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REPORT OF THE

WORKSHOP ON THE ESTIMATION OF SPAWNING STOCK BIOMASS OF SARDINE

Vigo, Spain
13–16 June 2000

International Council for the Exploration of the Sea

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1 INTRODUCTION

1.1 Terms of reference

At the ICES Annual Science Conference in Stockholm, Sweden, in September/October 1999 (87th Statutory Meeting) it was decided that (C.Res. 1999/2G07) a Workshop on Estimation of Spawning Stock Biomass of Sardine (WKSBS) (Chair A. Lago de Lanzós) would meet in Vigo, Spain from 13 – 16 June 2000 to:

1. Present and evaluate egg production estimates made by traditional and GAM methods using data from the 1999 egg surveys in Portugal (Area IXa) and Spain (Areas VIIIc and IXa);

2. Present and evaluate the batch fecundity and spawning fraction estimates from the same surveys;

3. Discuss spawning stock biomass estimates for sardine made by the DEPM method;

4. Coordinate planning for sardine surveys in 2001.

1.2 Participants

The Workshop met in Vigo (Spain) from 13-16 June 2000 with the following participation:

Bernal, Miguel	Spain
Cunha, Mª Emilia	Portugal
Franco, Concha	Spain
Lago de Lanzós, Ana (Chair)	Spain
Lopes, Plácida	Portugal
Pérez, José Ramón	Spain
Porteiro, Carmela	Spain
Silva, Alexandra	Portugal
Soares, Eduardo	Portugal
Stratoudakis, Yorgos	Portugal

2 DESCRIPTION OF 1999 SURVEYS

2.1 Ichthyoplankton survey

2.1.1 Portuguese survey

The 1999 egg production survey off the Portuguese coast and Gulf of Cadiz took place on board IPIMAR R/V "Noruega" from January 10th to February the 3rd.

A total of 417 egg samples spaced 6×6 nm apart (Figure 2.1.1.1) were obtained along 85 sections using a 150 µm mesh CalVET double net (Smith, Flerx and Hewitt 1985) towed vertically from 150m or close to the bottom. A flowmeter was used to detect clogging and nets were substituted whenever filtering inefficiency was noticed. Water column temperature was determined at every station using a Temperature/Depth Recorder coupled to the plankton net. Along selected transects a CTD was also used to obtain vertical profiles of temperature and salinity.

After towing samples from each net of the CalVET were immediately inspected for presence of sardine eggs and their number were recorded separately. Following three consecutive offshore stations without sardine eggs sampling was discontinued along the section and resumed at the most offshore station at the following section. Samples were preserved in 4% formaline and sorted at the laboratory.

2.1.2 Spanish survey

From 16th March to 11th April 1999 a Daily Egg Production Method survey was carried out off the north Atlantic Spanish coast with the purpose to evaluate the Spawning Stock Biomass (SSB) of sardine.

A total of 485 plankton stations were carried out. The stations were located along transects perpendicular to the coast, 15 miles apart in the West of Iberian Peninsula and 7,5 miles in the Cantabrian Sea with 3 miles distance between stations.

As in previous years, three different regions were defined; the Galician Region (Region I), the middle Cantabric Region (Region II) and the inner Bay of Biscay Region (Region III) (Figure 2.1.2.1). As seawater temperature and egg abundance in Region II were very low in comparison to the previous years it was decided to sample again region II. This was done at the end of the cruise. (Figure 2.1.2.2).

Vertical plankton hauls were obtained using a CalVET fitted with 150 µm mesh size net. Retrieving was 1m/second, the maximum depth was 100 m or 5 m above the bottom in shallower areas. A temperature and depth sensor fitted to the net cable was used to control depth of sampling and determine water column temperature.

Flowmeters were used to record the real distance towed during each haul. The volume filtered was calculated assuming a filtration efficiency of 100%.

The sample was fixed and sorted on board, and a preliminary analysis was conducted in order to obtain information on the egg presence. Following two consecutive offshore stations without sardine eggs, sampling was discontinued along the section and resumed at the nearest offshore station in the next section.

A CTD Seabird was use to obtain information on the hydrology of the area along sections spaced 30, 15 or 7,5 miles apart located and at 50, 100, 200, 500 and 1 000 m soundings in 131 stations.

2.2 Adult sampling

The planning and the methodology of the 1999 surveys for the sampling of sardine adults was based on the experience obtained in previous DEPM surveys undertaken by both countries in 1988 and 1997 (Cunha *et al.* 1992, Garcia *et al.* 1992, Cunha *et al* 1997, Lago de Lanzós *et al.*, 1998).

2.2.1 Portuguese sampling

In January-February 1999, samples of adult sardines were collected onboard R/V "Noruega" (Gulf of Cadiz Spanish IXa-South) and R/V "M. Costeiro" and from purse seiners and trawlers of the commercial fleet (Portuguese coast, ICES Sub-Area IXa Central-North and Central-South) (Table 2.2.1.1). The catch from each sampling station was inspected for the presence of hydrated females: whenever they were present up to 20 females was collected.

Fish samples from commercial vessels were generally transferred onboard the research vessel or on land very soon after the end of the fishing operation (generally less than 1 hour). Around 100 sardines were randomly subsampled from the catch of each haul and the gonads of females were immediately removed and stored in AFA solution. Gonad-free females and entire males were frozen and transported to the laboratory for further analysis.

Overall, 3940 fish were collected. In the laboratory at IPIMAR, Lisbon, all fish were defrosted and the following biological properties were obtained: sex, total length, total weight (only for males), gonad-free weight, gutted weight, gonad weight and macroscopic state of maturity (for males) and fat content level. All fish weights and male gonad weights were multiplied by 1.044 to obtain wet weights, since freezing and subsequent defrosting is known to affect weight measurements (Pérez *et al.*, 1992a). Macroscopic state of maturity and gonad weight for females were obtained from the gonads preserved in AFA (Bodian, 1937 *in* Lillie and Fullmer, 1976). For that, female gonad weights were multiplied by 0.852 to account for the weight gain due to the immersion in AFA (Pérez *et al.* 1992).

Random subsamples of 15 to 25 females per haul were selected for histological analysis. Among those fish, females macroscopically identified to be ready to spawn (maturity stage 4) were used to evaluate batch fecundity. To increase the number of fish used to obtain the batch fecundity model, some females at stage 4 non-randomly collected during the survey were also used. In 5 hauls from commercial vessels the sample was frozen for several hours prior to the extraction of gonads, and these hauls were excluded from the histological analysis, since Post Ovulatory Follicles (POFs) are known to degrade quickly without preservation.

2.2.2 Spanish sampling

During the acoustic survey Pelacus 0399, sardine samples were collected using the pelagic trawl net onboard the research vessel "Thalassa" and a purse seine net in the commercial fishing vessel "Gloria Belén". This purse seine was used to sample in shallow waters in the area between Galicia and Cantabria (Table 2.2.2.1).

Sardine captured by purse seine were measured onboard the fishing vessel. The biologic adult fish samples were transferred onboard R.V. "Thalassa" where they were weighted, measured, sexually identified, macroscopically maturity staged and the ovaries of hydrated females collected and preserved in 4% buffered formalin (Hunter, J.R. 1985). Later on at the laboratory these ovaries were histologically prepared for analysis.

3 RESULTS

3.1 Daily Egg Production

The parameter P_0A, the daily production of eggs in the sea, is an estimator of the total number of eggs released per day in the spawning area, assuming it to be constant over the range and duration of the survey. The ichthyoplankton samples of the 1999 survey were used to obtain data on the density of eggs at age. The age of the eggs for each of the spawning areas was determined taking into account the embryonic developmental stage, the water temperature, and the time of day when the sample was collected. Egg ageing procedure for Portugal is described in Cunha *et al.* (1999) and for Spain in Bernal (1999) and Bernal *et al.* (1999). Four cohorts were used in the analysis of Spanish data, as temperatures found in the survey were lower than in previous years (maximum temperature of 13.2°C) and sardine eggs were not expected to start hatching until the fifth day in those temperatures (Miranda *et al*. 1990).

The daily production of eggs, P_0, and its variance were estimated by regressing the counts of eggs on their age, using the negative exponential mortality model:

$$P_{ijk} = P_{0i}\, e^{-(Zt_{ijk})} + E_{ijk}$$

where P_{ijk} is the density of eggs in the kth hour age category from the jth station in the ith stratum; t_{ijk} is the age in hours measured as the elapsed time from the time of spawning for the kth hour category eggs to the time of sampling of the jth station in the ith stratum; P_{0i} is the daily production of eggs in a unit area of 0.05m^2 in ith stratum; Z is the hourly rate of instantaneous egg mortality; and E_{ijk} is an additive error term.

In Portugal the best model to fit the abundance at age data was a negative linear regression and therefore this was used. In Spain the negative exponential mortality model was used as in previous years.

Since a portion of the surveyed area was beyond the edge of spawning, post-survey stratification was used to reduce the variance of P_0 due to the presence of a large number of zero stations. Stratum 1 was defined as the geographic area of spawning, and stratum 0 was that area devoid of eggs.

The stratified estimate, P_0, for each region and total was calculated as the weighted average of P_{01} and P_{00}, where P_{00} is zero by definition, and the weights are the relative areas of the strata. Thus:

$$P_0 = A_1/A \times P_{01} + A_0/A \times P_{00}$$

and the variance, adjusted for post-survey stratification (Jessen, 1978):

$$Var(P_0) = (1 + 1/N) \times [(A_1/A) \times Var(P_{01}) + (A_0/A) \times Var(P_{00})]$$

where N is the total number of observations for each region and for the total area; A_1 is the area of stratum 1 for each region and total; A_0 is the area of stratum 0 for each region and total; A is the area of the two strata for each region and total; $Var(P_{01})$ is estimated for each region and total from the regression and; $Var(P_{00})$ is zero by definition.

3.1.1 Portuguese egg production estimate

In the case of Portugal there seemed to be two spawning areas and therefore two regions were created: north and south of 37° 30' latitude north. The area in km^2 and number of samples, n, for each stratum within each region and the total are shown in table 3.1.1.1. Two mortality models were determined for each area. However only the southern region present a regression model significant at $P<0.05$. The regression model for the northern region was non-significant. Therefore it was decided to determine a joint model for the total region that was also significant at $P<0.05$. The resulting estimates of Z ($hour^{-1}$), P_{01} (day^{-1} / $0.05m^2$) and their standard errors are presented in Table 3.1.1.2.

The estimates of P_0 per day per $0.05m^2$, the total number of eggs produced per day in the area, A_iP_0, and the variances of P_0 for the two regions and for the total area are presented in Table 3.1.1.3.

3.1.2 Spanish egg production estimates

Egg production estimates are provided by areas and by the total of the Northern Spanish Atlantic coast. (Figure 2.1.2.1). Egg production for the total area is estimated by using data from all regions in the mortality model used for estimating the egg production parameter. Egg production from the samples taken in the second leg of the survey (see section 2.1.2, Figure 2.1.2.2) was not used in this analysis, as no adult samples were taken at the same time, and thus the DEPM cannot be used (see section 4.5 for a more detailed explanation).

Table 3.1.2.1 shows the total and regional egg production estimates, together with the area of egg presence and the mortality estimates and their respective coefficient of variation (cv). Positive samples in Region I and II were scarce and that caused the egg production cv to be very large in that region. The cv of the total egg production is also high (44%) although common in this kind of analysis. Although the results were presented by region, in order to compare with previous years, it was decided that they were no reasons to maintain the separation by region. Separating by regions increased the cv of the method, while the original advantage of separating regions with different adult parameters was not accomplished, because it was not possible to obtain some of the adult parameters in neither Region I nor Region III.

3.2 Adult parameters

The estimation of spawning biomass through DEPM requires estimates of four adult parameters, namely mean batch fecundity, female spawning fraction, female proportion in the population (usually referred as sex ratio) and mean female weight. In the previous sardine DEPM surveys, the following general equations have been used to estimate each of the four parameters

(Eq. 3.2.1)

$$\hat{y} = \frac{\sum_i m_i y_i}{\sum_i m_i},$$

and their variance

(Eq. 3.2.2)

$$\hat{\mathrm{var}}(\hat{y}) = \frac{\sum_i m_i^2 (y_i - \hat{y})^2}{\left[\left(\sum_i m_i / n\right)^2 n(n-1)\right]},$$

where y is any of the four adult parameters and

m_i is the number of fish sampled in the *i*th haul,

n is the number of hauls with sardine, and

y_i is the mean of the parameter for the *i*th haul.

It should be noted that in Eq. 3.2.2 it is assumed that the total number of possible fishing stations is considerably larger than the number of sampled stations. For that, the finite population correction is considered to approximate 1 and the variation within station is considered to approximate 0. Also, since we have tried to maintain a fixed number of subsampled fish per haul, the above estimators practically reduce to the estimators of mean and variance under simple random sampling.

To maintain comparability with the previous DEPM estimates, all adult parameters for the Portuguese survey were estimated for the entire survey area, without considering post-stratification. In the Spanish survey, like in 1997, there was inadequate adult information to consider separate estimation per region, thus adult parameters were estimated for the entire survey area. Finally, to ensure that estimates refer to the "adult" or "mature" component of the population, only fish with macroscopic stage of maturation 2 and above were considered.

3.2.1 Batch fecundity

To estimate mean batch fecundity it is first needed to estimate the relationship between batch size (number of oocytes released in a single spawning event) and fish size. To estimate this relationship, fish in spawning condition without evidence of post-ovulatory folicles (POFs) in their gonads were used. For 55 fish in Portugal and 21 in Spain, hydrated oocytes were counted using the gravimetric method (MacGregor, 1957). It should be noted that the low number of fish in Spain was due to the presence of recent POFs in most of the 137 fish sampled with the intention to estimate the batch fecundity relationship.

Individual batch size was regressed against gonad-free fish weight using least squares. Preliminary inspection of the Portuguese data showed the variance to be an increasing function of fish weight, so the inverse of gonad-free weights were used as statistical weights. Using weighted least squares, the following relationship was obtained for the Portuguese data:

$$F = -89.3 + 437.1 w_{gonfree}, \qquad \text{(Eq. 3.2.1.1)}$$

The residual standard error for the fitted model was 907.2 on 53 degrees of freedom and the standard error of the slope parameter was 54. It should be noted that the intercept estimate was highly non-significant (t = -0.039, p >0.05) and the above relationship could be simplified by forcing it through the origin (in which case the estimated slope would have been 435.1). For the Spanish data, the relationship between batch fecundity and gonad-free fish weight was:

$$F = 9046 + 243.3 w_{gonfree}, \qquad \text{(Eq. 3.2.1.2)}$$

The intercept estimate in this case was significant (t = 2.62, p < 0.05), although it should be noted that only 21 observations were used. Figure 3.2.1.1 and 3.2.1.2 show respectively the Portuguese and Spanish data used to estimate the fecundity models, with the fitted lines superposed.

The relationships of Eq. 3.2.1.1 and 3.2.1.2 were applied to the gonad-free wet weight of all female fish sampled in the Portuguese and Spanish survey respectively, and Eq. 3.2.1 was then used to obtain the average batch fecundity for each survey. Like in previous DEPMs, Eq. 3.2.2 was slightly modified for the variance estimation of batch fecundity in order to account for the additional variation about the regression model:

$$\hat{\text{var}}(\hat{F}) = \frac{\sum_i m_i^2 \left\{ \left[\frac{\left(F_i - \hat{F}\right)^2}{n-1} + \frac{s_h^2}{n_h} + \left(w_i - w_h\right)^2 Var(\hat{b}) \right] \right\}}{\left[\sum_i \left(m_i / n\right) \right]^2 n(n-1)}, \qquad \text{(Eq. 3.2.1.3)}$$

where

w_i is the mean female gonad-free weight in the *i*th haul,

w_h is the mean gonad-free weight of the fish used to estimate the fecundity relationship

n_h is the number of fish used to estimate the fecundity relationship

s_h is the standard error about the regression line and

Var(b) is the variance of the regression slope parameter

The average batch fecundity was estimated to be 18416 eggs per fish (CV = 5%) for the Portuguese survey and 21874 eggs per fish (CV = 12%) for the Spanish survey.

3.2.2 Spawning fraction

In the Portuguese survey, spawning fraction was estimated using the average proportion of female fish with day-1 and day-2 post-ovulatory folicles (POFs) in the sample of all mature female fish examined histologically per haul. The selection of these POF age groups was to be in accordance with the previously adopted estimation procedure in Portugal and Spain (Cunha *et al*. 1992; Lago de Lanzós 1998). The mean spawning fraction for the entire study area was 0.101 (CV = 15%) using 643 mature female fish from 35 hauls.

The estimation of spawning fraction was not possible for the Spanish survey because appropriate samples were not collected. For this reason it was decided to assume S=0.18 (Cv = 15%) as the spawning fraction value estimated for the DEPM for the same area in 1997 (Lago de Lanzós, *et al*. 1998).

3.2.3 Sex ratio

Similar to the previous sardine DEPMs, sex ratio was estimated using the female proportion per haul based on weight. To avoid the problems with temporary weight increases due to imminent spawning, gutted weights were used. The sex ratio for the Portuguese survey was 0.61 (CV = 5%) using 3294 mature fish from 40 hauls. The estimated sex ratio for the Spanish survey was 0.55 (CV = 45%) based on 374 mature fish from 6 hauls.

3.2.4 Mean female weight

Mean female weight was estimated using only non-hydrated fish. The estimated mean female weight for the Portuguese survey was 44.42 gr (CV = 4.8%) based on 1801 female fish from 40 hauls. The estimated mean weight for the Spanish survey was 66.03 gr (CV = 41%) based on 195 female fish from 6 hauls.

3.3 Spawning Stock Biomass (SSB)

The 1999 sardine SSB estimates for Portugal and Spain are presented in Table 3.3.1. Overall, the 1999 estimate is 215 Ktonnes, with 95% of the total coming from the Portuguese survey (Portuguese waters and the Gulf of Cadiz). Although the Spanish estimate is not reliable as an absolute value due to the problems in estimating spawning fraction, there is no doubt that the general pattern observed in the recent years (with considerably higher SSB in the southern area of the stock) is also verified during spring 1999. It should also be noted that the most recent DEPM estimate remains once more well below the acoustic estimate of sardine biomass.

3.4 Comparison with previous estimates

The summary of parameters for estimating the sardine spawning stock biomass by the DEPM obtained during the previous years in Portugal and Spain are presented in Tables 3.4.1 and 3.4.2. Table 3.4.3 summarizes the SSB values and present for the first time the values for the Iberian Peninsula. It should be noted that the 1997 DEPM estimate for Portugal is now revised to account for all adult parameter estimates of the 1997 survey (Gordo *et al*. 1998) that were not available during the preliminary estimation made by Cunha *et al*. (1997). Unfortunately, although the 1997 adult estimates have been available for some time, the preliminary 1997 estimate for Portugal has been used in the assessment working group to this date. It should also be highlighted that the 1988 estimate excludes the Gulf of Cadiz, while in 1997 and 1999 this area is included.

In comparison to the previous Portuguese DEPM surveys, the Portuguese survey in 1999 provides a much higher SSB estimate (almost double the previous estimate). All other things being equal, the 1999 Portuguese estimate was expected to be slightly higher than the previous due to the higher mean female weight in January than in March. Exploring monthly estimates of the female condition factor in the recent years clearly suggests that the fish condition deteriorates from October to March (main spawning season in Portuguese waters) and then gradually increases until it reaches a maximum in August/September (end of summer feeding). As a result, DEPM estimation in January should be higher than in March due to the higher sardine weight for a given length distribution (mean female weight in January 1999 was almost 10% higher than in the previous two surveys).

However, the much higher Portuguese SSB estimate in 1999 is mainly due to the strange combination of higher egg production and lower spawning fraction than in the previous two surveys. In principle, shifting the timing of the survey to get closer to the peak of spawning should have only influenced the precision of the SSB estimate and not its absolute value, since the anticipated increase in egg production should have been counterbalanced by a similar increase in spawning fraction and (possibly) batch fecundity. The Portuguese 1999 results verified that egg production and batch fecundity were higher in January, but indicated a much lower spawning fraction. However, the spatial distribution of the spawning fraction estimates (see section 4.6) indicates that the overall reduction is due to the very low estimates observed in the region of Algarve and the Gulf of Cadiz, while spawning fraction in northern Portugal was higher than in the previous years. Given that adult sampling was considerably intensified in the 1999 survey, the current estimate appears more reliable than the previous (in 1988 spawning fraction was estimated based on data from 9 hauls mainly from northern Portugal), but problems related to adult survey design and post-stratification (see section 4.6) still need to be addressed.

In the case of the Spanish DEPM survey, the situation is the opposite. SSB estimates in 1999 are the lowest of all the available estimates. However, SSB estimates in 1999 rely on the spawning fraction estimate of 1997. Although variations in spawning fraction of sardine in Spanish Region III through years are small (WDb), the impact of small variations in any of the adult parameters in the final SSB estimate is large, as all the parameters enter the model in a multiplicative way. Therefore, the Spanish 1999 DEPM SSB estimate should be used with caution. Nevertheless, SSB estimates in 1997 and 1999 are clearly much lower than the ones in 1988 and 1990, and the SSB series shows a clear descending pattern. The Egg Production is the main parameter forcing this trend, as from 1990 all of the consecutive surveys gave an egg production estimate lower than half the one of the previous survey. Within the adult parameters, mean female weight shows a decreasing trend, although in the DEPM formula it is counterbalanced by batch fecundity, which also shows a decreasing trend.

4 METHODOLOGICAL AND PRACTICAL ISSUES

4.1 Egg staging

Due to the low abundance of sardine eggs found in stage III during surveys carried out in 1999 by Spain and Portugal, it was decided to do an intercalibration with scientists from both countries.

From the analysis of several sardine egg samples in different stages a consensus was observed between the two countries except for stages III and IV, where some difficulties in distinguishing them were found.

Both countries have decided to do an intercalibration of sardine egg stages assignment after each survey in order to standardise the staging criteria.

An extra effort should be paid in assigning stages to disintegrated sardine eggs as the number of these eggs from Portuguese data had considerably increased in the last survey.

4.2 Egg ageing

The assignment of ages to staged eggs is based on information coming from sardine daily spawning frequency and rearing experiments. Two different methods are nowadays available to automatically assign ages to staged eggs. Lo (1985) described the first automatic method to assign ages to staged eggs of anchovy, based on a temperature development model and on observations about the daily frequency of anchovy from adult ovaries. Using similar information for sardine (temperature development model (Miranda *et al*., 1990) and daily spawning frequency (Pérez, 1990), Bernal *et al*. (1999) developed a new automatic procedure to assign ages to staged eggs. The method of Lo assigns deterministic ages to each stage based on either the expected mean age given by the development model or the elapsed time between a fixed peak spawning and the survey time. On the other hand, the method of Bernal *et al*. relies on a probabilistic assignment of ages given the possible age each stage can have (obtained from a stochastic version of the temperature development model) and the probability density function (pdf) of the spawning time. The advantage of the method by Bernal *et al.* is that more flexible daily spawning frequency patterns can be used, and the error associated with the ageing procedure can be estimated and incorporated in the error estimation of the egg production.

After evaluation of the results from both methods (Bernal *et al*., 1999) and given the practical results obtained using the new method proposed by Bernal (Bernal, 1998; Bernal *et al*. 1999; Bernal, 1999; Stratoudakis *et al.*; 2000) the workshop recommends that the method of Bernal *et al.* (*opus cit.*) is used as the standard ageing method for future DEPM applications for Atlanto-Iberian sardine.

Both the daily spawning frequency and the temperature dependent development model define the degree of precision that can be acquired with the ageing method. Also, the information about each of the basic units can now be given to the model in a more detailed way, *via* the spawning time pdf and the stochastic temperature development model. Some problems associated with the temperature development of Miranda *et al*. (1990) were presented during this workshop (WDd). New data to refit an appropriate temperature development model will be thus desirable. Also, better data about the daily spawning frequency will be desirable in order to construct a robust empirical pdf of spawning times that can be used routinely in the ageing process.

4.3 Statistical independence of ichthyoplankton observations

The traditional egg production estimator assumes that the sampling units are independent. If the assumption is not met, then the variance estimation of the egg production is negatively biased. A solution to this problem is to aggregate the original sampling units into sampling units which meet the assumption of independence. This kind of procedure is common in other areas where DEPM is used, where stations located at distances smaller than the estimated maximum distance of spatial autocorrelation are aggregated in transects located at distances larger than the maximum autocorrelation distance. Information about the spatial autocorrelation of the data in the Atlanto-Iberian sardine DEPM surveys is scarce. Stratoudakis *et al*. (WDe) investigated the spatial structure of data from 1988 Portuguese DEPM survey using omni-directional and directional variograms. He found spatial structures of scales up to 50 km (Figure 4.3.1) and he also concluded that there is more variation in the inshore offshore direction rather than the along shore direction (Figure 4.3.2).

Using the data from the 1999 Spanish DEPM survey, a comparison between using stations and transects as the basic sampling unit was carried out (WDa). In this case data from all stations were used, including those sampled on the way back to Vigo’s port (i.e., Region II bis, see Section 2.1.2, figures 2.1.2.1 and 2.1.2.2). The results of this comparison are shown in tables 4.3.1 and 4.3.2. Egg production estimates from both methods are very similar in Region II bis and Region III, although different in Region I and II. Region I and II had only a few positive stations which cause the estimated CV to be very large in both methods, and that may also cause the differences between the methods. The area of the positive stratum is different in the two methods, as it is defined in a different way. When using stations as the sampling unit, the positive stratum includes the positive stations plus a few embedded negative stations, while when using transects as the sampling units, the full transect is included in the positive stratum if there are some positive stations inside the transect. Nevertheless, this shouldn’t cause bias, as the mean density in each transect is computed including both the positive and negative stations inside the transect. The coefficient of variation of both methods are similar, although slightly smaller in the method using transects. In the case of spatially correlated stations, but statistically independent transects, the expected result would have been the opposite, as the cv of the method using the stations is underestimated. Nevertheless, taking into account the work of Stratoudakis (2000, WDe) both stations and transects are correlated, and thus the coefficient of variation of both methods may be negatively biased.

Without further information on the spatial structure of the samples from the Spanish and Portuguese surveys and further comparisons of the results from stations or transects, decisions on which method to adopt is difficult to take. Thus, the workshop recommends that further analysis of the spatial structure of the data is carried out.

4.4 Identification and ageing of Post Ovulatory Follicles

The postovulatory follicle method for the estimation of spawning fraction requires a division of the processes of deterioration and resorption of the follicles into a series of distinct histological stages, each one with a specific age assigned (Hunter and Macewicz, 1985). This is the method that has always been applied for the estimation of sardine spawning fraction (Pérez *et al*, 1992).

In the Portuguese 1999 survey, sardines were sampled throughout the 24 hour period. The ageing of POFs was undertaken assuming 19:00H as the time of spawning (Ré *et al.*, 1988), and considering the time of capture and the morphological aspect of the POFs (size, presence of structures similar to cord-like cell layers, number and clearness of folds). The shorter the dimensions of the POF structure with less number and less defined folds, the older the POFs are. The POFs analysed were grouped into Day classes: Day 0 considers the oocytes released during a 24h period between 19h of the spawning day and 19h of the next day; Day 1 includes POFs with a minimum of 25h and a maximum of 48h in the ovary and Day 2 POFs includes oocytes released at a minimum time of 48h from sampling.

As an exercise, in order to detect any discrepancies between both countries readings, a sample of 20 microscope slides of Portuguese sardine ovaries were histologically re-analysed during this Workshop. POFs were identified and aged by the Spanish researcher. These slides were mounted in historesin.

In general, Portuguese slides of ovaries from sardines caught by commercial fishing vessels had a less clearly defined follicle structure, while the Spanish slides had a better general aspect despite being mounted in paraffin. The worse quality of the follicular tissue in the Portuguese slides from commercial samples is possibly due to the time interval between fish capture and gonad preservation in AFA. Although every effort was made to minimise the transport and sampling time, it is possible that gonads from commercial vessels stayed exposed for longer time intervals. It is known that the tissue deterioration is a quick and continuous process and it is vital to collect the gonads and preserve them very fast. In fact, some slides of gonads caught by the Portuguese research vessels had better quality of tissues structure, which probably confirms the above assumption.

From the analysis of the observations of the Spanish researcher some differences were detected between countries concerning the age assigned to follicles. Part of these differences may come from a different interpretation of the evolution of follicles degenerative process in each stage. It is difficult to delimit with precision each follicle stage as the degenerative process is continuous and these differences may partially being caused by this handicap. This problem could be overcome with a frequent exchange of slides between countries in order to intercalibrate the age assignments to follicles. In addition, POF stage duration needs to be standardised between the two countries before the next sardine DEPM survey takes place.

4.5 Survey timing

It is important to define the best moment to carry out the DEPM survey. Although the DEPM method does not assume that the survey is carried out in the peak of spawning, doing the survey near the time of maximum spawning has a series of advantages. First the assumption of all the spawning population being in the survey area is easier to meet. Also the number of adult samples with mature ovaries that can be used to estimate some of the adult parameters are usually larger. The distribution of eggs is also more representative of the spawning areas that the species used. Due these reasons, the workshop has decided that the DEPM survey should be carried as close to the peak of spawning as possible.

In Spanish waters the best period for the DEPM survey is defined to be spring. Nevertheless, in the last sardine DEPM survey in Spanish waters (April1999) some problems associated with the survey timing were found (WDa). The survey was carried following the same sampling procedure as in previous years, but low abundances of eggs were found. As some extra time was saved because of the low densities found, one of the Regions defined in Figure 2.1.2.1 was covered twice, one in West-East direction and the other in East-West direction in the way back to port. In the second leg, a larger amount of eggs was found (Tables 4.3.1 and 4.3.2). The survey was probably carried out slightly earlier in the spawning season of sardine in the Cantabric waters. Thus it was decided to use all of the historical data available to try to fine-tune the best survey period for the 2002 survey.

In Portugal, unlike the previous two DEPM surveys, the survey in 1999 took place in January. Despite losing continuity and coordination with the Spanish survey (March) and the Portuguese acoustic surveys (November and March), the shift in timing was considered necessary in view of recent evidence from biological market samples of sardine. The monthly distribution of fish maturity and gonadosomatic index for sardines caught off Portugal suggest that peak spawning should occur sometime between December/January (Figure 4.5.1). However, by shifting the timing of the survey to January, biological samples collected during the acoustic survey were not available. This imposed a modification on the general sampling plan for adult, as it was decided to rely partly on samples from commercial boats. As explained in section 4.4, this may have been responsible for the lower quality of some histological preparations. This, together with the unexpectedly low spawning fractions observed in the southern area of the Portuguese survey, suggest that the timing of the next Portuguese survey needs to be reconsidered based on all the available biological information.

4.6 Area stratification for adult sampling

In standard DEPM methodology, design-based estimators are used to obtain adult parameter estimates. This means that the estimated expected value and variance of each parameter is entirely determined by the sampling probabilities, which are in turn defined by the adopted sampling design. Piquelle and Stauffer (1985) discuss several sampling designs and the appropriate estimators of adult parameters and their variance for a DEPM survey. A major assumption under any of these designs is that all parameters are constant over the range and duration of the survey. When this assumption is violated, Piquelle and Stauffer (1985) recommend post-stratification, where a series of strata is determined *a posteriori* and estimation is performed independently for each stratum.

When post-stratification is used, Piquelle and Stauffer (1985) suggest that SSB (and its variance) for the entire survey area is obtained by summing the strata SSBs (and their variances). However, this is true only when stratification is decided *a priori*, in which case stratum sample size is fixed (Thompson 1992). When post-stratification is used, stratum sample size is a random variable, and variance estimation should include an additional term that accounts for random sample sizes within strata. In that sense, even if “judgement” sampling (Piquelle and Stauffer 1985) can effectively approximate proportional effort allocation to strata defined *a posteriori*, variance estimation that is only based on the sum of the strata variances is going to underestimate the true SSB variance. An additional problem with judgement sampling (where information independent of DEPM fishing stations is used to place more samples to areas with high fish abundance) is that the criteria for sample allocation are often based on qualitative information obtained during the DEPM survey (i.e. echogram registers of a simultaneous acoustic survey or observed egg densities from the ichthyoplankton survey). As a consequence, the resulting distribution of sampling effort often deviates considerably from the relative abundance of the sampled population.

In the case of Atlanto-Iberian sardine, adult sampling in DEPM surveys has generally been considered to follow the principles of judgement sampling, although proportional allocation of sampling effort is rarely achieved. Post-stratification has been used in the Spanish DEPM surveys of 1988 and 1990 (Garcia *et al*. 1992; Garcia *et al*. 1991), where considerable differences in mean weight and spawning fraction were observed between Galician and Eastern Cantabrian. In the 1997 and 1999 Spanish DEPM surveys, adult post-stratification was not considered due to the small number of fishing stations available per region. On the other hand, Portuguese DEPM estimates have up to now been always based on the entire survey area. However, in 1999 adult sampling effort was considerably higher, thus allowing for the first time meaningful comparison of adult parameter estimates over the survey area (Figure 4.6.1). Observing the spatial distribution of spawning fraction (Figure 4.6.1a) it is obvious that a higher proportion of fish were in spawning condition off the western Portuguese coast than in Algarve and Cadiz during January 1999. Given the lack of proportional effort allocation, applying the post-stratification criteria to the 1999 Portuguese survey would have resulted in a higher SSB estimate due to the very low (and imprecisely estimated) spawning fraction in the southern part of the survey.

To bypass the problems in sampling effort allocation that judgement sampling can involve and the problems of variance underestimation with post-stratification, a possible solution would be to adopt a classical stratified random design based on the results of recent surveys (Thompson 1992). In stratified random sampling with *L* strata and a fixed total sample size of *n*, allocation of sampling effort in the strata can be based either on proportional allocation (where the aim is to maintain a steady sampling fraction throughout the population) or on optimum allocation (where the aim is to minimise the variance in the estimation of one parameter). Under proportional allocation, the sample size for stratum *h* is:

$$n_h = \frac{nN_h}{\sum_{k=1}^{L} N_k} \qquad \text{(Eq. 4.6.1)}$$

while, under optimum allocation, the sample size for stratum *h* is:

$$n_h = \frac{nN_h\sigma_h}{\sum_{k=1}^{L} N_k\sigma_k} \qquad \text{(Eq. 4.6.2)}$$

where N_h is the stratum total units, and σ_h is the stratum population standard deviation. When the stratum population standard deviation is not available, it can be replaced by sample standard deviations (s_h) from past data (Thompson 1992).

In the case of sardine DEPM surveys, the availability of regional estimates of sardine abundance from acoustic surveys and the information from recent DEPM surveys permit the use of either of the above estimators. The decision on whether to rely on proportional or optimum allocation should depend on the confidence that can be placed on the most recent DEPM information. Given that the most important adult parameter for DEPM estimation is spawning fraction, optimum allocation would be the natural option when the most recent estimates of the standard deviation of spawning fraction per stratum are expected to reflect the situation during the new survey. In that case, the appropriate stratum sample size for a new DEPM survey is given by Eq. 4.6.2, where the most recent acoustic estimate of sardine abundance in the stratum (N_h) and the most recent DEPM estimate of standard deviation of spawning fraction in the stratum (s_h) are used. When a similar regional pattern in the standard deviation of spawning fraction cannot be assumed for the new survey, then Eq. 4.6.1 could be applied to the most recent acoustic data, in order at least to guarantee proportional allocation of adult sampling effort during the DEPM survey. In either case, *a priori* determination of stratum sample size will facilitate estimation of sardine SSB and its variance for the entire survey area, without having to consider the complicated variance estimators that are imposed by post-stratification.

4.7 GAM-based estimation of sardine egg production

One of the original aims of this workshop was to provide GAM-based estimates of sardine egg production for the 1999 DEPM survey. Although considerable work has already been undertaken in this direction, it was decided not to present GAM-based estimates of sardine egg production in this workshop. Stratoudakis (1999), Bernal (1999) and Bernal *et al*. (2000) have already applied binomial GAMs to sardine egg presence/absence data from a series of ichthyoplankton surveys from 1986 to 1999. These studies, apart from showing decadal changes in sardine egg distribution, also provide the starting point for obtaining a two-step estimate of sardine egg production. The natural next step would have been to fit an abundance model (with a negative binomial error distribution) conditioned on egg presence and then integrate numerically the product of predicted egg presence and abundance along the prediction grid. However, preliminary runs of abundance models have demonstrated that the partial age effect (which should decrease monotonically over time, thus reflecting egg losses due to mortality) was often appearing to be a non-monotonic function of age: in several cases a temporary increase during the second day was observed before sampled counts dropping again in the third day (Bernal 1999). Since it is counter-intuitive for egg counts to be a non-monotonic function of age, this observation forced a rethinking of the sardine egg sampling, staging and ageing methodologies. During this workshop it became evident that the problem was most likely related to the existing sardine egg development model of Miranda *et al*. (1990) that does not seem to capture adequately the relative duration of several stages. Finally, an additional reason for postponing the presentation of GAM-based egg production estimates for sardine is that a new EU project that has started in April 2000 is expected to provide soon methodological improvements in the GAM fitting and model selection procedures.

5 FUTURE SURVEYS

DEPM estimates of sardine spawning biomass are now incorporated in the annual analytical assessment of the Atlanto-Iberian stock as an absolute fisheries independent estimate of SSB. There is consensus between Portugal and Spain that these surveys should continue with regularity in the near future, while reinforcing the level of co-operation between the two countries in the planning of the surveys and the analysis of results. However this workshop identified several biological and methodological questions that need to be answered with some urgency in order to improve the performance of the method. In the next sections we describe the proposed course of short and medium term action in relation to future sardine DEPM surveys.

5.1 Next surveys

In general, it seems reasonable to expect that after a DEPM survey, final egg production estimates can be made available within 3-4 months and final adult parameter estimates within a year. With these figures in mind, and considering that acoustical estimation of sardine abundance is available on an annual (Spain) or semestral (Portugal) basis, a co-ordinated sardine DEPM survey every 3 years seems a realistic solution. Ideally, we would want to establish one “sardine DEPM year” every 3 years, the way there currently is a “mackerel-horse mackerel AEPM year”. For the immediate future, this would mean that the next sardine DEPM survey will be planned for early 2002 and the following one for early 2005.

On a related issue ichthyoplankton monitoring surveys have recently been incorporated in routine acoustic surveys for sardine in both IPIMAR and IEO. During the sardine acoustic surveys of November 1999 (Portugal) and March 2000 (Portugal and Spain), approximately 130 CALVET stations were sampled during the night, covering the continental shelf of the entire survey area. Temperature/depth profiles were obtained in all ichthyoplankton stations and a considerable volume of additional oceanographic information was obtained during March 2000 as part of the EU project PELASSES. This coupling is anticipated to continue in future acoustic surveys, thus providing the means to monitor the distribution and abundance of sardine eggs and larvae in the years between DEPM surveys.

5.2 Preparation before the next survey

Planning the next sardine DEPM survey for 2002 allows the time for background work in order to answer some important methodological and biological questions identified during this Workshop. Some of the basic problems that need to be solved in order to improve the quality of sardine DEPM estimates under the traditional or the GAM estimation methodology are:

1. obtain more reliable information on egg ageing and diurnal synchronicity of spawning
2. validate the ageing criteria for post-ovulatory follicles
3. compare macroscopic with microscopic maturation
4. identify the best timing for future surveys
5. understand the spatial structure of egg patches

In relation to (1), a new sardine egg rearing experiment would permit consolidating the information obtained by Miranda *et al*. (1990) and improving the temperature-dependent ageing model that, as it has been shown during this Workshop, describes inadequately the relative duration of several egg stages. Related to this and to (2), a new experiment on sardine spawning under laboratory conditions would extend the work by Olmedo *et al*. (1990) by obtaining more information on the mean time of spawning and its variance, and also on the ages of post-ovulatory folicles (POFs). A better egg development model and a more realistic probability density function for daily spawning frequency could easily be incorporated to the new sardine egg ageing technique of Bernal *et al*. (1999) that has been recommended by the Workshop. Also, validating the ageing criteria for POFs and identifying the impact of water temperature on follicle deterioration would greatly improve the reliability of future spawning fraction estimates.

In relation to (3), a comparison of macroscopic/microscopic maturity stage identification would test the current assumption that all fish with a macroscopic stage of maturation of 2 and above are contributing to the SSB. In relation to (4), analysing existing biological and ichthyoplankton data at the finest possible spatio-temporal resolution would provide information on the duration and intensity of sardine spawning by region, thus allowing the best timing of future surveys. Finally, in relation to (5) performing Continuous and Underway Fish Egg Sampler (CUFES) sampling on a very fine spatial scale (1 mile) during future acoustic surveys would provide data to study the spatial structure of individual sardine egg patches, in order to evaluate the level of spatial autocorrelation between neighbouring ichthyoplankton stations during the DEPM surveys.

6 RECOMMENDATIONS

1. It is recommended that a Planning Group for the 2002 sardine DEPM survey should meet during November 2001 in order to design the forthcoming survey.

2. A common maturity scale and the same definition of mature fish have been used by Portugal and Spain within DEPM. It is however advisable to intercalibrate periodically the criteria used for classification of macroscopic maturity and to agree on common criteria if and where discrepancies are found. Furthermore, the group agrees that the correspondence between macroscopic and microscopic maturity stages needs further clarification and recommends the development of a histological study with this goal.

3. Based on sections 4.1 it is recommended to:

   To carry out an exchange of egg samples after each survey in order to uniformize the staging criteria.

   To organize a reference collection of egg stages.

   To put additional effort on the staging of disintegrated eggs.

4. Taking into account the discussion on the methodology for egg ageing (section 4.3), participants agreed to adopt the method of Bernal (1999). Furthermore, it is strongly recommended to conduct new laboratory experiments on the development of sardine eggs.

5. Taking into account the methodological issues regarding the ageing of POFs (section 4.4) it is recommended:

   To carry out an intercalibration exercise on the ageing of POFs. From this exercise, a reference collection should be organized to guide further work on this area.

   To carry out a laboratory experiment in order to validate the criteria for ageing POFs.

6. A considerable amount of data has already been collected within DEPM surveys. There is a need to compile these data on a common database to ensure that it is easily and rapidly accessible and to simplify the exchange and/or collation of data for analysis of the whole area.

7. Based on point 4.6, we recommend that all relevant information is reviewed before the meeting of the Planning Group for the next sardine DEPM survey, at which time a final decision on the appropriate design for adult sampling should be taken.

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8 WORKING DOCUMENTS

Bernal, M., Franco, C., Pérez, J.R. and Lago de Lanzós, A. 2000. Daily egg production method estimates of sardine (*Sardina pilchardus*) SSB off the Spanish coast in March 1999. (WDa).

Pérez, J.R., Peleteiro, E., García, M.Sánchez, M.E. 2000. Parameters of sardine (*Sardina pilchardus*, Walb.) adults for the application of the Daily Eggs Production Method in 1999 in Spain. (WDb).

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Zwolinski, J., Stratoudakis, Y., Soares, E., Morais, D. and Godinho, S. 2000. Diurnal, seasonal and inter-annual variation in biological properties of female sardine (*Sardina Pilchardus* Walb.) spawning off Portugal. (WDf).

Note that the abstracts of the above WDs are presented in Annex 1.

ANNEX 1

Daily Egg Production Method estimates of sardine (*Sardina pilchardus*) SSB off the Spanish coast in March 1999

M. Bernal, C. Franco, J.R. Pérez and A. Lago de Lanzós.

Abstract: In this Working Document we estimate the SSB of sardine (*Sardina pilchardus*) off the Spanish coast in March 1999 using the Daily Egg Production Method. Comparison between the traditional way of obtaining egg production estimates and some changes of the method are carried out, namely: 1) using transects instead of stations as the sampling unit; 2) excluding egg in stage I from the analysis and 3) using an extra cohort in the analysis. Changes 2) and 3) are based on biological and environmental information from 1999 and previous surveys, while change 1) is based on bibliography. Also, one of the sampling regions (middle Cantabric Region – Region II) was sampled twice in the 1999 survey, and egg production estimates are obtained for each of the sampling legs. Results from both the traditional egg production estimator and the new one are similar. Both methods indicate a decrease in egg production in comparison with the 1997 estimator, although the results are slightly different when using the first or the second time Region II was sampled. No adult sampling occurs at the time the second survey in Region II was carried out, and so this data was not used in the DEPM Spawning Stock Biomass estimator (SSB). SSB estimates in 1999 are obtained using 1997 spawning fraction, as this parameter is not available in 1999. The 1999 SSB estimate obtained using the 1997 spawning fraction value is lower than the SSB estimate of 1997, mainly due to the reduction in the egg production. Investigations of sardine egg spatial structure are recommended in order to further test the differences between using transects or stations as the basic DEPM sampling units.

Parameters of sardine (*Sardina pilchardus*, Walb.) adults for the application of the Daily Eggs Production Method in 1999 in Spain.

J.R. Pérez,. E. Peleteiro, M.E. García and M. Sánchez

Abstract: In March 1999, the IEO in the Northern Coast of Spain (ICES Division IX a North and VIIc) carried out the Acoustic Survey Pelacus 0399 in order to estimate the adults parameters for the sardine spawning stock biomass by the Daily Eggs Production Method (DEPM). The sampling was carried out with a trawler an commercial purse seiner. To obtain the batch fecundity a total of 137 slides corresponding to 4 different hauls, 2 hauls in the Region I, 1 in Region II and 1 in Region III were undertaken was obtained between batch fecundity and weight without ovary F = 243,33 W* + 9046 (Figure 3) obtaining a partial fecundity of 21,874 hydrated oocytes, that corresponds to an average relative fecundity of 415 hydrated oocytes/gram of mature female without ovary (CV 12 %). The spawning fraction was not estimated because samples were not collected in appropriate conditions. For this reason was decided to assume S=0.18 as the spawning fraction value estimated for the DEPM for the same areas in 1997 Lago de Lanzós, A. *et al*. 1998. The sex ratio in weight was R=0,55, CV= 45%. Average weight of mature females estimated considering195 non hydrated females from the ordinary biological samplings, was 66,03 g, CV= 41%.

Diurnal, seasonal and inter-annual variation in biological properties of female sardine (*Sardina pilchardus*, Walb.) spawning off Portugal

Juan Zwolinski, Yorgos Stratoudakis, Eduardo Soares, Delfina Morais and Susana Godinho

Abstract: Female sardines ready to spawn were collected off Portugal and the Gulf of Cadiz at various times of day during November 1998, January, March and November 1999 with the main aim to study variation in batch fecundity. We combine these data with information from previous DEPM surveys and biological market samples to describe diurnal, seasonal and inter-annual variation in several biological properties of female sardine spawning off Portugal. The density of hydrated oocytes in the gonad demonstrated a negative relation with the time of sampling, while gonadosomatic index and mean oocyte diameter showed a positive relation. This provided another independent source of evidence for the daily synchronicity in sardine spawning towards dusk. Total number of oocytes per fish was regressed against gonad-free weight using a linear model whose parameters were estimated using weighted least squares. Comparing the batch fecundity/fish weight relationship during the 1998/1999 spawning season showed that there was no statistically significant difference between November 1998 and March 1999 but the model had a higher intercept in January 1999. Relative fecundity and gonadosomatic index was higher in January (mid spawning season) than in November (start) and March (end of spawning season), while the condition factor of spawning females

gradually deteriorated along the spawning season. Extensive market samples demonstrate a very similar monthly evolution of female gonadosomatic index and condition factor during the 1998/1999 spawning season. A plausible interpretation of these results is that up to mid spawning season (January) the energy allocated to reproduction gradually increases leading to larger batches of eggs, while after January the deterioration in condition possibly starts to confine the reproductive output. Finally, comparing the March/November model with those obtained during March 1988, March 1997 and November 1999 suggests that there is sufficient interannual variation in sardine batch fecundity to justify separate estimation at each DEPM survey, but the observed variation is smaller than that reported in the literature for similar species.

DEPM estimate of sardine spawning biomass in the area 36º - 41º 50´N during January 1999

Yorgos Stratoudakis, Eduardo Soares, Manuela Garção, Fernando Mota, José Albuquerque, Susana Godinho, Delfina Morais and Emilia Cunha

Abstract: In this working document we estimate the spawning biomass of sardine (*Sardina pilchardus*) in Portuguese waters and the Gulf of Cadiz based on the DEPM survey of January 1999. The egg production estimate is derived from the aged sardine eggs obtained in 417 ichthyoplankton stations, while adult parameters are estimated from 40 fishing stations along the survey area. Applying the methodology used in the previous two Portuguese DEPM surveys, the estimated spawning biomass of sardine for January 1999 is 197.6 thousand tonnes with a coefficient of variation of 34%. This estimate is 55% higher than the corrected estimate of March 1997 (127.2 Kt, CV = 57%) and 72% higher than the estimate of March 1988 (115.1 Kt, CV = 34%), although the latter only refers to Portuguese waters. The above estimates are obtained assuming that adult parameters and egg density are constant over the range and duration of the survey. The relatively large number of fishing stations in January 1999 allowed to explore spatial effects in adult parameter estimation, but also introduced possible systematic variation due to the use of two sampling methods (pelagic purse seining and demersal trawling). In the last part of the document, area and gear effects are explored, but final decisions on the appropriate methodology for SSB estimation are left for the meeting. Overall, area stratification leads to a considerably higher SSB estimate (337.3 Kt, CV = 37%), mainly due to the marked regional difference in spawning fraction between areas (lowest spawning fraction in the area with the highest egg production). Gear effects in the properties of the sampled population were also evident, mainly affecting sex ratio and spawning fraction estimation. The area stratified SSB estimate using only data from bottom trawls was 284.6 Kt (CV=52%) while the estimate for purse seines was 360.6 Kt (CV=47%).

Thoughts on the planning of future DEPM surveys for Atlanto-Iberian sardine

Yorgos Stratoudakis, Emilia Cunha, Fatima Borges, Eduardo Soares and Catarina Vendrel
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Abstract: In this working document we introduce and discuss several problems related to the application of DEPM for the Atlanto-Iberian stock of sardine. Using the existing information and experience, we discuss each of the identified problems and when possible propose solutions. This WD is meant to work as a basis for discussion during the Study Group meeting, in order to improve the planning and performance of future DEPM surveys in Iberian waters.

The Spawning Fraction of Sardine (*Sardina pilchardus,* Walb.) off Portuguese coast (January-February 1999)

Eduardo Soares and Leonel Serrano Gordo

Abstract: Spawning fraction, i.e. the fraction of mature females with postovulatory follicles <48 hours old, of Sardine (*Sardina pilchardus*, Walb.) off the Portuguese coast (January-February 1999) was estimated based on the ageing of postovulatory follicles by histological analysis.

Table 2.2.1.1 – Portuguese Sampling fishing stations in January 1999 (Gear: BT-Bottom Trawl, **PT**-Pelagic Trawl, **PS**-Purse Seine **Origin**: **RV** Research Vessel, **CF**-Commercial Fleet).

Sample	Gear	Origin	ICES Sub-Div.	Date	Time (hh:mm)	Depth (m)
AF2	BT	RV	Gulf of Cadiz	99-01-09	10:35	36.0
AF3	BT	RV	Gulf of Cadiz	99-01-09	13:05	37.5
AF4	BT	RV	Gulf of Cadiz	99-01-10	7:43	31.0
AF5	BT	RV	Gulf of Cadiz	99-01-10	10:05	16.0
AF6	BT	RV	Gulf of Cadiz	99-01-10	12:20	45.0
AP1	PT	RV	Gulf of Cadiz	99-01-09	7:59	20.0
AP7	PT	RV	Gulf of Cadiz	99-01-01	17:10	51.0
ARR1	BT	RV	IXa-South	99-01-23	12:00	55.0
AVE1	BT	RV	IXa-C.North	99-02-02	1:30	49.0
AVE2	PS	CF	IXa-C.North	99-02-01	20:00	43.0
AVE3	PS	CF	IXa-C.North	99-02-01	20:00	45.0
CAM1	BT	CF	IXa-C.North	99-02-01	9:00	60.0
CAS1	BT	RV	IXa-C.South	99-02-05	15:00	35.0
CAS2	BT	RV	IXa-C.South	99-02-05	12:50	32.0
FIG1	BT	RV	IXa-C.North	99-02-03	8:30	
FIG3	BT	RV	IXa-C.North	99-02-03	11:30	
FIG4	BT	RV	IXa-C.North	99-02-03	9:30	30.0
LIS1	BT	RV	IXa-C.South	99-02-02		
MAT2	BT	CF	IXa-C.North	99-01-29		
MAT3	BT	CF	IXa-C.North	99-01-27		
MAT4	BT	CF	IXa-C.North	99-01-28		
MIL1	BT	RV	IXa-South	99-01-24	8:30	60.0
MIL2	BT	RV	IXa-South	99-01-24	10:00	62.0
OLH1	PS	CF	IXa-South	99-01-19	7:00	45.0
PEN1	PS	CF	IXa-C.South	99-02-04	19:30	41.0
PEN2	BT	RV	IXa-C.South	99-02-04	9:30	35.0
PEN3	PS	CF	IXa-C.South	99-02-04	17:15	40.0
POR1	PS	CF	IXa-South	99-01-20	7:30	37.0
POR2	PS	CF	IXa-South	97-01-21	9:00	36.0
SAG1	PS	CF	IXa-South	99-01-22	9:00	48.0
SAG2	PS	CF	IXa-South	99-01-22	8:15	46.0
SES1	PS	CF	IXa-C.South	99-01-26	19:15	78.0
SET1	PS	CF	IXa-C.South	99-01-25	20:30	40.0
SET2	PS	CF	IXa-C.South	99-01-25	21:00	38.0
SIN1	BT	RV	IXa-South	99-01-25	9:30	30.0

Table 2.2.2.1 Spanish sampling fishing stations in March in 1999.

Vessel	ICES Division	Haul	Date	latitude	longitude	GMT Time	Biologic Sample
Purse Seine	IXa North	12*	14/03/99	42º15'	8º47'	21	72
	IXa North	19*	18/03/99	43º45'	7º35'	21	20
	IXa North	28*	21/03/99	43º35'	6º03'	20	37
Pelagic Trawl	VIIc	3	6/03/99	44º02,12'	1º39,407'	14:21	82
	VIIc	4	7/03/99	44º11,67'	1º34,67'	14:56	80
	VIIc	7	9/03/99	45º15'	1º14'	7:31	80
	VIIc	8	9/0399	45º15'	1º33,25'	9:38	80
	VIIc	9	9/0399	45º15,016'	2º4,875'	14:04	104
	VIIc	10	10/03/99	45º5,010'	2º5,355	7:11	90
	VIIc	42*	23/0399	43º27,998'	3º15,767'	20:05	8
(*) used for histology						**Total**	**524**

Table 3.1.1.1 Area and number of ichthyoplankton at each stratum in the Portuguese DEPM 1999 survey

	REGION		
STRATUM	NORTHERN	SOUTHERN	TOTAL
1			
Area:	8,217	7,967	16,184
n:	66	63	129
0			
Area:	20,837	15,007	35,844
n:	134	47	181
Total			
Area:	29,054	22,974	52,028
n:	234	183	417

Table 3.1.1.2 Estimates of the mortality model parameters and their standard errors in the Portuguese DEPM 1999 survey.

	REGION		
	NORTHERN	SOUTHERN*	TOTAL*
Z ($hour^{-1}$):	-0.059	-0.346	-0.177
Standard error:	0.0911	0.1647	0.0869
$P_{01}(d^{-1}/0.05m^2)$:	11.32	22.40	16.19
Standard error:	3.486	5.638	3.171
n:	187	157	345

*- an extremely high value of 1305 eggs with an age of 28 hours found at just one station was considered an outlier and was not included in the regression

Table 3.1.1.3 Daily egg production during the Portuguese DEPM 1999 survey.

	REGION		
	NORTHERN	SOUTHERN	TOTAL
P_0 (eggs.$0.05m^{-2}$)	3.200	7.766	5.037
Standard error:	1.8583	3.3292	1.7705
CV:	0.58	0.43	0.35
A_iP_0 (.eggs10^{-12})	1.860	3.568	5.242

Table 3.3.1 Spawning biomass (Kt)

	Portugal	Spain	Total
Parameters	January 1999	April 1999*	
Egg production (eggs10^{-12})	5.24 (35)	0.34 (44)	
Female weight (g)	44.42 (5)	66.03 (41)	
Sex ratio	0.61 (5)	0.55 (45)	
Batch fecundity	18416 (5)	21800 (12)	
Spawning fraction	0.101 (15)	-	
Spawning biomass (Kt)	**205.1** (39)	**10.4** (77)**	**215.5** (86)

* Adult parameters correspond to the values obtained in Region III
** Spawning fraction obtained in 1997

Table 3.4.1 Summary of parameter estimates (coefficient of variation in brackets) for the three Portuguese DEPM surveys. Parameters estimates for 1988 from Cunha *et al* (1992), egg production for 1997 from Cunha *et al.* (1997) and adult parameters from Gordo *et al.* (1999).

Portugal			
	March 1988*	March 1997	January 1999
Egg production (eggs10^{-12})	2.87 (22)	4.41 (49)	5.24 (35)
Female weight (g)	40.94 (6)	41.28 (5)	44.42 (5)
Sex ratio	0.52 (11)	0.61 (22)	0.61 (5)
Batch fecundity	15581 (8)	17914 (3)	18416 (5)
Spawning fraction	0.13 (18)	0.13 (19)	0.101 (15)
Spawning biomass (Kt)	115.1 (34)	127.2 (57)	205.1 (39)

* Estimates do not include the Gulf of Cadiz

Table 3.4.2 Summary of parameter estimates (coefficient of variation in brackets) for the four Spanish DEPM surveys. Parameters estimates for 1988 from Garcia *et al.* (1992), for 1990 from Garcia et al. (1991), from 1997 from Lago de Lanzós *et al.* (1998), egg production from 1990 from Lago de Lanzós *et al.* (1999)

Spain				
	April 1988	April 1990	April 1997*	April 1999*
Egg production (eggs10^{-12})		1.78 (58)	0.72 (82)	0.34 (44)
Female weight (g)		-	70.05 (6)	66.03 (41)
Sex ratio			0.52 (11)	0.55 (45)
Batch fecundity			26563 (5)	21800 (12)
Spawning fraction			0.18 (15)	-
Spawning biomass (Kt)	180.2 (50)	77.7 (50)	20.7 (84)	10.4 (77)**

* Adult parameters correspond to the values obtained in Region III
** Spawning biomass calculated using the spawning fraction obtained in 1997

Table 3.4.3 Comparison of SSB obtained with DEPM

Year	Portugal	Spain	Total
1988	115.1 (34)	180.2 (50)	295.3 (33)
1997	127.2 (57)	20.7 (84)	147.9 (51)
1999	205.1 (39)	10.4 (77)	215.5 (39)

Table 4.3.1: Egg production estimates and SSB estimates using stations as the sampling unit. Regions I to II are defined as in Figure 2.1.2.1 and Region II bis is Region II covered on the way back.

	A0 (Km^2)	A1(Km^2)	Z (se)	P_{01} (eggs / 0.05 m^2) (se)	P_0	Egg production [% CV]
Region I	12579	694	0.01 (0.01)	1.82 (1.09)	0.09 (0.24)	$2.526\ 10^{10}$ [267]
Region II	5402	2161	0.01 (0.01)	2.45 (0.71)	0.70 (0.37)	$1.059\ 10^{11}$ [53]
Region II bis	2546	4665	0.009 (0.005)	6.27 (1.75)	4.06 (1.41)	$5.850\ 10^{11}$ [35]
Region III	1852	3010	0.002 (0.004)	5.14 (1.21)	3.18 (0.96)	$3.094\ 10^{11}$ [30]
Total (excluding Reg II bis)	19833	5865	0 (0.003)	2.91 (0.60)	0.66 (0.29)	$3.415\ 10^{11}$ [44]
Total (excluding Reg II)	16978	8369	0.006 (0.004)	5.27 (1.04)	1.74 (0.60)	$8.821\ 10^{11}$ [34]

Table 4.3.2: Egg production estimates plus SSB estimates using transects as the sample unit. Regions I to II are defined as in Figure 2.1.2.1 and Region II bis is Region II covered on the way back.

	A_0 (Km^2)	A_1(Km^2)	Z (se)	P_0 (eggs / 0.05 m^2) (se)	P (eggs / 0.05 m^2) (se)	Egg production [% CV]
Region I	11190	2084	0.03 (0.02)	1.19 (0.70)	0.19 (0.28)	$4.960\ 10^{10}$ [147]
Region II	695	6868	0.02 (0.01)	1.25 (0.33)	1.13 (0.32)	$1.717\ 10^{11}$ [28]
Region II bis	0	7211	0.006 (0.007)	3.77 (1.34)	3.77 (1.40)	$5.437\ 10^{11}$ [37]
Region III	0	4862	0.002 (0.004)	3.04 (0.82)	3.04 (0.85)	$2.956\ 10^{11}$ [28]
Total (excluding Reg. II bis)	12502	13814	0.004 (0.005)	1.64 (0.44)	0.86 (0.32)	$4.532\ 10^{11}$ [37]
Total (excluding Reg. II)	11190	14157	0.005 (0.004)	3.07 (0.69)	1.71 (0.52)	$8.692\ 10^{11}$ [30]

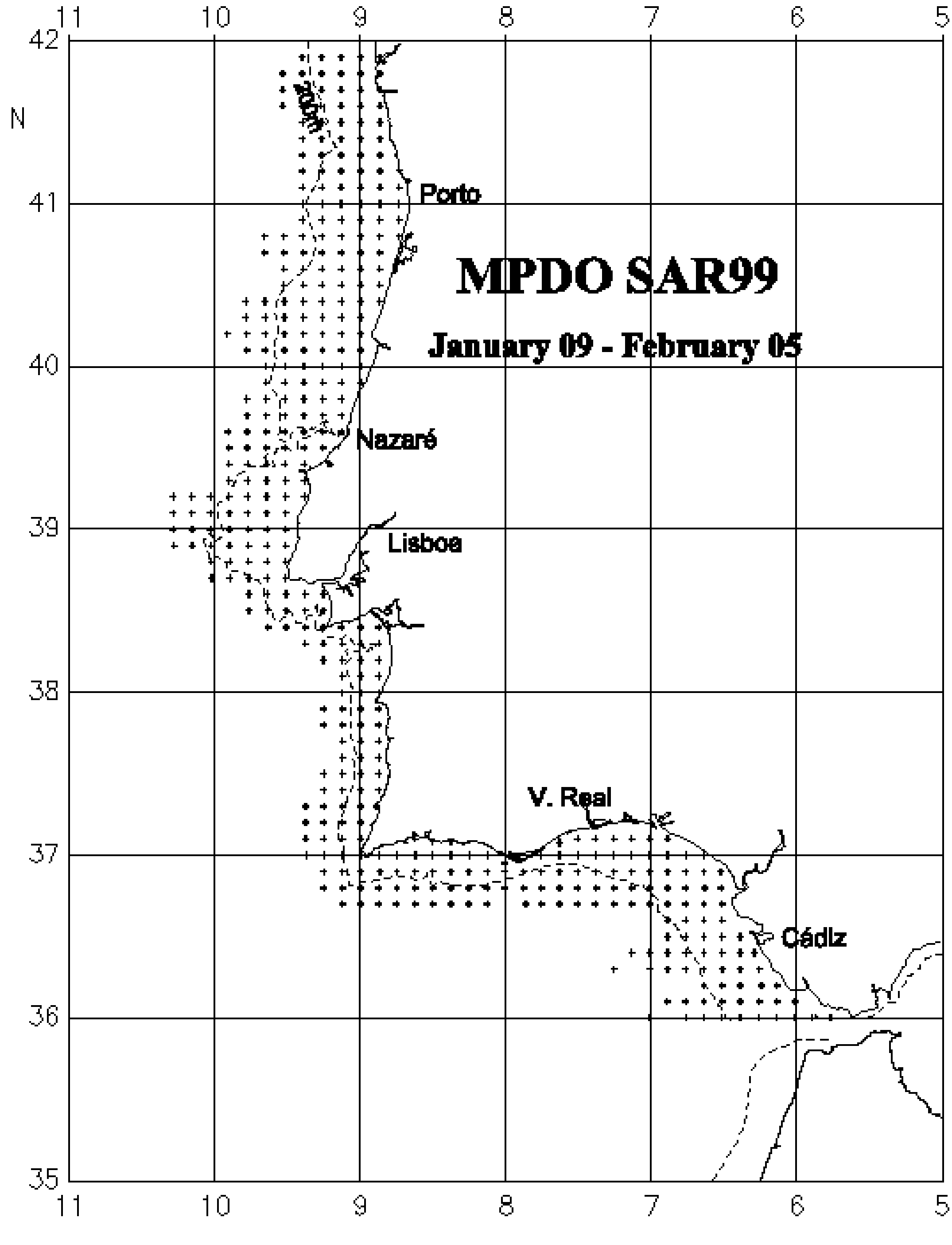


Figure 2.1.1.1: Location of CalVET stations off the Portuguese coast

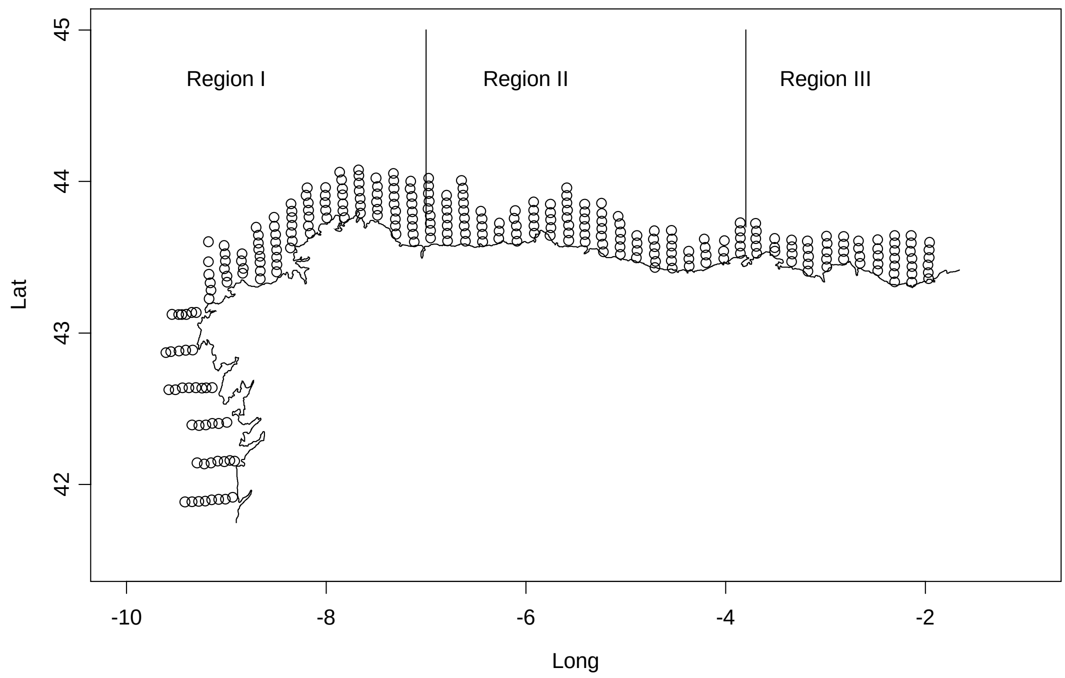


Figure 2.1.2.1: Station location and Regions off the Spanish coast. .

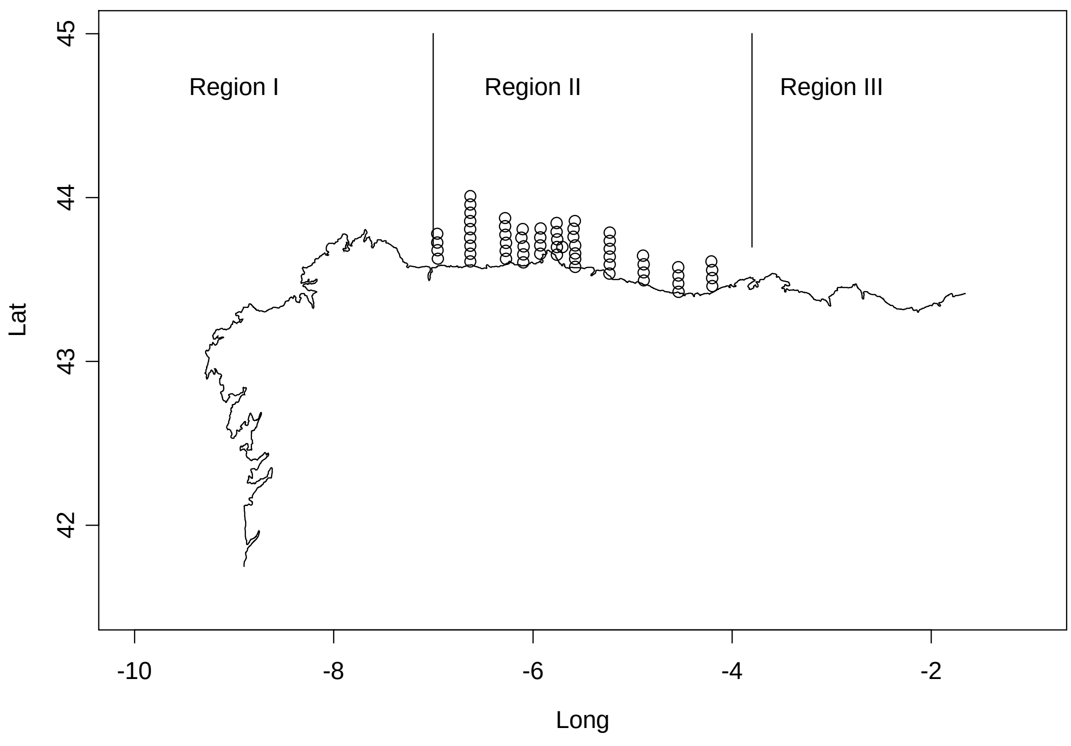


Figure 2.1.2.2: Location of the sampling stations off the Spanish coast in Region II bis.

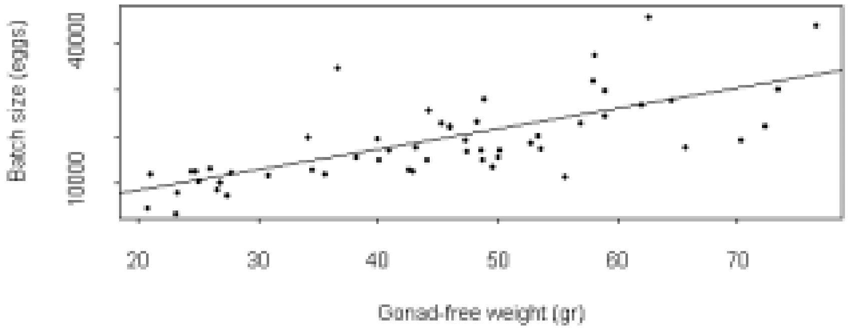
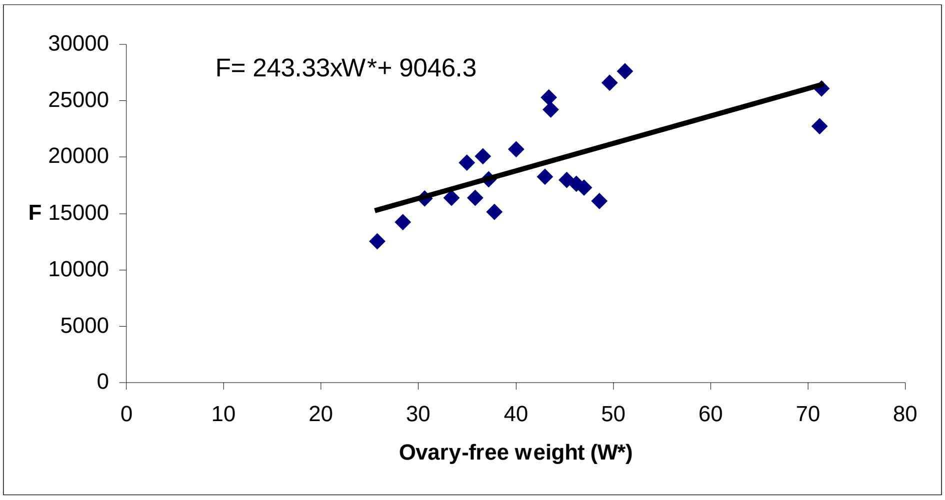


Figure 3.2.1.1: Batch size/fish gonad-free weight relationship for the Portuguese 1999 DEPM survey



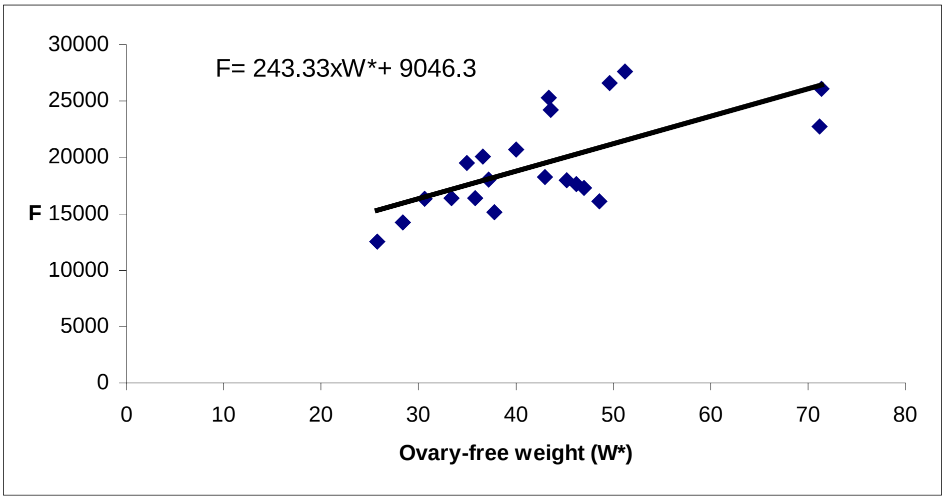


Figure 3.2.1.2: 1999 Sardine batch fecundity F / gonad free weight 1999

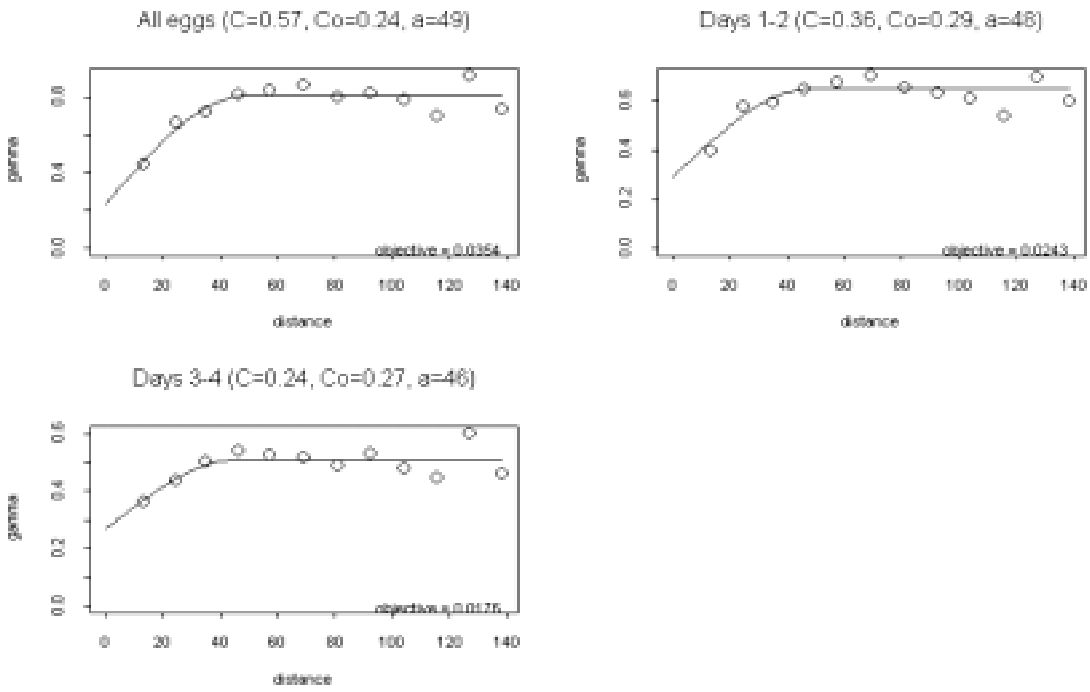


Figure 4.3.1: Experimental and theoretical omni-directional variograms for sardine egg abundance in northern Portugal during the 1988 survey (a: total, b: cohorts 1 and 2 days old, c: cohorts 3 and 4 days old). The fitted parameters of the spherical models fitted to each variogram are given on the title of each pannel.

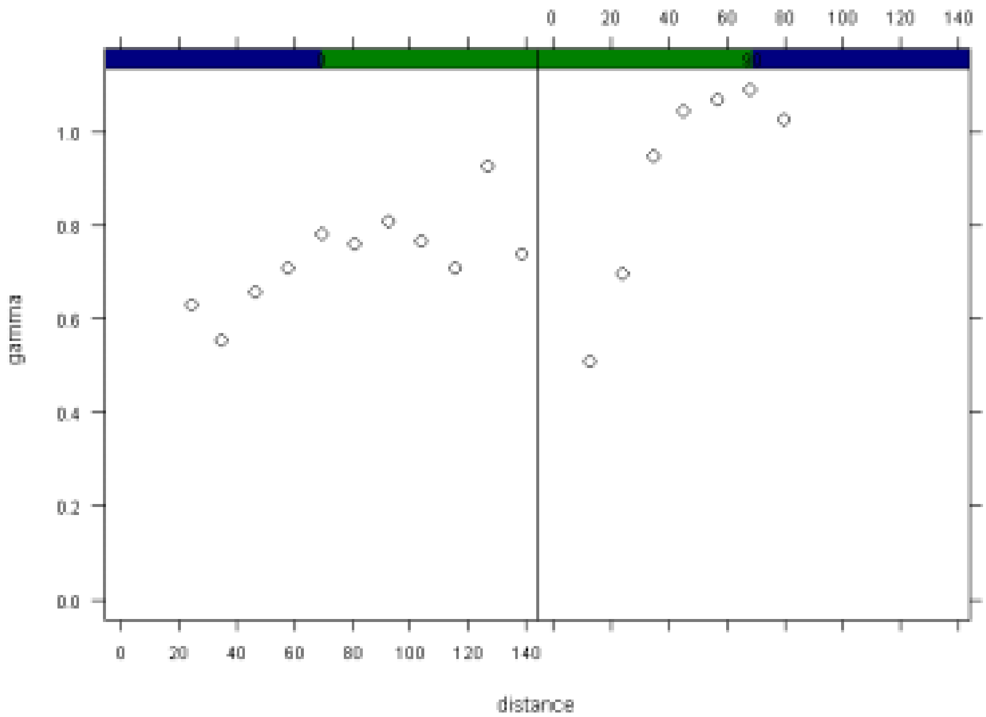


Figure 4.3.2: Directional experimental variograms of sardine egg abundance (fourth root transformation) in northern Portugal during the 1988 DEPM survey. Left pannel: N-S; right pannel: E-W.

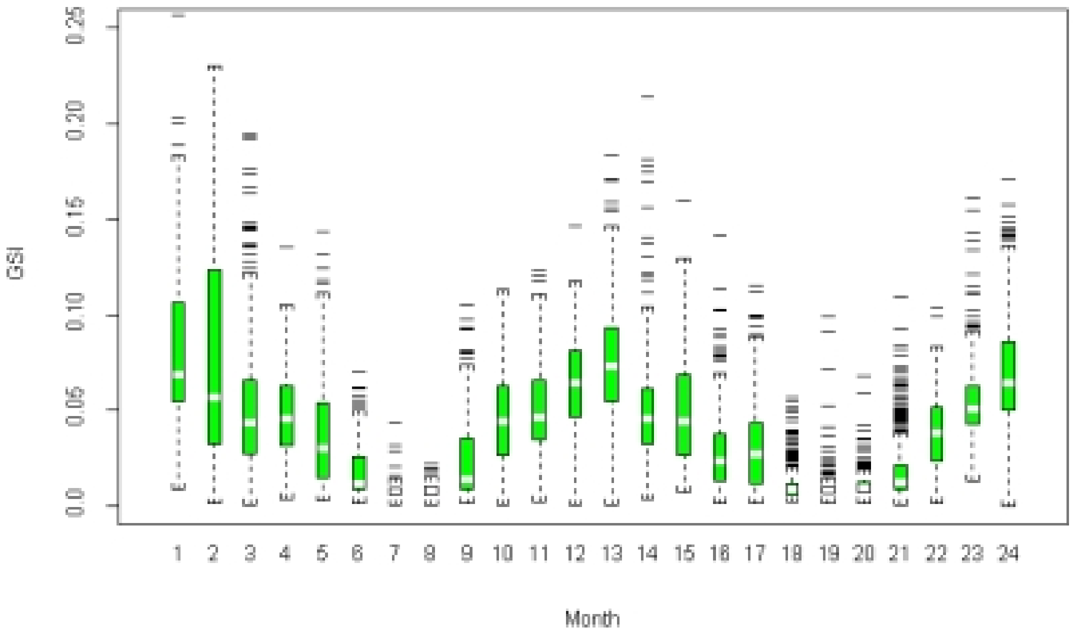


Figure 4.5.1: Monthly distribution of female gonadosomatic index from sardine market samples off Portugal during 1998 and 1999.

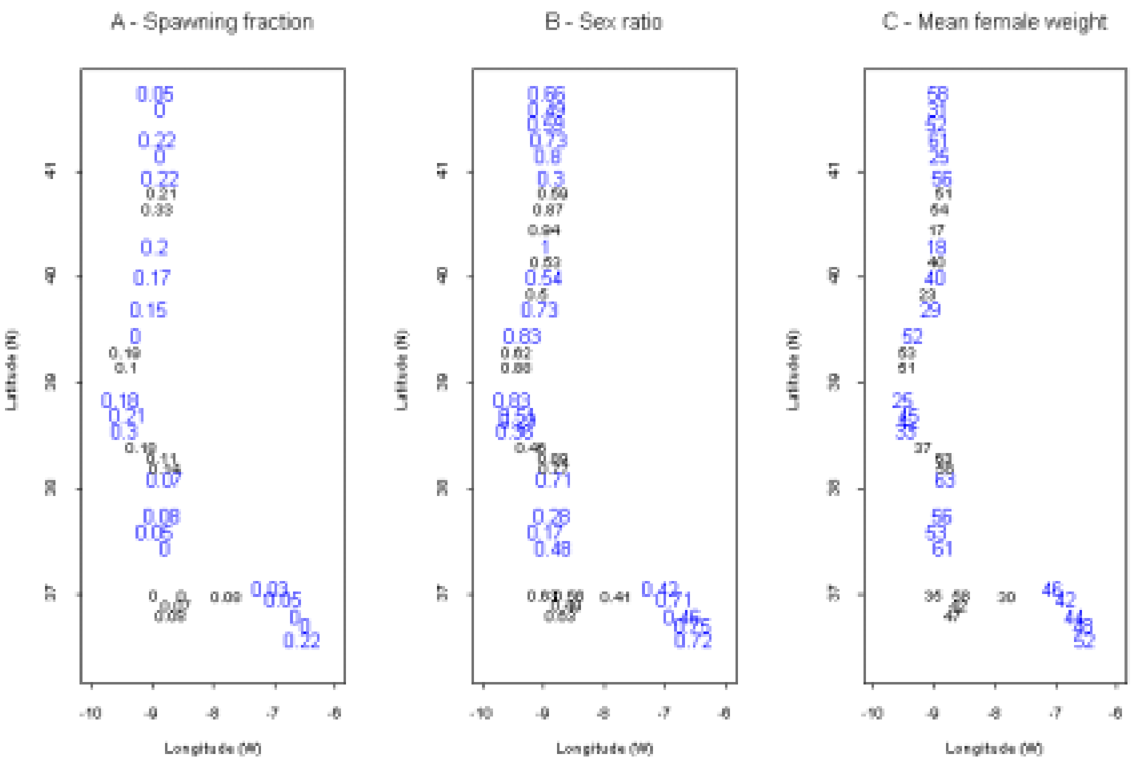


Figure 4.6.1: Spatial distribution of spawning fraction (a), sex ratio (b) and mean weight estimates per haul along the Portuguese DEPM survey area during January 1999. The location of the sampled hauls is slightly altered to reduce overlay. Values in larger font (or colour blue) correspond to demersal trawls, while values in normal font correspond to pelagic purse seines or pelagic trawls.