length is also significant. For example, in 2005, the probability of a 14 cm sar-

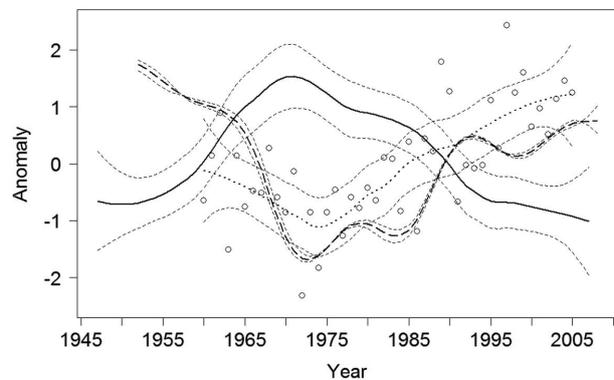


FIG. 7. – Long-term trends in sardine L_{50} (continuous line) in 1947–2007, condition (dashed line) in 1952–2007 and sea surface temperature (SST, black symbols and dotted line) in 1960–2005. All trends are presented as anomalies from the long-term mean. Trends in L_{50} and SST are represented by a loess smoother with a span of 0.5. Mean SST values are calculated from ICOADS at $41^{\circ}30'N$ latitude and $9^{\circ}30'W$ longitude (northern Portugal). The trend in condition corresponds to predicted values for a 17 cm sardine of the generalized additive model fit to the percentage of fat individuals (see Table 2). Thin dashed lines show ± 2 standard errors.

dine being fat was 17% in June, 77% in August and 53% in October, while that for a 17 cm sardine was 38%, 91% and 79%, respectively.

Sardine condition declined sharply from the 1950s to the early 1970s and increased thereafter, with periods of steeper change in the second half of the 1980s and in the early 2000s (Fig. 6). Gutted weight data since 1986 supports the increasing trend in condi-

tion with steep changes from 1988 to 1993 and from 1999 to 2007. The gutted weight model predicts that a 17-cm sardine sampled in July increased from 34.6 g to 36.8 g (6%) between 1988 and 1993 and from 36.8 g to 38.0 g (3%) between 1999 and 2007 (3%), with a 0.7 g increase taking place as of 2004. Approximate confidence intervals for predicted weights suggest that increases over those periods are significant at the 5% level.

Relationship between maturation and condition

There is a good match between the long-term tendency in sardine maturation, as shown by trends in L_{50} , and those in the proportion of fat fish in the previous growing season (Fig. 7). In particular, the fact that both variables show a reversal of the trends around 1970 is noteworthy. Point estimates of L_{50} are significantly correlated with the percentage of fish with fat in the previous feeding season (Spearman $\rho = -0.60$, $p < 0.001$). On the other hand, the correlation between L_{p50} and the % fish with fat is not significant (Spearman $\rho = -0.30$, $p = 0.12$).

DISCUSSION

The data used to examine long-term changes in sardine maturation and condition were collected over a period of 60 years, during which methodological changes took place and different persons were involved in biological sampling. The involvement of different persons has certainly contributed to the uncertainty of our results because of the subjectivity inherent to visual assessment of macroscopic maturity and fat, but there is no a priori reason to suspect that it could have biased the observed temporal trends. As of 1957 maturity at length data do not raise serious concerns because they were all collected using the same criteria. From 1986 onwards, the consistency of maturity data over time is supported by the significant correlation between L_{50} and the gonadosomatic index of maturing individuals (Silva *et al.* 2006) and changes in fish condition are corroborated by comparable trends in the proportion of fat fish and gutted weight at length (this study). Unfortunately, there is no way to cross-check data from the earlier period, in particular the equivalence of maturity stage data collected before and after the change of the maturity key in 1957. Nevertheless, data on actively spawning females (not shown), for which visual maturity staging is relatively trustworthy due to the presence of hyaline oocytes, provide some support to the trends in maturation described below; the smallest stage 4 females in the 1950s, 1970s and 2000s were in the 11, 14 and 12 cm length classes, respectively.

Our results demonstrate that sardine maturation probability has undergone pronounced changes over the past 60 years, showing a decreasing trend from the early 1950s to the early 1970s, an increasing trend until the mid-1990s, and annual fluctuations since then

(Fig. 7). Changes in maturity at length reflect mainly changes in the length of individuals maturing in their first year of life (old age 0 and young age 1 sardine). Although maturation changes of second- and third-year spawners are not examined here, the positive correlation between L_{p50} and L_{50} suggests that they may be parallel.

The significant negative correlation between L_{50} and the percentage of fat fish indicates that sardines mature gradually at smaller size as their condition in the preceding summer improves. As both increasing and decreasing trends in maturation/condition were seen over time, the reverse is also true, i.e. poor condition in the growing season leads to maturation at greater lengths. Trends in sardine maturation and condition are concurrent with those in sea surface temperature since the 1960s (Fig. 7). There is evidence that surface waters off Portugal cooled during the 1960s (Fig. 7) as part of a long-term trend of pronounced cooling which began in the late 1940s and affected the whole northeast Atlantic (Lemos and Sansó 2006). This trend reversed in the early 1970s and since then the western Iberian waters have undergone a general warming of 0.015°C to 0.037°C per year (Relvas *et al.* 2009).

There are several ways in which sardine maturation, condition and temperature might be linked. An obvious link in an upwelling system would be that lower temperature reflects an intensification of upwelling and thus higher food availability, which improves condition, and this in turn enhances maturation of smaller individuals (Parrish *et al.* 1995, Watanabe and Yatsu 2006). However, the parallel trends observed in water temperature and sardine condition are contrary to what would be expected in this case. There is strong evidence that the long-term warming of coastal waters off Portugal reflects a weakening of the upwelling regime (Lemos and Pires 2004, Lemos and Sansó 2006, Relvas *et al.* 2009). However, if food has not become a limiting factor, other mechanisms, such as higher metabolism and higher rate of food intake with increasing temperature (Handeland *et al.* 2008), may explain a relationship between temperature and sardine condition. It is also possible that temperature directly affects the onset of maturation in sardine as seen in experimental studies. For example, Yoneda and Wright (2005) show that low temperatures led to an arrest in the onset of vitellogenesis in Atlantic cod and significantly affected the number of females that matured. Dhillon *et al.* (2004) and Tobin and Wright (2011) prove that increased temperature enhance maturation at a lower size independently of growth. They further showed that above a certain threshold temperature gonad development was disturbed, leading to a reverse effect on maturation length and age. Finally, as discussed in Pankhurst and Porter (2003), it is also plausible that temperature and condition have a combined influence on sardine maturation.

Alternatively, changes in food availability per capita could result from changes in population abun-

dance, i.e. changes in maturation could be a density-dependent response (Engelhard and Heino 2004, van der Lingen *et al.* 2006, Watanabe and Yatsu 2006). In the earlier period, coincident with substantial changes in sardine maturation and condition, there were striking changes in LPUE (landings per unit of effort), with a sharp decline during the 1960s and a sharp increase during the 1970s (Fig. 1B), possibly associated with recruitment dynamics (Jorge 1972). However, if density-dependent effects on maturation were to be expected, changes in abundance over this period should have been the opposite. The increase in maturation probability since the early 1980s might have some relationship with the decreasing trend in stock abundance (Fig. 1C). However, along with this trend there were substantial fluctuations in stock biomass which apparently have no parallel in maturation changes.

The possibility that changes in sardine maturation had an evolutionary component cannot be ruled out but appears unlikely. First, fisheries-induced evolution does not provide a consistent explanation for long-term changes in sardine maturation, and especially for the reversal of maturation trends in the 1970s. Moreover, the sardine fishing history provides no basis for the hypothesis of fisheries-induced evolution. Although sustained high catches during the 1960s may have led to intense fishing mortality, evidence of good recruitments in the early 1970s and stable catches suggest that the situation reversed rapidly. Since 1978, fishing mortality has fluctuated inversely to stock biomass, within the range 0.17–0.40 year⁻¹, being below natural mortality (0.33 year⁻¹) in more than 80% of the years (Fig. 1C). The biomass shows a downward trend but no signs of juvenescence (ICES 2012).

Finally, the decline in sardine maturation length suggests that the proportion of spawners increased and the average size of spawners in the population declined. Several reproductive traits of sardine, such as the duration of the spawning season, batch and total annual fecundity and probably also spawning fraction, increase with body size (Silva *et al.* 2006, Nunes *et al.* 2011a, b). The decline in length at maturation may influence sardine egg production and early life survival negatively and ultimately affect the success of recruitment, as observed in other fish species (Enberg *et al.* 2009, Óskarsson and Taggart 2010). This might happen if the decline in maturation length impairs growth and therefore lifetime reproduction or if there is juvenescence. Otherwise, more spawners and egg production may be added to the population and the overall reproductive potential increases. Moreover, compensatory mechanisms, such as increased fecundity associated with improved condition, may counteract potential effects of early maturation on reproductive success (Rochet 2000, Yoneda and Wright 2004).

In summary, sardines have shown considerable changes in maturation over the past 60 years, including a tendency to mature at larger lengths until the early 1970s, followed by a tendency to mature at smaller

lengths until the mid-1990s and thereafter random fluctuations in maturation length. The results of this study, combined with historical data on sardine abundance and fishing intensity, suggest that density-dependent effects and fisheries-induced evolution are unlikely to have caused maturation changes. A more plausible explanation is that sardine maturation—and possibly also body condition—change in response to temperature. An increase/decrease in temperature may induce maturation at smaller/larger length directly via physiological processes, indirectly by increasing/decreasing condition, or both. The consequences of maturation changes to sardine lifetime reproductive potential and productivity deserve further investigation.

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