The Egg Production Method applied to the spawning biomass estimation of sardine, *Sardina pilchardus* (Walb.), on the North Atlantic Spanish coast

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ABSTRACT

During April-May 1988, an Egg Production Method Cruise was carried out with the purpose of evaluating the spawning biomass of the sardine, Sardina pilchardus *(Walb.), from the Galician and Cantabrian coasts of Spain. This cruise was coordinated with the acoustical evaluation of this stock on board the R/V Cornide de Saavedra.*

The area covered extends from the Spanish-Portuguese border (41° 55' N) to the Spanish-French border (1° 58' W). Vertical plankton tows at 524 stations were distributed over the continental shelf and slope water in a 6×6 mile grid, with a 6×3 mile grid in areas of more intense spawning, to estimate the daily egg production parameter.

A total of 44 epipelagic trawls were done in order to estimate the biological parameter relative to the adult population, that is, average female weight, batch fecundity, spawning fraction and sex ratio.

The survey area was post-stratified into three regions and the spawning biomass was estimated for each region. The sum of the three spawning biomass estimates was the total spawning biomass for the survey area. Three regions were established by differentiated adult parameters, such as average female weight in conjunction with the considered acoustical divisions. These regions correspond to the Galician coast (I), the western Cantabrian area (II) and the eastern sector of the Cantabrian coast (III).

The biomass estimates with their respective coefficient of variation of each region were as follows:

	Galicia I	W Cant. II	E Cant. III	Total Areas
Spawning Biomass (B)	134 195	33 503	12 467	180 165
CV	0.66	0.30	0.56	0.50

Maximum biomass estimates were found on the Galician shelf, although the stock was mainly concentrated in the northern sector of Galicia and the westernmost part of the Cantabrian shelf. Biomass estimates decreased towards the east. The trend in spawning biomass was similar to the total biomass estimate from the acoustic survey, with a total estimate of 174 016 t.

Key words: sardine, evaluation, egg production, fecundity, Cantabrian Sea, Spain.

RESUMEN

El Método de Producción de Huevos aplicado a la estimación del stock re-
productor de sardina, *Sardina pilchardus* (Walb.), en aguas noratlánticas espa-
ñolas.

*Desde abril a mayo de 1988 se aplicó el Método de Producción Diaria de Huevos con
el objetivo de estimar la biomasa reproductora de la sardina noratlántica española,
Sardina pilchardus (Walb.), comprendida en el área de las costas de Galicia y del
Cantábrico. Esta campaña fue realizada coordinadamente con la campaña de evalua-
ción acústica de pelágicos a bordo del B/O Cornide de Saavedra.*

*El área de cobertura se extiende desde la frontera luso-española (41° 55' N) hasta la
frontera con Francia (1° 58' O). Se muestrearon 524 estaciones para la recogida de
muestras planctónicas a lo largo de la plataforma y talud en un mallaje de estaciones de
6×6 millas náuticas, y en zonas de mayor abundancias de huevos en 6×3, con el
propósito de estimar la producción diaria de huevos.*

*Se llevaron a cabo un total de 44 pescas epipelágicas con el fin de estimar los pará-
metros biológicos y reproductivos de la población de adultos, como son el peso medio de
las hembras, fecundidad parcial, fracción de hembras en puesta y proporción de sexos.*

*El área muestreada fue post-estratificada en tres regiones y, consiguientemente, la
biomasa reproductora estimada para cada una de las mismas. La biomasa reproductora
total se logra con la suma de cada una de las biomasas por región. El criterio de
estratificación por regiones se basa en la diferenciación zonal de los parámetros de adul-
tos, como es el peso medio de las hembras, coincidiendo con las divisiones acústicas
preestablecidas. Estas regiones corresponden a las costas de Galicia (I), Cantábrico occi-
dental (II) y Cantábrico oriental (III).*

*El resultado de las estimaciones de la biomasa reproductora de sardina junto con sus
respectivos coeficientes de variación figuran a continuación:*

	Galicia *I*	Cant. oeste *II*	Cant. este *III*	Total *Areas*
Spawning Biomass (B)	134 195	33 503	12 467	180 165
CV	0.66	0.30	0.56	0.50

*El máximo de biomasa se produce en las costas norte de Galicia y la parte más
occidental del Cantábrico. La biomasa tiene una tendencia decreciente hacia el este,
igualmente acusables por acústica, que estima el stock en 174 016 t.*

INTRODUCTION

Fisheries assessment and management on the Iberian pilchard, *Sardina pilchardus* (Walb.), from the North Atlantic coast have been used with two stock evaluation methods: VPA (since 1976) and acoustic survey evaluations (since 1983). Within the framework of a SARP project on sardine recruitment variability, another stock estimation procedure, the Daily Egg Production Method (DEPM), was introduced (Lasker, 1985). The DEPM has been used for the spawning biomass estimates of northern anchovy, *Engraulis mordax*, off California (Picquelle and Hewitt, 1983; Picquelle and

Hewitt, 1984; Bindman, 1986; Smith and Hewitt, 1985). It has also been used for related species, such as the Peruvian anchovy, *Engraulis ringens* (Santander; Altheit and Smith, 1984), and the southern Benguela anchovy, *Engraulis capensis* (Armstrong *et al.*, 1988). In the ICES area, efforts are being made to apply fishery-independent estimates such as the DEPM. Alheit (1985) described the method at the ICES 73rd Statutory Meeting. Santiago and Sanz (1989) applied DEPM to the Bay of Biscay anchovy *Engraulis encrasicolus*.

The purpose of this paper is to present the first experience of DEPM on the Iberian sardine *(Sardina pilchardus)*. A DEPM cruise

combined simultaneously with an acoustic survey covered the whole of the Galician and Cantabrian waters. This survey was coordinated with another DEPM survey along the Portuguese coast on the same species (Cunha *et al.*, 1989).

To conduct a successful DEPM cruise, the following information is required: the spatial and/or temporal distribution of the egg stages of pilchard and the delimitation of the spawning areas (Smith and Hewitt, 1985).

The survey was conducted during the peak spawning period (from March to April) (Dicenta, 1984; Solá, 1987; Lago de Lanzós, Franco and Solá, 1988; Pérez *et al.*, 1985; García *et al.*, 1988).

The knowledge of the reproductive biology of pilchard and the correct histological techniques are also essential for obtaining accurate estimates of the reproductive parameters.

MATERIAL AND METHODS

Survey description

The combined DEPM and acoustic survey of 1988 (MPH Saracus 0488) was conducted aboard the R/V Cornide de Saavedra from March 29 to May 6. The cruise began in the southern area of the Galician shelf next to the Spanish-Portuguese border (41° 55' N, 08° 55' W) and ended in the transect close to the Spanish-French border (43° 43' N, 1° 58' W) (fig. 1).

A 6 X 6 mile grid was the basic plankton station scheme. Transects were spaced every 6 miles and acoustic tracking was carried out following the stations' numerical order. To achieve a more precise estimate of egg production, other plankton tows were conducted in the western and part of the eastern Cantabrian area, where high spawning activity occurred. The 6 X 6 mile grid was modified to a 6 X 3, that is, adding up one station every 3 miles within the same transect. There were 524 plankton stations representing a coverage of 51 736 km² of sea surface. Sardine eggs were present at 283 stations.

Plankton samples were taken using a 25 cm diameter net of 150-micron mesh (Smith,

Flerx and Hewitt, 1985) retrieved vertically from a depth of 100 m at a speed of approximately 1 m/s. The mean volume filtered was 5.09 m³ with a standard deviation of 1.05 m³.

In every station, surface temperature, wind speed and direction were recorded. Salinity, temperature, depth (CTD) (77) and expendable bathythermography (XBT) (50) casts were conducted at about 25 % of the stations sampled.

Adult sardine were sampled with an epipelagic trawl at 30 sampling stations out of 44 tows (fig. 2), and 7 of them showed hydrated females. The sampling strategy followed a judgment sonar sampling of sardine schools along the coastline. We estimated the total number and weight of sardine caught.

Fish were processed on board with random sampling, in each trawl: 50 fish served to record sex and maturity and to measure standard length to determine sex ratios. Gonads of 25 obvious females and 10 males were frozen. The gonads of all hydrated females captured ($n = 116$) were preserved and the ovary-free body frozen.

Egg production estimation model

The spawning biomass estimate was based on Parker's (1980) equation on biomass estimation, modified by Stauffer and Picquelle (1980) for the northern anchovy, *Engraulis mordax*:

$$B = \frac{kAP_oW}{RFS}$$

where,

B = spawning biomass in metric tons

P_0 = daily egg production (number of eggs per sampling unit, 0.05 m².

W = average weight of mature females (g)

R = sex ratio (fraction of mature females by weight)

F = batch fecundity (mean number of eggs per mature female per spawning)

A = total survey area (in 0.05 m² sampling units)
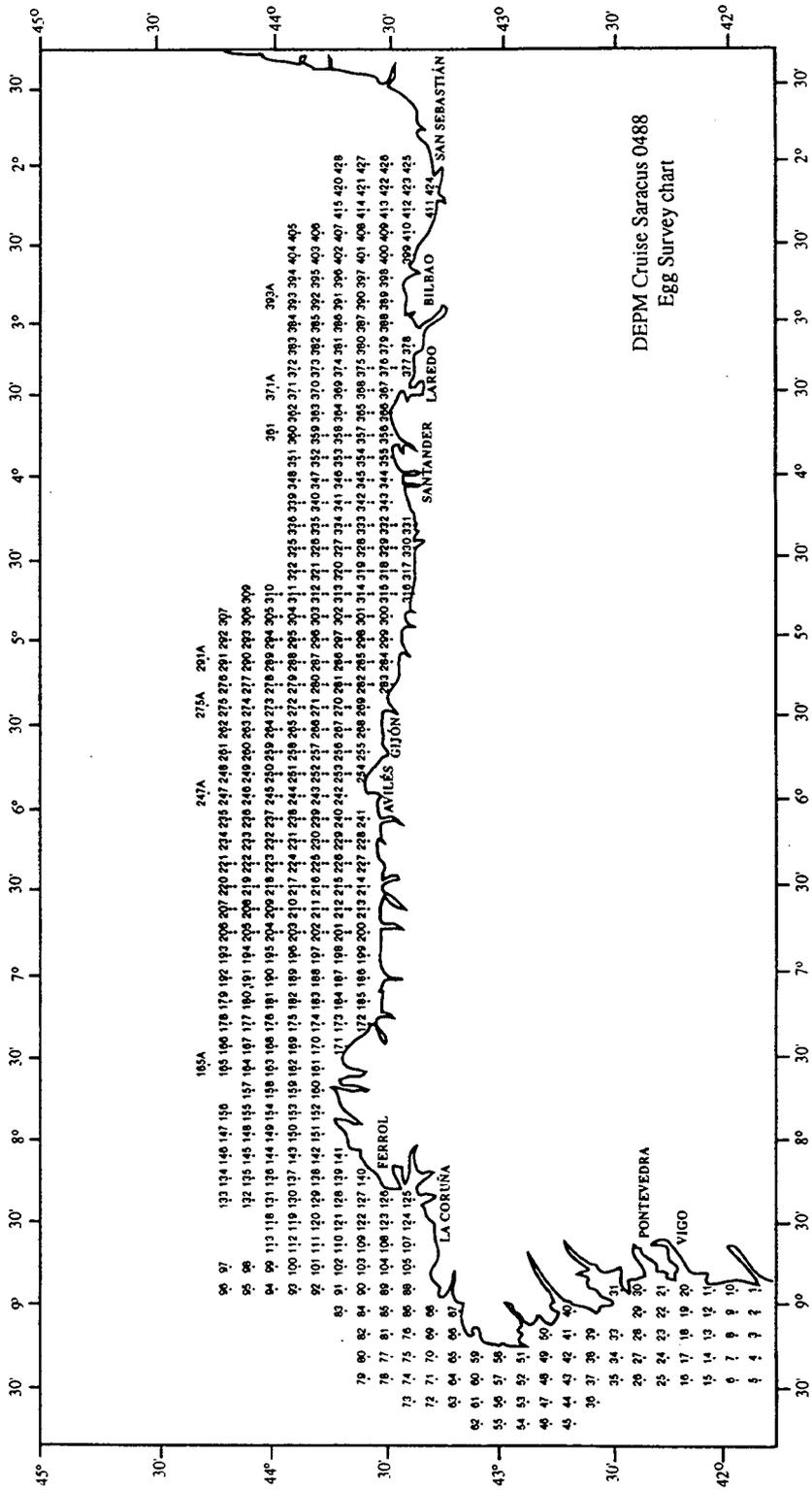
Fig. 1.—DEPM Egg Survey chart.

DEPM Cruise Saracus 0488
Egg Survey chart
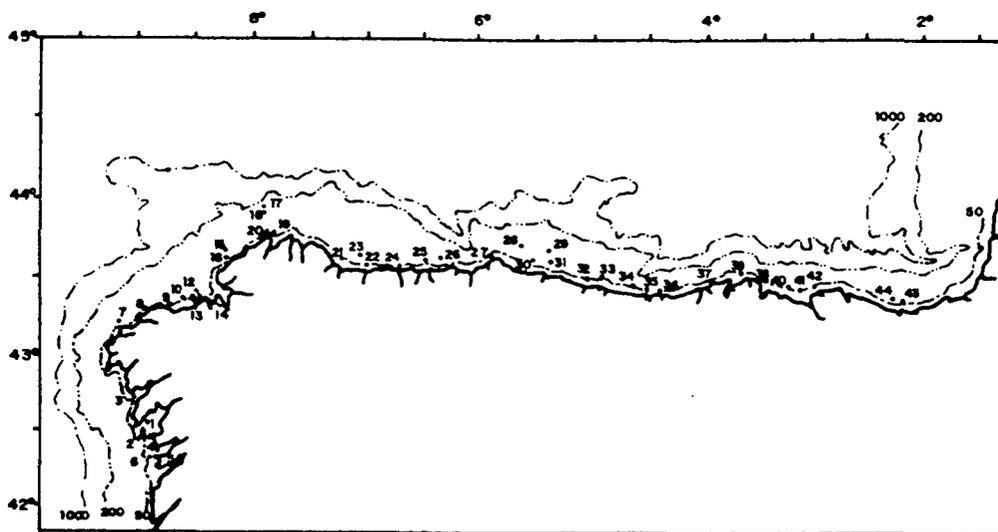
Fig. 2.—Adult trawl survey chart.

Fig. 2.—Mapa de estaciones de muestreos de adultos.

S = fraction of mature females spawning per day

k = conversion factor from grams to metric tons

The variance of the biomass estimate through this method is calculated by the delta method (Seber, 1973), as a function of variance and covariance of the estimates of parameters:

$$Var(B) = B^2 \cdot \left[\frac{Var(P_0)}{P_0^2} + \frac{Var(W)}{W^2} + \right.$$

$$+ \frac{Var(R)}{R^2} + \frac{Var(F)}{F^2} + \frac{Var(S)}{S^2} +$$

$$+ 2 \left(\frac{Cov(P_0, W)}{P_0 W} - \frac{Cov(P_0, R)}{P_0 R} - \right.$$

$$- \frac{Cov(P_0, F)}{P_0 F} - \frac{Cov(P_0, S)}{P_0 S} - \frac{Cov(W, R)}{WR} -$$

$$- \frac{Cov(W, F)}{WF} - \frac{Cov(W, S)}{WS} + \frac{Cov(R, F)}{RF} +$$

$$\left. \left. + \frac{Cov(R, S)}{RS} + \frac{Cov(F, S)}{FS} \right) \right]$$

Daily egg production in the survey area, $P_0 A$, is based on the egg sampling and a temperature-dependent model of the sardine egg developmental rate, while the biological parameters of the adults are estimated from the adult sampling trawl survey.

RESULTS

Egg distribution pattern

Sardine fish egg distribution is shown in figure 3. No spawning activity was observed in the southernmost area of the Galician region. Spawning was first registered south of Cape Finisterre in the mouth of Ría de Arosa (transect lines 31-35), and continued toward the northwest-east direction. Actually, the area of these first egg catches coincided with the first adult catches, which contained the first samples of females with hydrated oocytes.

The distributional pattern indicates regional differences, which were taken into account in this study. In the Galician region, spawning areas of pilchard are restricted to the coastal areas, whereas in the western and central Cantabrian (Asturian region)

pilchard eggs are widespread and extend to offshore waters (García, Franco and Solá, 1992) and in some transects (between Avilés and Gijón), the spawning area was not encompassed with negative egg hauls. The eastern Cantabrian seems to show an intermediate situation, with a higher predominance of a more littoral type of distribution of pilchard eggs.

In general, the distribution of adults — whether measured by sonar mapping or by the size of sardine catches — coincides with that of the eggs, except in offshore areas of the western Cantabrian.

Significant differences in the adult biological parameter estimates and differences in egg distribution and the preestablished acoustical divisions led us to post-stratify the survey area into three regions (I, II and III), and the DEPM spawning biomass estimate was obtained for each region. Region I corresponds to the Galician area, extending from the south (41° 55′ N) to the north, including a section of the western Cantabrian (transect lines 179-185).

Region II covers the western Cantabrian; egg presence dominates most of this area. This region includes from transect lines 186-192 to transect lines 351-355.

Region III, the eastern Cantabrian, was the smallest area.

DEPM Parameters Estimate

Daily Egg Production, P_0

The parameter P_0A, the daily production of eggs in the sea, is the total area multiplied by the number of eggs spawned per day per unit area, averaged over the range and duration of the survey, or in this case, within each of the regions considered.

Positive plankton tows provided the sardine egg data and eggs were staged and aged. Egg production, P_0, is estimated by fitting an exponential mortality function to the data of eggs at age. Time-zero intercept of the fitted function is the estimate of egg production at spawning.

Following the sampling scheme used by Picquelle and Hewitt (1983) for northern anchovy, the total area was first determined by the sampled surfaces in terms of 6×6 n mi grid. A 0.05 m^2 sampling unit represents the center of this block. This sampling design assumes that the distribution of eggs within one block is independent of the distribution within the adjacent blocks. In the areas of expected spawning intensity, sampling intensity increased to a 6×3 n mi grid. In order to compensate for this uneven sampling intensity, each station was assigned a weighing factor, proportional to the area which the station represents.

Secondly, the stations were stratified by location in order to decrease variance. Many stations were located beyond the spawning range of pilchard. These contribute a large number of stations with 0 egg counts. To reduce their impact on variance, the total survey area was poststratified into two strata: stratum 0 contains the geographical area where no spawning occurs (thus, daily egg production = 0) and stratum 1 includes the stations where positive egg counts occurred, along with the few negative stations imbedded in this area. These two strata were created for each region (fig. 3).

Their respective areas (km^2) and the number of stations (n) comprising each stratum by region were as follows:

Stratum	Galicia I	W Cant. II	E Cant. III	Total areas
1 Area (km^2)	6 915	13 829	5 680	26 424
n	56	186	41	283
0 1 Area (km^2)	15 188	6 668	3 457	25 313
n	123	81	37	241
Total Area	22 102	20 497	9 137	51 736
n	179	267	78	524
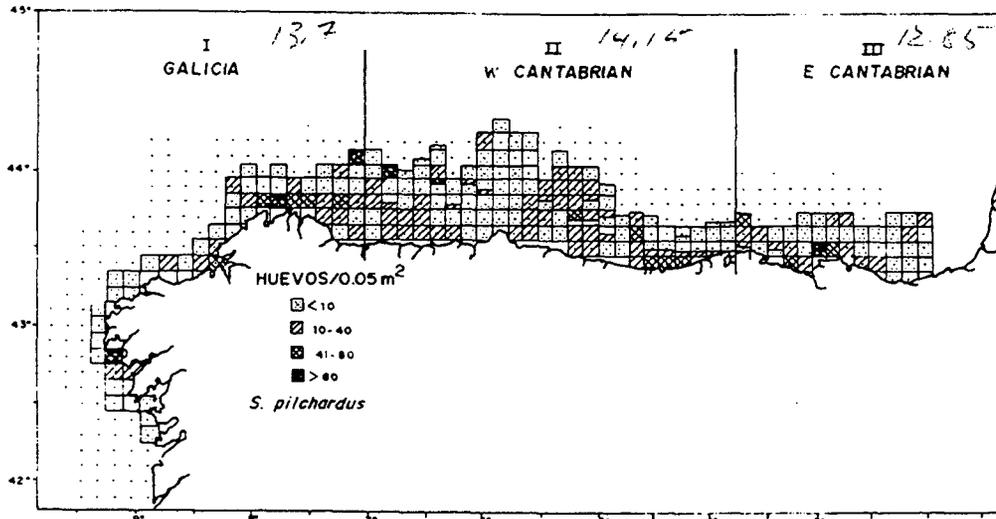
Fig. 3.—Sardine egg abundances (eggs/0.05 m²) by regions. Shaded blocks represent the positive stratum.

Fig. 3.—Abundancias de huevos de sardina (huevos/m²) por regiones. Bloques sombreados corresponden al estrato positivo.

Sardine eggs from each sample were counted and staged according to their degree of embryonic development. These were classified into the 11 stages established by Gamulin and Hure (1955), complemented with criteria described by Moser and Ahlstrom (1985) and Ahlstrom (1943). These stages of eggs were then aged.

The stage-to-age procedure was based on the results of a laboratory experiment, in which the induction-spawned eggs were incubated at five temperatures (11° C, 13° C, 15° C, 18° C and 20° C) and the elapsed times were recorded (Miranda, Cal and Iglesias, 1989).

Analysis of the empirical data on the rate of development indicated that the best fit of development curves was the combination of an exponential and power function, described in Lo (1985).

The resulting equation was:

$$Y_{i,t} = 17.515 \ e^{-.13621(t) - .1734(i)} \ i^{2.222}$$

where,

$y_{i,t}$ corresponds to the average age of the egg at stage i and temperature t. It showed a very good fit; r^2 was 0.986.

Thus, for each of the 11 stages, the average age of sardine eggs can be estimated

from the above equation for a given temperature.

The program STAGEAGE described in Hewitt, Bindman and Lo (1984) and in Lo (1985) was modified according to 1) a model for a temperature-dependent egg development, 2) the distribution of age within each stage, and 3) a daily peak spawning time (19:00 GMT), in order to acquire an automated ageing procedure.

The STAGEAGE, a FORTRAN program, was compiled to run on a compatible PC. It was modified and named SSTAGEAG, with reference tables that allow the assignment of ages to the egg data set according to the station temperature and the time of tow. These reference tables were created assuming peak spawning time at 19:00 GMT, and thus measure the time elapsed between spawning and catch.

STAGEAGE outputs several data files (Lo, 1985), among which FOR038.DAT gives the results of two variables, number of eggs and age in day categories, A, B, and C-day eggs. Thus, the data is tabulated by age for each station, with each one accounting for up to three observations (one for each day category). This file was used directly for the regression estimates of egg production and egg mortality rates.

The exponential mortality model

$$P_t = P_0 e^{-zt}$$

was then fit to the data using a weighted nonlinear least squares regression (Dixon and Brown, 1979), where,

P_t = number of eggs per 0.05 m^2 in age category t

t = age in days measured as the elapsed time from the spawning to the time of sampling

P_0 = daily egg production per sampling unit (0.05 m^2)

z = daily rate of instantaneous mortality

This model was fit to the data from stratum 1 for each of the three regions. In consequence, each region has an estimate of P_{01} (intercept) and a corresponding egg mortality, z (slope) (fig. 4).

The final stratified estimate of P_0 by regions was calculated as the weighted average of the two strata, where the strata weights u_i are proportional to A_i, the area of the ith stratum and P_{00} is zero by definition,

and the weights are the relative areas of the two strata, that is,

$$u_i = \frac{A_i}{A_1 + A_0}$$

$$P_0 = u_1 P_{01} + u_0 P_{00} = u_1 P_{01}$$

and the variance, adjusted for the postsurvey stratification (Jessen, 1978), is:

$$Var(P_0) = \left(1 + \frac{1}{n}\right) \left[\left(\frac{A_1}{A}\right) Var(P_{01}) + \right.$$

$$\left. + \left(\frac{A_0}{A}\right) Var(P_{00}) \right]$$

where, A_i is the area of stratum i for each region, n is the total number of observations by region, $Var(P_{01})$ is the estimated variance of stratum 1 for each region as calculated from the regression, $Var(P_{00})$ is zero by definition.

Figure 5 represents the fitted curve of egg
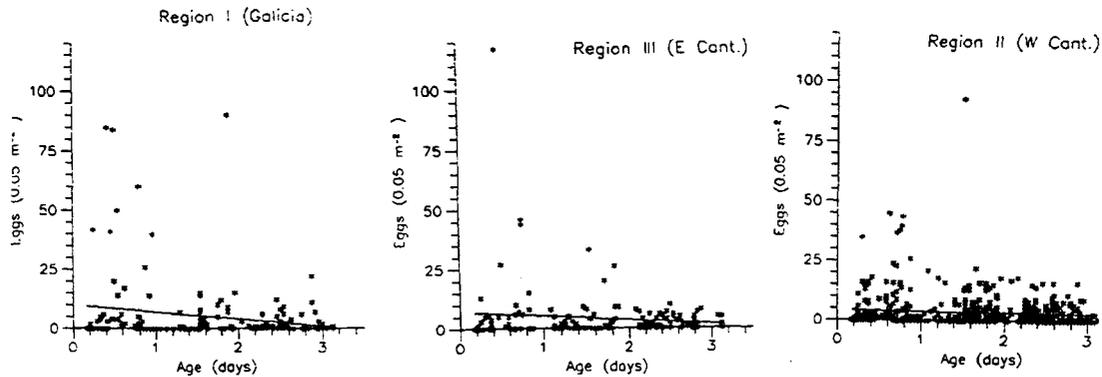
Fig. 4.—Exponential mortality model for estimating P_{01} plotted with egg abundances for each region, where P_{01} is the daily egg production at Day-0 in stratum 1.

Fig. 4.—Curvas de ajuste a función exponencial para la estimación de P_{01} con la abundancia de huevos por región, en la que P_{01} representa la producción diaria de huevos en el día 0 en estrato 1.

Sardine egg mortality curve
Galicia (Region I)

W Cantabrian(Region II)

E Cantabrian (Region III)
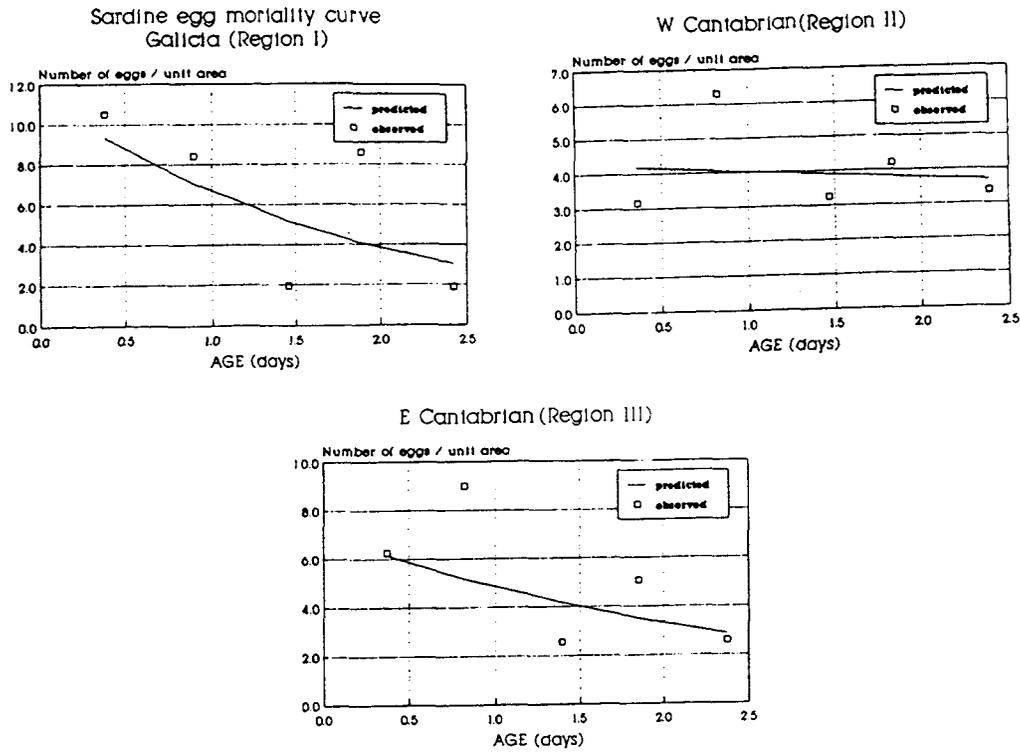
Fig. 5.—Egg mortality curves for each of the regions plotted as mean abundance by half-day intervals.

Fig. 5.—Curvas de mortalidad de huevos por región representados por la abundancia media en intervalos de medio día.

production rates for each of the regions computed in mean half-day frequencies of age.

In summary, the results related with the estimates of P_{01} and P_0, for the three regions were:

Region I had the highest egg densities, P_{01}, due to the high egg counts that occur on the northern coast of Galicia and the westernmost coast of the Cantabrian region.

On the other hand, in region I, P_0 drops to values similar to those produced in re-

Stratum	Galicia I	W Cant. II	E Cant. III
P_{01} (eggs/0.05 m^2) strat-I Standard error	11.52 3.78	4.29 0.86	7.08 3.02
z (egg mortality) Standard error	0.55 0.33	0.062 0.14	0.38 0.38
u_0 u_1	0.69 0.31	0.33 0.67	0.40 0.60
Mean Temperature (°C)	13.7	14.15	12.85
P_0 (eggs/0.05 m^2) Standard error	3.5712 2.1046	2.8743 0.7039	4.248 2.3392

gions II and III, because in this region, 31 % of the weighted areas account for the positive stratum, whereas in regions II and III, this relationship is over 60 %. Intense spawning in the Galician area was confined to a rather small area in relation to the total surveyed area. These concentrated high egg densities resulted in the high standard error of the estimate.

In constrast, in the western Cantabrian (II), spawning showed a widespread distribution and the resulting egg counts data set was much more homogeneous.

Adult Parameters

The adult parameters, *W*, *F*, *S* and *R*, were estimated through the epipelagic trawl samples and the biological sampling of sardines done during the cruise.

Three main problems have to be noted in the adult sampling survey: 1) difficult fishing operations in rocky areas of the northern Galician shelf, 2) inaccessibility to areas where fishing gear of fixed nature was installed (western and eastern Cantabrian) and, 3) hydrated females had to be fished during daylight hours, moments at which sardine are most likely to avoid the net.

In all regions, sardine are restricted to coastal areas, except where high egg abundances occurred in offshore areas, such as the western Cantabrian; but, generally the geographic distribution of adult fish schools observed by the sonar mappings or the adult sardine catches agreed with the distribution of eggs.

For each of the parameters, mean and variance were estimated following Picquelle and Stauffer's (1985) procedure, calculating weighted averages (since the number of sampled individuals were not equal in each of the tows), where,

$$\bar{\bar{y}} = \frac{\sum_{i}^{n} m_i \bar{y}_i}{\sum_{i}^{n} m_i} \qquad (1)$$

$$\widehat{Var}(\bar{\bar{y}}) = \frac{\sum_{i}^{n} m_i^2 (\bar{y}_i - \bar{\bar{y}})^2}{\left[\sum_{i}^{n} m_i / n\right]^2 \cdot n / (n - 1)} \qquad (2)$$

$\bar{\bar{y}}$ = the estimate of the population mean

n = number of collections

$\bar{y}_i$ = $\sum y_{ij} / m_i$ observed mean value in collection i

y_{ij} = observed value for the jth fish sampled and i^{th} trawl

m_i = the number of fish subsampled from the ith catch

Average Female Weight, W

This parameter was calculated as the mean weight of mature females per trawl, using a maximum number of females as subsample target, which was 25 mature females per trawl and a total of 563 females. However, this number was not always reached, because either few fish were caught or most of the catch were immature females.

In the estimation of this parameter y_{ij} of equation (1) becomes W_{ij}, that is, the whole body weight of the jth mature female from the ith trawl. This observed weight has to be adjusted for those females which were in hydrated condition, due to the water retention during hydration. This adjusted W_{ij} was estimated through a linear regression of whole body weight to ovary-free weight for females which had no hydrated oocytes.

The resulting linear regression is:

$$W = -2.051 + 1.079 \ W^*$$

where,

W is the whole body weight of mature females and,

W* is the ovary-free weight of females without hydrated eggs.

The following data on this parameter represents the average weight of mature females by regions with their corresponding coefficients of variation, showing a clear increase of this parameter in an eastward direction, in accordance with the differential distribution of the adults mentioned by Porteiro *et al.* (1986).

Av. Female Weight	Galicia I	W Cant. II	E Cant. III
W (g)	64.93	79.34	86.31
CV	0.06	0.08	0.03

Batch Fecundity, F

Hydrated females were collected throughout the survey, and the number of eggs per batch (F) was the basic data set for this parameter. Batch fecundity was estimated by regressing batch fecundity (F) on ovary-free weight of those females which had hydrated oocytes (W^*) and without postovulatory follicles, as shown through histological analysis, indicating that spawning had not begun.

A total of 126 females with hydrated oocytes, obtained from the DEPM survey carried out by Spain and Portugal, were analyzed in order to have a higher number of ovaries for regression analysis, because there were no significant differences in the number of oocytes per gram of fish between specimens from Spain and Portugal (Pérez *et al.*, 1989).

A weighted linear regression was used to estimate batch fecundity of all the mature females up to maximum number of 25 per catch.

The resulting linear regression was:

$$F = -1260.8 + 444.43\ W^*$$

The average batch fecundity was calculated from equation (1), but in this case, variance is estimated as described in Draper and Smith (1966), where,

$$Var(\bar{\bar{F}}) = \frac{\sum_i m_i^2 \cdot \dfrac{(\bar{F}_i - \bar{\bar{F}})^2}{n-1} + \dfrac{S_h^2}{n_h} + (\bar{W}_i - \bar{W}_h)\ \hat{Var}\ (\beta)}{\left[\sum_i \dfrac{m_i}{n}\right]^2 n}$$

where,

$\bar{\bar{F}}$ = the estimate of batch fecundity for the whole population of mature females

$\bar{F}_i$ = the average batch fecundity

S_h^2 = the variance in regression

n_h = the number of hydrated females used in the regression

$\bar{W}_i$ = average ovary-free weight for the ith trawl

$\bar{W}_h$ = average ovary-free weight for the n_h hydrated females

$\hat{Var}(\beta)$ = the variance of the slope of the regression.

The results of the analysis of this parameter were:

Batch fecundity	*Galicia* I	*W Cant.* II	*E Cant.* III
F	27274.80	33801.80	33910.70
CV	0.06	0.09	0.03

Because batch fecundity is high for larger females, it is expected to observe higher batch fecundity in eastern regions: regions II and III.

Spawning fraction, S

This parameter represents the fraction of mature females that have spawned per day.

Since no validated system existed to classify sardines postovulatory follicles by age, these were assigned to follicles taking into account their histological characteristics, the sample collected time, and maximun intensity of spawning hours. This time was delimited at 19:00 GMT, by the frequency of females with hydrated and postovulatory Day-0 follicles, at different times in the sample caught.

The analysis of the histological age structure of postovulatory follicles of female field samples had revealed the possibility of determining four different ages. An artificial spawning technique for aging sardines' postovulatory follicles (Pérez *et al.*, 1989) was used to confirm the histological criteria applied to the postovulatory follicles of the sea-caught females sampled during the DEPM survey held in 1988. With this experiment, we know that the degeneration of follicles in pilchard has been shown to be very slow; and therefore, follicles are clearly differentiated with ages reaching 80 hours. Females with follicles from 0:00 to 6:00 hours have been classified as Day-0, from 18:00 to 30:00 hours as Day-1, from 42:00 to 54:00 as Day-2 and finally as Day-3, those follicles with ages over 66:00 hours.

The term y_{ij} of equation (1) here is equal to the average proportion of mature females

in the ith trawl which have Day-1 and Day-2 postovulatory follicles. Day-2 postovulatory follicles have been included in the estimate, since the degeneration of follicles in pilchard is very slow.

There was an oversampling of follicles under 18 hours (Day-0), thereby causing a bias in the calculation of the spawning fraction. In this manner, the total number of mature females has been calculated following the expression,

$$m_i = \frac{Day\text{-}1 + Day\text{-}2 + Day\text{-}3}{3} +$$

$$+ Day\text{-}1 + Day\text{-}2 + Day\text{-}3 + Mature\ females$$

in which each term represents the number of females in a particular age of postovulatory follicles.

The following table shows the spawning fraction values by the different postovulatory follicle ages by the different regions and their respective coefficients of variation.

The coefficient of variation in relation to the spawning fraction increases as we use the Day-1 or Day-2 postovulatory follicles, instead of their mean value.

Follicle age	*Galicia* I	*W Cant.* II	*E Cant.* III
Day-0 CV	0.64 0.58	0.15 0.68	0.10 0.47
Day-1 CV	0.04 0.55	0.09 0.24	0.23 0.20
Day-2 CV	0.11 0.24	0.17 0.22	0.19 0.20
Day-3 CV	0.05 0.60	0.23 0.14	0.13 0.23
Day-1 + Day-2 CV	0.08 0.20	0.13 0.11	0.21 0.13

The values of spawning fraction by regions also show an increasing trend towards the east, indicating a higher spawning frequency in the older females.

Spawning fract.	*Galicia* I	*W Cant.* II	*E Cant.* III
S CV	0.8 0.20	0.13 0.11	0.21 0.13

Sex ratio, R

This parameter was calculated as the fraction in weight of mature females of the population. The equation used is also (1), but in this case, m_i is the weight of the subsample instead of the number of fish and y_i is the fraction of the weight of the subsample that corresponds to female fish.

Mature and immature male specimens were included in the analysis. The maximum number of specimens per trawl was 50, from which only the weights of the first 10 males and 25 females were measured.

The total weight for each sex was obtained from Picquelle and Stauffer (1985), where,

$$m_i = W_i^F + W_i^M$$

m_i is the weight of the subsample,
W_i^F is the total estimated female weight and,
W_i^M is the total estimated male weight, and, y_i is the estimated total weight divided by m_i,

$$y_i = \frac{W_i}{m_i}$$

The results of the estimates of this parameter were:

Sex ratio	*Galicia* I	*W Cant.* II	*E Cant.* III
R CV	0.35 0.12	0.65 0.10	0.66 0.08

Region I registered low sex ratio values whereas regions II and III recorded estimates of more than 50%. If sex ratio were calculated for the entire survey area, it would be 55%.

Biomass Estimate

All the required parameters for estimating the spawning biomass are summarized in the following table. The biomass estimates

The sample covariances were calculated for the adult parameters, since P_0 was derived from the plankton survey, whereas the adult parameters were derived from the trawl survey. Thus the sample covariance

DEPM Parameters	Galicia /ᵃ 7 I	W Cant. II	E Cant. III
P_0	3.57	2.87	4.25
CV	0.59	0.25	0.55
A (in 0.05 m²)	4.420×10^{11}	4.099×10^{11}	1.827×10^{11}
W (g)	64.93	79.34	86.31
CV	0.06	0.08	0.03
F	27274.8	33801.8	33910.7
CV	0.06	0.09	0.03
S	0.08	0,13	0.21
CV	0.20	0.11	0.13
R	0.35	0.65	0.66
CV	0.12	0.10	0.08
Biomass (B)	134 195	33 503	12 467
CV	0.66	0.30	0.56
TOTAL BIOMASS =	180 165		
CV =	0.50		

of each region were calculated by the egg production estimation equation, and the variances were calculated using the delta method (Seber, 1973).

between P_0 and the adult parameters was assumed to be 0.

The sample covariances calculated for the adult parameters were:

Sample covariance	Galicia I	W Cant. II	E Cant. III
Cov (W, R)	0.01227	0.142	0.0719
Cov (W, F)	5833.1	17783.2	2047.1
Cov (W, S)	0.0156	0.0276	− 0.0408
Cov (R, F)	5.484	63.162	30.076
Cov (R, S)	0.00029	0.00024	− 0.000469
Cov (S, F)	7.0029	12.31	− 17.818

DISCUSSION AND CONCLUSIONS

The DEPM assumes that the parameters in the model are constant over the range and duration of the survey. Since this assumption does not occur in this paper, the spawning biomass of the Galician and Cantabrian pilchard has been evaluated by regions.

One of the clearest differentiations was observed in the mean weight of mature females. An increment towards the east of this parameter is. very clear; and it has been demonstrated by Porteiro, Álvarez and Pereiro (1986) that the oldest age classes of pilchard (over 5 years old) mainly reside in the eastern sector of the Cantabrian coast. Different age composition of adult fish affects other parameters, such as batch fecundity, which in the distributional areas of the young age-classes (Galicia) is quite lower.

In the case of sex ratio, the differences between regions are quite pronounced. In Galicia (I), this value is 0.35, in contrast to the two other established regions, where

there is a predominance of females in the population. Different fishing times by regions may ultimately have altered the number of females in the different areas. However, no clear relationship exists between the fraction of reproductive females and trawl time for any of the three regions (fig. 6). The explanation of this phenomenom would be that in part of region I (northern Galician shelf), the difficult fishing operations in rocky areas were bigger than in other regions, and the sardine outside these areas were more vulnerable to the fishing gear. Most of these sardine were spawning, and the proportion of sex at that moment was less, because many males and few females are more effective for reproduction.

Comparing the biomass estimate by the DEPM acoustic evaluation, there is a remarkably slight difference in the total biomass of approximately 6000 t. Porteiro, Miquel and Carrera (1989) had made acoustic estimates of 102394 t for region I, 58010 t for region II and 13612 t for region III.

The general trend among regions be-
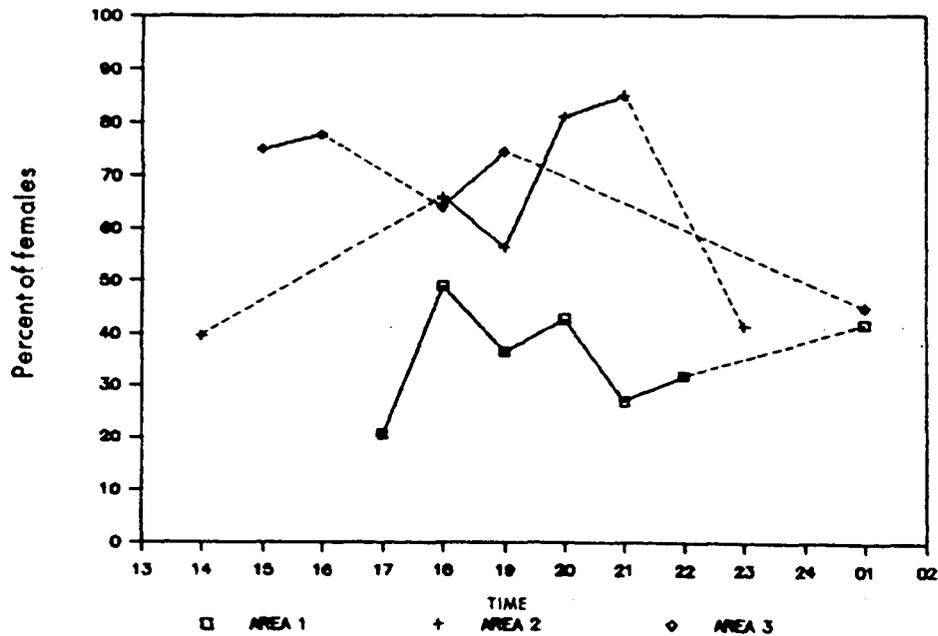
Fig. 6.—Proportion of females by times and regions.

Fig. 6.—Proporción de hembras de sardina por horas y regiones.

tween DEPM and acoustics is similar, showing an increment of biomass from east to west. The adult parameters estimate shows concordance between both methods. For example, acoustic sampling revealed that region I had the principal fraction of age classes 0 and 1, while the DEPM estimates indicated that in this region, batch fecundity, average female weight and spawning fraction were lowest. In the eastern sectors, a greater representation of the older age classes from the acoustics survey was reflected by the estimate of the adult parameters.

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REFERENCES

Ahlstrom, E. H. 1943. Studies on the Pacific pilchard or sardine, *Sardinops caerulae*. 4. Influence of temperature on the rate of development of pilchard eggs in nature. *US Fish Wildl Serv Spec Sci Rep:* 23-26.

Alheit, J. 1985. Egg Production Method for spawning biomass estimates of anchovies and sardines. *ICES C. M.* 1985/H:41.

Armstrong, M. 1988. Egg production estimates of anchovy biomass in the Southern Benguela System. *CalCOFI Rep* Vol. XXIX: 137-157.

Bindman, A. G. 1986. The 1985 spawning biomass of the Northern Anchovy. *CalCOFI Rep* Vol. XXVII: 16-24.

Cunha, E., I. Figueiredo, A., Farinha and M. Santos. 1989. Estimation of sardine spawning biomass off Portugal by Egg Production Method. In: *C.M. SARP Seminar on research conducted by the SARP projects off Spain and Portugal, 1987-1989* (November 1-3, 1989. La Jolla, California, USA). *NOAA Admin Rep.*

Dicenta, A. 1984. Aportación al conocimiento del ictioplancton de la costa vasca. *Bol Inst Esp Oceanogr* 1(2): 94-105.

Dixon, W. J. and M. B. Brown, eds. 1979. *Biomedical*

Computer Programs. (P-series). University of California Press. Berkeley.

Draper, N. R. and H. Smith. 1966. *Applied Regression Analysis.* John Wiley and Sons. New York: 407 pp.

Gamulin, T. and T. Hure. 1955. Contribution a la connaissance de l'ecologie de la ponte de la sardine, *Sardina pilchardus* (Walb.), dans l'Adriatique. *Acta Adriat* 7(8): 1-22.

García, A., C. Franco and A. Solá. 1992. Sardine, *Sardina pilchardus* (Walb.), egg and larval distribution off the Spanish North Atlantic coast (Galician and Cantabrian areas) in April 1987. (In this volume.)

García, A., C. Franco, A. Solá, M. Alonso and J. M. Rodríguez. 1988. Distribution of sardine, *Sardina pilchardus* (Walb.), egg and larval abundances off the Spanish North Atlantic Coasts (Galician and Cantabrian Areas) in April, 1987. *ICES C.M.* 1988/H:21.

Hewitt, R. P., A. Bindman and N. Lo. 1984. Procedures for calculating the egg production estimate of spawning biomass. *Natl Mar Fish Serv, NOAA Southwest Fish Cent, La Jolla, CA, Admin Rep LJ-84-19:* 46 pp.

Jessen, R. J. 1978. *Statistical Survey Techniques.* John Wiley and Sons. New York: 520 pp.

Lago de Lanzós, A., C. Franco and A. Solá. 1988. Comparative study of the annual variation in the spawning intensity of sardine, *Sardina pilchardus* (Walb.), of the Cantabrian and NW Spain. *ICES C.M.* 1988/H:73.

Lasker, R. (ed.). 1985. *An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy,* Engraulis mordax *NOAA Tech Rep NMFS 36:* 99 pp.

Lo, N. C. H. 1985. A model for temperature-dependent northern anchovy egg development and an automated procedure for the assignment of age to staged eggs. In: Lasker, 1985: 43-50.

Miranda, A., R. Cal and J. Iglesias. 1989. Effect o temperature on the development of eggs-and larvae of sardine, *Sardina pilchardus* (Walb.), in captivity. In: *C.M. SARP Seminar on research conducted by the SARP projects off Spain and Portugal, 1987 1989* (November 1-3, 1989. La Jolla, California USA). *NOAA Admin Rep.*

Moser, H. G. and E. H. Ahlstrom. 1985. Staging Anchovy Eggs. In: Lasker, 1985: 37-41.

Parker, K. 1980. A direct method for estimating northern anchovy, *Engraulis mordax,* spawning biomass. *Fish Bull* US 78: 541-544.

Pérez, N., I. Figueiredo and N. C. H. Lo. 1989. Batc fecundity of *Sardina pilchardus* in the Iberian Peninsula. In: *C.M. SARP Seminar on research conducted by the SARP projects off Spain and Portuga 1987-1989* (November 1-3, 1989. La Jolla, California, USA). *NOAA Admin Rep.*

Pérez, N., I. Figueiredo and B. Macewicz. 1992. Th spawning frequency of pilchard, *Sardina pilchardus* (Walb.), on the Atlantic Iberian coast. (In th volume.)

Pérez, N., C. Porteiro and F. Álvarez. 1985. Contr

bución al conocimiento de la biología de la sardina de Galicia. *Bol Inst Esp Oceanogr* 2(3): 27-37.

Picquelle, S. J. and R. P. Hewitt. 1983. The northern anchovy spawning biomass for the 1982-1983 California fishing season. *CalCOFI Rep* Vol. XXIV:16-28.

Picquelle, S. J. and R. P. Hewitt. 1984. The 1983 spawning biomass of the northern anchovy. *Cal-COFI Rep* Vol. XXV:16-27.

Picquelle, S. J. and G. Stauffer. 1985. Parameter Estimation for an egg production method of anchovy biomass assessment. In: Lasker, 1985: 7-16.

Porteiro, C., F. Álvarez and J. A. Pereiro. 1986. Sardine, *Sardina pilchardus* (Walb.), stock differential distribution by age class in Division VIIIc and IXa. *ICES C.M.* 1986/H:28.

Porteiro, C., J. Miquel and P. Carrera. 1989. Acoustic stock estimation of sardine, *Sardina pilchardus* (Walb.), in the Galician and Cantabrian waters - March 1988. In: *C.M. SARP Seminar on research conducted by the SARP projects off Spain and Portugal, 1987-1989* (November 1-3, 1989. La Jolla, California, USA). *NOAA Tech Rep.*

Santander, H., J. Alheit and P. E. Smith. 1984. Estimación de la biomasa de la población desovante de la anchoveta peruana, *Engraulis ringens*, en 1981 por aplicación del Método de Producción de Huevos. *Inst Mar del Perú*, 8 (n.º 6).

Santiago, J. and A. Sanz. 1989. Egg production of the Bay of Biscay anchovy in 1987 and 1988, and spawning stock size estimates. In: *Preliminary Rep., Work. Doc. on the Assessment of Pelagic Stocks in Div. VIIIc and IXa and Horse Mackerel* (May 1989. Copenhagen).

Seber, G. A. F. 1973. *The Estimation of Animal Abundance and Related Parameters.* Hafner Press. New York: 506 pp.

Solá, A. 1987. Yearly variation of the ichthyoplankton of commercial interest species in the Santander coast (north of Spain). *ICES C.M.* 1987/L:32.

Smith, P. E., W. Flerx and R. P. Hewitt. 1985. The CalCOFI Vertical Egg Tow (CalVET) Net. In: Lasker, 1985: 27-32.

Smith, P. E. and R. P. Hewitt. 1985. Sea survey design and analysis for an Egg Production Method of anchovy biomass assessment. In: Lasker, 1985: 17-26.

Stauffer, G. D. and S. J. Picquelle. 1980. Estimates of the 1980 spawning biomass of northern anchovy. *Natl Mar Fish Serv, Southwest Fish Cent, La Jolla, CA Admin Rep LJ-80-09:* 41 pp.