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The DEPM Estimation of Spawning-Stock Biomass for Sardine and Anchovy

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Contents

1   Introduction

1.1   Background

Lasker (1985) provides the first and most thorough report on the use of the Daily Egg Production Method (DEPM) for the estimation of the spawning biomass of small pelagic fish stocks. An update to this report is provided by Anonymous (1997), a compilation of research papers resulting from an EU concerted action on DEPM techniques. The present Cooperative Research Report is intended to complement to the above publications, reviewing the use of the method for sardine and anchovy in European waters and describing very recent developments in specific areas of DEPM sampling and estimation. Its contents are predominantly drawn from the most recent report of the ICES Study Group on the Estimation of Sardine and Anchovy Spawning Biomass (SGSBSA) that met in Malaga (Spain) during the summer of 2003 (ICES, 2003b). In addition, it includes sections from the first SGSBSA report that resulted from a meeting in Lisbon (Portugal) during the autumn of 2001 (ICES, 2002).

1.2   Contributions

The SGSBSA reports were based on valuable contributions from several national and international research projects, as well as the personal experience of the 24 scientists that participated in the two group meetings. Statistical methodology for the most appropriate use of egg incubation data in stage/age models, the ageing of staged eggs and the modelling of daily egg production and mortality through GAMs were developed as part of an EU project on GAMs (Study 99/080, http://www.ruwpa.st-and.ac.uk/depm/index.html) that was concluded within 2003. Additional research in the use of CUFES in ichthyoplankton surveys and a workshop for the calibration of sardine and anchovy egg staging were performed within the EU project PELASSES (EU Study 99/010). Most surveys presented in this report took place with the financial support of the EU. A sardine incubation experiment was performed by IEO (Spain) as part of national research activities, while most of the recent activities in Portugal (IPIMAR) were performed as part of the research project PELAGICOS (http://ipimar-iniap.ipimar.pt/pelagicos). Miguel Bernal (Spain), Emilia Cunha (Portugal), Leire Ibaibarriaga (Spain), Concha Franco (Spain), Paz Jimenez (Spain), Ana Lago de Lanzós (Spain), José Ramon Pérez (Spain), Luis Quintanilla (Spain), Maria Santos (Spain), Alexandra Silva (Portugal), Yorgos Stratoudakis (Portugal) and Andres Uriarte (Spain) contributed to the preparation of both SGSBSA reports, while Pablo Carrera (Spain), Kostas Ganias (Greece), Alberto García (Spain), Daniel Gaughan (Australia), John Hunter (USA), Mike Lonergan (UK), Placida Lopes (Portugal), Immaculada Martin (Spain), Cristina Nunes (Portugal), Eduardo Soares (Portugal), Yolanda Vila (Spain), and Juan Zwolinski (Portugal) contributed in one of the two reports. Finally, most of the methodological developments presented in Section 3 resulted from the valuable contribution of Simon Wood,

David Borchers, Mike Lonergan and Camila Dixon (all from the University of St Andrews, Scotland).

1.3   Report structure

Section 2 summarizes existing information on DEPM applications for sardine and anchovy in European waters. Sampling and estimation is described in more detail for the most recent surveys (2002) performed in Atlantic waters for sardine and anchovy and already reported to the SGSBSA. Section 3 is dedicated to the application of GAMs in DEPM estimation, summarizing and extending the findings of the EU project on GAMs that most Study Group members participated in. The underlying theory is briefly reported and the method is illustrated through worked examples, based on the sardine and anchovy surveys presented in the previous section. Section 4 considers advances in other methodological aspects of DEPM estimation. The first part of this section is dedicated to issues related to egg production sampling and estimation (use of CUFES, staging and ageing, etc.), while the second part is dedicated to adult parameters, with emphasis on sardine. Section 5 provides a comprehensive (but not exhaustive) reference list, while Annexes 1–4 provide illustrations from reference collections of egg and post-ovulatory follicle (POF) stages for sardine and anchovy.

2   DEPM surveys for sardine and anchovy

2.1   Introduction

The first part of the Section (Sections 2.2–2.3) summarizes the DEPM surveys that have been performed for sardine and anchovy in European waters. Emphasis is given to applications in Atlantic waters (where DEPM estimates are used routinely in stock assessment), but a brief description of known applications in the Mediterranean are also provided. The second part of the chapter (Sections 2.4 and 2.5) describes the most recent surveys in Atlantic waters (2002) in more detail, in order to demonstrate the survey and estimation methodology applied. Estimates are based on the traditional methods (Lasker, 1985; Hunter and Lo, 1997), which continues to provide the standard estimates of spawning-stock biomass for the purposes of stock assessment. However, results for 2002 should be compared to those obtained by the application of GAMs (Sections 3.3 and 3.4 for sardine and anchovy respectively), although GAM estimates of adult parameters and SSB are necessarily provisional (given that they were applied for the first time during the course of the most recent SGSBSA meeting). In the case of sardine, estimates based on mean survey values are compared to post-stratified and GAM-based estimates to clarify whether inappropriate sampling design under spatial structure in abundance and adult parameters can lead to biased biomass estimates (Stratoudakis and Fryer, 2000; ICES, 2002). In the case of anchovy, the presence of sufficient spatial structure to justify post-stratification for 2002 is explored and the results are compared with the long series of DEPM

long series of DEPM estimates and the acoustic results for 2002.

2.2 DEPM surveys for sardine (*Sardina pilchardus*)

2.2.1 Atlantic waters (Iberian Peninsula)

The method was first used to estimate the spawning biomass of the Atlanto-Iberian sardine stock in 1988 (Cunha *et al.*, 1992; García *et al.*, 1992) and then repeated in 1990, 1997, 1999 and 2002, based on coordinated surveys by Portugal and Spain (García *et al.*, 1991; García *et al.*, 1993; Cunha *et al.*, 1997; Lago de Lanzós *et al.*, 1998; Stratoudakis *et al.*, 2000; Bernal *et al.*, 2000; ICES, 2000; ICES 2002; ICES 2003b). Up to 1999 the surveys were based on informal contacts between IPIMAR (Portugal) and IEO (Spain). Since 2000 surveys are planned and executed under the auspices of ICES on a triennial basis (the next survey is planned for 2005 with financial support from the EU). Tables 2.2.1.1 and 2.2.1.2 provide sampling details for the sardine DEPM surveys performed until now by Portugal and Spain respectively, while Table 2.2.1.3 summarizes the coverage, sampling and estimation characteristics of each survey. The latter table demonstrates that the entire distribution area of the Atlanto-Iberian stock of sardine has only been sampled since 1997 (in 1988 the Gulf of Cadiz was not sampled and in 1990 there was no survey in Portugal). Further, the only survey where sampling intensity was good throughout the stock area, both for eggs (see Figure 2.4.1.1 for distribution of plankton samples) and adults (see Figures 2.2.1.1 and 2.2.1.2 for spatial distribution of adult samples in all Portuguese and Spanish DEPM surveys respectively) was the most recent one (2002). Tables 2.2.1.4–2.2.1.9 provide the estimates of egg production, mean female weight, batch fecundity, sex ratio, spawning fraction and spawning biomass respectively for all surveys (details on sampling and estimation in ICES 2000; ICES, 2002; ICES, 2003b and Section 2.4 of this report).

2.2.2 Mediterranean waters

The DEPM was recently applied to estimate the spawning biomass of sardine in the central Aegean and Ionian Seas (Somarakis *et al.*, 2001; Ganias, 2003). This was the first reported application of the DEPM to Mediterranean sardine, and presented particular interest and difficulties due to the peculiar topography of the survey area (many small-sized semi-enclosed gulfs), the biological heterogeneity and the small size of the sardine populations. Table 2.2.2.1 shows the spawning biomass and DEPM parameter estimates. Since the stocks of sardine in the central Aegean and Ionian Seas exhibited different spawning peaks, the survey area was geographically stratified.

Daily egg production was estimated using both eggs and yolk-sac larvae in order to improve the precision of the estimate. Batch fecundity was measured in hydrated, tertiary-yolk globule and migratory nucleus females because in Mediterranean sardine there exists a well-defined hiatus between the advanced batch and the stock

of smaller oocytes in the tertiary yolk-globule stage (Ganias *et al.*, 2004). The histological examination and comparative analysis of sardine follicles revealed three classes of POFs: day-0 (0–9.5 hrs), day-1 (24–33.5 hrs) and day-2 (48–57.5 hrs). Despite differences in season and temperature regimes, batch fecundity and spawning fraction estimates from the Aegean and Ionian Sea were similar. Compared to existing values for the Atlanto-Iberian sardine stock, the estimates of spawning fraction and relative fecundity were slightly lower in the Mediterranean, despite considerable differences in the mean female weight between Mediterranean and Atlantic populations.

2.3 DEPM surveys for anchovy (*Engraulis encrasicolus*)

2.3.1 Atlantic Waters (Bay of Biscay)

The DEPM has been regularly applied to the Bay of Biscay anchovy to estimate its biomass and population numbers at age (Motos *et al.*, 1991; Motos and Uriarte 1991; Motos 1994; Motos and Uriarte 1994; Motos 1996; Uriarte 2001). The series of DEPM estimates spans the period 1987–2003 (with a single gap in 1993) and is routinely used by ICES for the assessment of the Bay of Biscay anchovy stock (e.g. ICES 2003a). These surveys have been undertaken by AZTI in cooperation with the Spanish (IEO) and French (IFREMER) institutes of marine research (Uriarte *et al.*, 1998; Uriarte *et al.*, 1999a; Uriarte *et al.*, 1999b).

In order to obtain estimates of daily egg production and specific fecundity, two surveys (an egg and an adult cruise) have usually been carried out at peak spawning time (May/June) over the expected spawning area of the Bay of Biscay anchovy population. This area extends over the Southeast area of the Bay of Biscay, with limits at 5°W in the Iberian Coast and at 47°N in the French Coast (e.g. Figure 2.5.1.1). Adult sampling during the survey (e.g. Figure 2.5.1.2) is usually complemented with samples taken opportunistically on board the Spanish and French commercial fishing fleets of purse seiners and pelagic trawlers (Uriarte *et al.*, 1996). In 1987, 1988, 1996, 1999 and 2000 no adult surveys took place and in those cases the opportunistic sampling obtained through the commercial fleet was the only source for adult information: in the first two years, commercial samples were used to derive the daily fecundity estimates of the population, whereas in the latter three years those samples were not used at all and a regression method assuming constant daily fecundity were used instead. The total set of DEPM estimates is presented in Tables 2.3.1.1 and 2.3.1.2.

Egg sampling is based on the CalVET net (Smith *et al.*, 1985) and follows a systematic central sampling scheme. Eggs from both CalVET samplers are used in the analysis (Uriarte and Motos, 1998), often giving rise to the term PAIROVET to distinguish from applications where only one CalVET sampler is used. From the whole set of adult samples gathered during the adult survey, a subset is chosen for final processing with the criterion of the capture date being within ±5 days of the egg sam-

pling in the same area. The opportunistic adult samples from the fleet permit to expand the area of sampling coverage. In general, a broad spatial structure is evident in the adult population, with smaller fish tending to be closer to the shore. This leads generally to post-stratification of the DEPM estimation procedure (for P0 and adult parameters), with two or three spatial strata being defined according to depth. Adult parameters are unweighted averages of the strata. An extensive review and description of DEPM adult parameter estimation is provided in Uriarte et al. (1999a). Finally, the DEPM formulation has been extended to provide spawning-stock population at age (SSPa) estimates with variances inferred from the delta method (Uriarte, 2001). Sensitivity analyses on the influence of the stratification and weighting factors are routinely performed. Regression methods for the estimation of SSB in the absence of adult sampling have been applied since 1996 (Uriarte et al., 1999a, b) based on the relationships between spawning area, daily egg production per unit surface and the biomass obtained in years where complete DEPM is applied.

2.3.2 Mediterranean waters

The DEPM has been used to evaluate the anchovy spawning biomass of the Catalan Sea in 1990 (Palomera and Pertierra, 1993), Catalan Sea-Gulf of Lions in 1993 and 1994 (García and Palomera, 1996; Olivar et al., 2001), Ligurian-North Tyrrhenian seas in 1993 (García and Palomera, 1996), Aegean sea in 1993 (Tsimenides et al., 1995) and 1999 (Somarakis et al., 2002), Ionian Sea in 1999 (Somarakis et al., 2002), south-western Adriatic sea in 1994 (Casavola, 1998), and Sicilian Channel in 1998, 1999 (Quintanilla and García, 2001) and 2000 (Quintanilla, pers. comm.).

Spawning biomass and DEPM parameters estimates in the Mediterranean show high variability both within (inter-seasonal and inter-annual variations) and between regions (Table 2.3.2.1). Different methodologies can partially explain these variations. In the Aegean Sea, where exceptionally high egg production estimates were found in 1993, oblique Bongo tows were used instead of the vertical CalVET tows and the spawning area was not entirely covered. Also, different temperatures were used to assign ages to eggs in different regions (sub-surface temperature in the Aegean Sea, mean temperature of the first 10 m or 20 m in the Sicilian Channel and Catalan Sea respectively). Sampling with purse seiners instead of pelagic trawls, and commercial instead of research vessels in the Aegean and Catalan Seas, could restrict the sampling to commercial fishing grounds and explain some adult parameter differences. The use of the methodology of Laroche and Richardson (1980) instead of the hydrated oocyte method (Hunter et al., 1985) may explain the high relative fecundity in the Catalan Sea. Differences in spawning fraction estimation method could also explain some inter-regional differences in this parameter.

Overall, the parameters with the highest variance are the daily egg production (P) and the spawning fraction (S), while the large variation in egg mortality rates should also be noted. The temperature range during the peak spawning period may vary from 16° to 25°C in some Mediterranean areas. Egg development duration and post-ovulatory follicle degeneration can present great differences within this temperature range, thus affecting these parameter estimates.

2.4 The 2002 Atlanto-Iberian sardine survey

2.4.1 Survey details

The most recent DEPM survey for Atlanto-Iberian sardine took place in 2002, covering the entire distribution area of the Atlanto-Iberian stock. The region from the Gulf of Cadiz to the northern Portugal/Spain border (Minho River) was surveyed by IPIMAR, while IEO sampled the north and north-western Iberian Peninsula and the Bay of Biscay (up to 45°N). The Portuguese survey (7 January – 8 February 2002) was carried out onboard RV "Noruega", while the Spanish survey (18 March – 6 April 2003) was conducted onboard RV "Cornide de Saavedra" for the plankton component and RV "Thalassa" for the adult component.

Both national surveys consisted of ichthyoplankton sampling on fixed (CalVET) and underway (CUFES) stations (Figure 2.4.1.1). The CalVET hauls were performed using a net with 150 μm mesh size, operating vertically from 150 m (Portugal) or 100 m (Spain) to the surface. In shallower areas, the net was towed from 5 m above the bottom to the surface. CUFES samples were used to delimit sardine spawning grounds and to modify adaptively the intensity of CalVET sampling. In the Portuguese survey, sampling depths and towing efficiency of the hauls were controlled with a sensor (Minilog) fitted on the net line; while sampled volume was calculated from towing length and stray angle (see ICES, 2002). Sea surface (3 m depth) temperature, salinity and fluorescence were determined using sensors located at the entrance of the CUFES concentrator, while broad indications about the thermal structure of the water column were obtained by a Minilog sensor coupled to the CalVET net. In the Spanish survey, General Oceanics Flowmeters were used to record the towing length and estimate the sampled water volume (assuming a filtration efficiency of 100%), while a Minilog was used to record maximum sampling depth. A continuous record of temperature and salinity was obtained from a thermosalinometer coupled to CUFES, while CTD profiles were obtained in each CalVET station.

Sardine eggs were identified and counted on board immediately after collection. All samples were fixed in 4% buffered formaldehyde solution for subsequent verification of egg counts and staging in the laboratory. The decision on the distance between CalVET stations was based on presence or absence of sardine eggs on the previous CalVET and/or CUFES stations. In total, 769 CalVET (473 in Portugal and 296 in Spain) and 1185 CUFES (546 in Portugal and 639 in Spain) samples were obtained during the surveys. Daily egg production was determined using data from the CalVET performed along transects spaced 8 nm apart. Within the same transect the distance between stations was 3 nm for CUFES sampling and varied between 3 and 6 nm for CalVET hauls. In the Spanish survey (that used for the first time CUFES on-

board RV "Cornide de Saavedra") a calibration exercise was carried out in French waters (see Section 4.1.3) to test the performance of CUFES as a quantitative sampler. Finally, sardine egg incubation experiments were attempted in both surveys, but with poor results. In the Portuguese survey the eggs did not develop (showing morphological characteristics of unfertilized eggs), while in the Spanish experiment eggs only developed up to stage VI. In both cases mature sperm seemed to be a limiting factor, with few ripe males and small quantities of sperm being collected in hauls with large number of hydrated females.

Adult fish samples (Figure 2.4.1.2) were obtained by demersal and pelagic trawls (research vessels) and purse seining (commercial vessels). Overall, 32 samples were obtained in the Spanish survey (4 in the French coast) and 74 in the Portuguese (roughly proportional to regional sardine abundance), providing the most comprehensive adult DEPM sampling of the series. Most samples were obtained in the inner shelf, with a mean fishing depth of 45 m. Random samples of 80 and 100 fish were aimed in the Spanish and Portuguese survey respectively (although most commercial samples off Portugal only contained 50 fish). For fish collected onboard the research vessels, biological sampling was immediately performed and gonads of macroscopically identified mature females were preserved in individual jars with formaldehyde solution for further processing in the laboratory. Fish collected onboard commercial Portuguese vessels were immediately preserved and on land the abdomen was lightly slit to allow better fixation of the gonad. In the latter case, biological sampling was performed on preserved fish and conversion factors were applied to transform preserved to fresh weight. For the estimation of batch fecundity, extra hydrated females were collected in several hauls performed by the research vessels. Preserved female gonads were treated histologically for the estimation of spawning fraction (Pérez *et al.*, 1992a; Quintanilla and Pérez, 2000a) and the elimination of gonads with POFs from the estimation of batch fecundity. Batch fecundity was estimated using the gravimetric method (MacGregor, 1957) by counting the hydrated oocytes (Hunter *et al.*, 1985; Pérez *et al.*, 1992b; Quintanilla and Pérez, 2000b; Zwolinski *et al.*, 2001).

2.4.2 Egg production estimation

Egg production estimates from the 2002 sardine surveys have already been reported (see Table 2.2.1.4 and ICES, 2003a). These estimates were used to obtain spawning biomass estimates for the Spanish and Portuguese surveys and to compare with GAM-based production estimates (see Section 3.3.1). However, to explore the impact of spatial structure in the 2002 survey, post-stratified estimates of egg production were obtained in the course of the last SGSBSA meeting (ICES, 2003b). These results are reported in Section 2.4.4.

2.4.3 Adult parameter and spawning biomass estimation

Sardine adult DEPM parameters and spawning biomass are separately estimated for the Portuguese and Spanish

survey of 2002, without considering their spatial structure (i.e., without post-stratification), in line with the estimates that have been provided so far for the 1997 and 1999 surveys (however, see Section 2.4.4). All estimates refer to mature fish (i.e., maturity stage II and above, according to the rationale described in Section 4.3.1.4), including those inactive. Estimation for the Spanish survey excludes the 4 adult samples that were collected in the French coast (outside the stock area).

Mean weight (W): In the Spanish survey, female weight was estimated from gonad-free weight using the linear model $W = -1.304 + 1.094 \ W^*$ ($R^2 = 0.98$). The model was fitted using data from 520 non-hydrated females collected during the 2002 survey. Mean female weight in the Spanish survey was 75.0 gr (CV = 5%), using data from 28 hauls. The 2002 estimate is higher and considerably more precise than the 1999 one (66.0 gr, CV = 41%), when data from only 6 hauls were used. In the Portuguese survey, mean female weight was estimated from the observed female weight of non-hydrated fish (for rationale see ICES, 2003b). Mean female weight in the Portuguese survey was 44.3 gr (CV = 5%), using data from 70 hauls. The 2002 estimate is very similar to the 1999 one (44.4 gr, CV = 5%, n = 40). The lack of improvement in the precision of the 2002 estimate (despite the duplication of sampling effort) is largely due to the presence of very large fish in a single commercial haul off central Portugal. Removing this haul from the estimation leads to a slightly lower estimate of mean weight (42.9 gr) and increases its precision (CV = 3.7%, n=69). However, this haul was maintained in the final estimation, since there was nothing apparently erroneous with this outlier.

Batch fecundity (F): In total, 113 hydrated females without post-ovulatory follicles (POFs) were available for batch fecundity estimation in Portugal and 73 in Spain. In Spain, estimation followed the standard weighted linear regression model (batch fecundity as a function of gonad-free weight (W^*), weighted by the inverse of W^*) and the following relationship was obtained:

Spanish 2002 survey: $F = -3255 + 436.25 \ W^*$ ($R^2 = 63\%$)

The standard error was 713 for the intercept and 38.9 for the slope. The intercept estimate was non-significant (t =-1.113, p> 0.05). If the relationship is forced through the origin, the slope (which then provides an estimate of relative fecundity) is 394.2 with a standard error of 9.4. Following the above model, mean batch fecundity for the 2002 Spanish survey was estimated to be 26089 (CV = 6%).

In Portugal, 2 linear (with and without weighing) and two generalised linear models (with a Gamma or a negative binomial error distribution and an identity link) were considered (Table 2.4.3.1). The model parameters and the resulting mean estimates of batch fecundity were very similar in all cases (the largest discrepancy in mean batch fecundity was <0.5% among the four models considered). However, the two GLMs led to higher proportions of explained variation, had smaller standard errors associated to the parameter estimates and provided considerably improved residual inspection plots (Figures

2.4.3.1 and 2.4.3.2). For estimation purposes, the GLM with a Gamma distribution and an identity link was chosen, given that a model with the same parameterisation has also been successfully used to describe mackerel fecundity (Darby, pers. comm.):

Portuguese 2002 survey: $F = -4286.2 + 464.3\ W^*$ ($R^2 = 81\%$).

Following the above model, mean batch fecundity for the 2002 Portuguese survey was estimated to be 14 255 (CV = 6%). Figure 2.4.3.3 shows the relationship between female weight and batch fecundity in the 2002 survey in comparison to all previous sardine DEPM surveys. It clearly shows that relative fecundity in 2002 was significantly lower than in previous years, and relative fecundity was the lowest ever reported off Portugal. For the Portuguese 2002 survey, this agrees with other indicators (see Section 4.3.2.2) to suggest that the survey took place during uncharacteristic conditions for sardine spawning. However, relative fecundity was also low in the March 2002 Spanish survey, probably indicating bioenergetic limitations in sardine reproduction during that year.

Spawning fraction (S): Spawning fraction was estimated using the composite sample of day 1 and day 2 POFs. In total, 352 ovaries from 19 hauls were used in the Spanish survey and 1350 ovaries from 67 hauls in the Portuguese survey. The estimated spawning fraction for the Spanish survey was 0.127 (CV = 21%) and for the Portuguese survey 0.030 (CV = 21%). In both cases, these are the lowest spawning fractions ever reported. In the Portuguese case, this is probably the lowest S estimate that has ever been reported for a sardine species during peak spawning. A consequence of this very low estimate is that the precision of the Portuguese estimate remains low, despite the effective duplication of the number of histologically examined ovaries. It should be noted that if S in 2002 had remained at the levels observed in 1999 (around 0.10), the increased level of adult sampling would have reduced the CV of this parameter estimate to around 10–12% (Picquelle, 1985).

Sex ratio (R): Sex ratio was estimated as the weight ratio of females in the mature population. Given that male gonads were only classified macroscopically, sex ratio was estimated based on individuals that were macroscopically identified in a maturity stage larger than I (i.e., traditional definition of mature fish for DEPM purposes). In total, 2222 mature fish were used for the estimation of sex ratio in the Spanish survey and 4481 in the Portuguese survey. The estimated sex ratio for the Spanish survey was 0.542 (CV= 9%) and for the Portuguese survey 0.611 (CV = 3%). These estimates are very similar to those obtained in 1999, but in both surveys the 2002 estimates are more precise due to the larger number of independent samples.

Spawning-stock Biomass (SSB): Table 2.4.3.2 summarizes the DEPM parameter estimates for the Portuguese and Spanish 2002 surveys respectively and calculates the resulting estimate of spawning biomass. Overall, the 2002 DEPM survey for the Atlanto-Iberian stock leads to an SSB estimate of 382.3 Ktonnes, with a CV of 37%. Despite the lowest ever egg production in sardine DEPM surveys, the 2002 estimate of SSB is the highest of the existing estimates, due to the particularly low daily

fecundity observed in that year. Also, despite the considerable intensification of sampling in both national surveys, the precision of the biomass estimate remains relatively low (for assessment purposes), mainly due to the low precision in the egg production and spawning fraction estimates.

The low precision in the egg production estimates seems to be partly inherent to the use of the traditional estimator. Section 3.3.1 shows that considerable improvements in the precision of this parameter can be achieved through the use of GAMs, where CVs in the order of 20% or below are achieved without evidence of bias. This was also the case for 2002, where the GAM-based estimate reduced the CV of the Iberian egg production estimate to 16%. The precision in the spawning fraction estimate of the Spanish survey was close to that anticipated (Picquelle, 1985) for the observed level of sampling effort and spawning activity (S = 0.13, n = 19). However, the extremely low estimate of spawning fraction in the Portuguese survey was something that could have not been anticipated during the planning of the survey, given that estimates below 6% had never been reported for sardine in peak spawning. The large sampling effort in the 2002 Portuguese survey and the wealth of auxiliary information collected along it, leave little doubt that the very low spawning fraction in 2002 resulted from unfavourable conditions to sardine spawning. Despite the low levels of precision, the large sampling effort in 2002 precludes the presence of sampling artefacts, suggesting that large inter-annual variations in spawning activity is an inherent feature of the Atlanto-Iberian stock of sardine. Further, the information collected in that survey can contribute to improve the understanding on sardine spawning dynamics. For example, Figure 2.4.3.4 (left) shows that spawning activity during the Portuguese 2002 survey was very patchy, mainly concentrated in small areas of high phytoplankton densities (data obtained from CUFES), while Figure 2.4.3.4 (right) suggests that the smooth relation between chlorophyll fluorescence and observed spawning fraction (GAM with binomial error distribution) is significant.

2.4.4 Spatial structure in recent sardine DEPM surveys and comparison with acoustics

Traditional estimation of spawning biomass in the DEPM is entirely based on the selected survey design, using design-based estimators. Judgement sampling has been recommended as a way of achieving sampling proportional to local fish densities and reliable estimation of spawning biomass when there are spatial differences in abundance and in the DEPM adult parameters. DEPM surveys for adult sardine parameters have been considered to follow the principles of judgment sampling, using acoustic density as an indicator of local fish densities (Cunha *et al.*, 1992; García *et al.*, 1991; García *et al.*, 1992; Cunha *et al.*, 1997). However, the exact procedure for allocating sampling effort according to the acoustic signal has never been described, and in most surveys the regional allocation of sampling effort does not reflect the estimates of regional abundance obtained from the DEPM (Spain) or from acoustic surveys (Portugal). In 1999, the Portuguese DEPM survey further deviated

from the principles of judgement sampling since, to increase sampling effort, additional samples were collected opportunistically from commercial vessels fishing near the research vessel.

In addition, a major assumption in DEPM estimation 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 an estimation is performed independently for each stratum. Post-stratification has been used in the Spanish DEPM surveys of 1988 and 1990 (García *et al.*, 1992; García *et al.*, 1991), where considerable differences in mean weight and spawning fraction were observed between Galician and Eastern Cantabria. In 1999 post-stratification was not considered in the Spanish DEPM surveys due to the small number of fishing stations available per region. On the other hand, post-stratification has never been used in the Portuguese DEPM surveys. In 1988 and 1997 there was insufficient information to stratify (in 1988 there were only three fishing stations south of Lisbon). In 1999, post-stratification was not used for comparability with the previous two surveys (ICES, 2000).

Under an adequate survey design (i.e., sampling effort proportional to local abundance), post-stratification should only lead to more precise estimates. Stratoudakis and Fryer (2000) demonstrated the impact of inadequate survey design and post-stratification on the DEPM estimation of sardine spawning biomass off Portugal in 1999. Post-stratifying the Portuguese 1999 DEPM survey into two strata (western and southern) increased the SSB estimate by nearly 50%. The origin of this large difference was explored in a simulation exercise. A series of populations consisting of two strata were constructed, in which fish abundance and mean spawning fraction in each stratum were allowed to vary widely, and where egg production, sex ratio and batch fecundity were assumed known without error. Each population was sampled using simple random sampling and various forms of stratified random sampling (allocation proportional to survey area, to fish abundance, and optimal allocation). Ignoring spatial structure in spawning fraction led to very biased and imprecise estimates of fish abundance. In the population scenario that most closely resembled the 1999 Portuguese DEPM survey, the bias was −25%, suggesting that unstratified estimation underestimates the true SSB. Stratified random sampling with allocation proportional and optimal allocation outperformed allocation proportional to area and were robust to moderate levels of misallocation.

To evaluate the impact of sampling effort allocation and spatial structure in the 2002 survey, estimation was repeated using post-stratification (Figure 2.4.4.1). Post-stratification in Portugal considered the two strata used by Stratoudakis and Fryer (2000), where the survey was divided into a western and a southern stratum. Post-stratification in Spain used the three strata previously considered by García *et al.* (1991 and 1992). Non-linear weighted least squares (*nls* library in R, weights to account for the uneven spacing of samples) were used to obtain post-stratified estimates of egg production for the Portuguese 2002 survey. It should be noted that this es-

timator is not the one proposed by the Study Group (GLM estimator), but was maintained to obtain comparable results and concentrate on the impact of spatial structure in daily fecundity. Post-stratified estimates of egg production in the Spanish survey were obtained using the recommended estimator (GLM with negative binomial error distribution, an offset accounting for the effective area of the sampler and weights to account for the uneven spacing of samples). To test the significance of post-stratification linear and generalized liner models (GLMs) were used for the 4 adult parameters, with stratum being the explanatory variable (2 and 3 level factor for the Portuguese and Spanish survey respectively). A linear model was used for female mean weight and batch fecundity, where observations were weighed by the number of mature females in each sample. A GLM with a binomial error distribution was used for spawning fraction and sex ratio, where the binomial denominator was the number of histologically examined females and the number of mature fish in the sample respectively.

Table 2.4.4.1 shows the post-stratified estimates of egg density, mortality and production for the Spanish and Portuguese 2002 surveys. Results are not provided for Galicia, since very few stations with eggs were observed, not permitting the fitting of a GLM. Also, the unstratified Spanish estimate is slightly higher than that reported in ICES (2003), due to modifications in the estimation of positive area and the use of stations rather than transects in estimation. Post-stratification led to an overall estimate of egg production 8% higher than under no stratification (6% higher in Portugal and 13% higher in Spain), but the two estimates are not significantly different. Table 2.4.4.2 shows the significance of the stratum effect in the models fitted to each adult parameter from the Portuguese and Spanish 2002 surveys. In the Portuguese survey, there is a significant spatial effect in mean female weight, which is also reflected in batch fecundity. In the Spanish survey, female weight and batch fecundity do not differ significantly among strata, but on the other hand significant differences among strata were found in spawning fraction and sex ratio.

Table 2.4.4.3 shows the estimates of all DEPM parameters and spawning biomass in each stratum for the 2002 survey. Overall, the post-stratified estimate of sardine SSB is 441.6 thousand tonnes (CV=28%), which is 16% higher than the unstratified estimate of Table 2.4.3.2. The post-stratified estimate also leads to a 9% reduction in the estimated CV. This estimate is very close to the GAM-based estimate for 2002 (466.2 thousand tonnes, see Section 3.4.1). Although the stratified estimate is not significantly different from the unstratified one, the close agreement with the GAM estimate and the evidence of significant spatial structure within the survey area suggest that the former provides a more reliable estimate of sardine abundance. Further, the post-stratified Portuguese estimate and the GAM estimate for Portugal are for the first time in relatively close agreement with the spawning biomass estimate from the March 2002 Portuguese acoustic survey (Table 2.4.4.4). However, considerable work is still needed in the comparison between DEPM and acoustic estimates. For example, the discrepancy between the DEPM and the November acoustic survey is still large (the latter being al-

most double), while the Spanish DEPM estimate is considerably lower than the Spanish acoustic one (about one third). In the future, such comparisons would be facilitated if estimates of spawning biomass would be routinely provided for acoustic surveys.

2.5 The 2002 Biscay anchovy survey

2.5.1 Survey details

The most recent DEPM survey for anchovy in the Bay of Biscay that has been reported to the SGSBSA took place in May 2002, using distinct research vessels for ichthyoplankton and adult sampling. The egg survey was undertaken by AZTI (6/5 –21/5/2002) on board RV "Investigador" (Figure 2.5.1.1). In total, 376 vertical plankton hauls were performed using a PAIROVET net (2-Calvet nets, of a mouth aperture of 0.05 m² each, Smith *et al.*, 1985). The frame was equipped with nets of 150 µm. The net was lowered to 100 m, or 5 m above the bottom in shallower waters, left at maximum depth during 10 seconds (for stabilisation), then retrieved to the surface at a rate of approximately 1 m/sec. A 45 kg depressor was used to allow for correctly deploying the net. A flowmeter (G.O. 2030R) was used to estimate the volume of water sampled during the tow.

The strategy of egg sampling was identical to that used in previous surveys (Uriarte *et al.*, 1999a), i.e., a systematic central sampling scheme with random origin and with different sampling densities according to egg abundance. Sampling stations (3 miles apart) were located along transects (15 miles apart) perpendicular to the coast. Concurrently with each PAIROVET station date, GMT time, position and variables such as surface temperature, surface salinity, wind direction and force were recorded. Temperature, salinity and chlorophyll profiles were obtained in selected stations by means of CTD casts. Around 1000 underway CUFES samples were collected during the survey. They were collected for 1.5 nm before and after each PAIROVET station, each PAIROVET sample thus being associated to 2 CUFES samples. Immediately after the haul, the net was washed and the content of both nets was concentrated and fixed in a 4% buffered formaldehyde solution, in seawater and kept at 50 ml jars. Before reaching the end of each transect, samples were checked under the microscope to identify the presence/absence of anchovy eggs. This information was used to continue/discontinue the sampling schedule or to intensify/relax the sampling intensity by doing stations 3/6 miles apart or increasing the number of transects by adding inter-transects (7.5 miles apart).

Egg samples were analysed onboard for sorting, identification and counting of anchovy eggs, after leaving them at least 6 hours of fixation. Afterwards, in the laboratory, the sorting made at sea was checked and completed when necessary and anchovy eggs were staged (Moser and Ahlstrom, 1985). The spawning area was delimited with the outer zero anchovy egg stations and it contained some inner zero egg stations embedded on it (Picquelle and Stauffer, 1985). Following the systematic central sampling scheme (Cochran, 1977) each station

was located in the centre of a rectangle. Egg abundance at a particular station was assumed to represent the abundance in the whole rectangle. The area represented by each station was calculated. A standard station has a surface of 45 squared nautical miles (154 km²) = 3 (distance between two consecutive stations) x 15 (distance between two consecutive transects) nautical miles. Since sampling was adaptive, station area changed according to sampling intensity. Processing methods used in egg samples followed standard procedures (Lasker, 1985) and are described in detail in previous papers (see for example, Motos *et al.*, 1991; 1994).

Adult anchovy samples for DEPM purposes were obtained from pelagic trawl hauls during the 2002 Bay of Biscay acoustic survey (IFREMER) onboard RV "Thalassa". Additional adult anchovy samples were collected onboard commercial purse-seiners in an opportunistic manner during the time of the egg survey (Figure 2.5.1.2). Onboard the research vessel, immediately after fishing, anchovy were sorted from the bulk of the catch and a sample of around 2 kg was randomly chosen. Sampling finished as soon as a minimum of 1 kg, or 60 anchovies were sexed, and 25 non-hydrated females (NHF) were preserved. Sampling was also stopped when more than 120 anchovies had to be sexed to achieve the target 25 NHF. Samples collected on board commercial vessels were also selected at random immediately after the catch, put into jars filled with 4% buffered formaldehyde and afterwards they were sent to the laboratory for further processing. Adult samples from the commercial fleet were selected according to their concurrence in space and time with egg sampling. All adult samples collected in a particular area three days before or after egg sampling in the same area were rejected. In total, 35 adult samples were processed, 24 from the specific adult survey and 11 from the commercial fleet.

2.5.2 Egg production estimation

The total area was calculated as the sum of the representative area of each station. The spawning area was delimited with the outer zero anchovy egg stations. It contained some inner zero egg stations embedded on it (Picquelle and Stauffer, 1985) and three stations with eggs were encountered out of this area. (Figure 2.5.2.1) The spawning area was calculated as the sum of the representative area of those stations. The total sampling area was 56 176 km² and the spawning area was 35 980 km². Staged eggs were classified into daily cohorts using the traditional method by Lo (1985) and the new stage-to-age method described in Section 3.2.1. The egg mortality exponential curve was fitted to the daily cohort abundances and mean ages as a weighted non linear regression model (as traditionally done) and as a generalised linear model (GLM) with negative binomial error distribution and log link (as recommended by SGSBSA in 2002). In all cases only stations in the positive stratum were used and eggs with an assigned age lower than 4 h and higher than 90% of the incubation time (94.32 hours.) were removed to avoid possible bias on the final daily egg production and mortality rate estimates. Figures 2.5.2.1 and 2.5.2.2 show the fitted curves, whereas total daily egg production estimate for each method, with

the correspondent coefficient of variation in brackets, are shown in Table 2.5.2.1.

2.5.3 Adult parameters and spawning biomass estimation

Mean female weight (W): Body weight of anchovies was corrected for weight gain due to conservation in formaldehyde by multiplying it by 0.98 (taking into account the elapsed time between preservation and processing). Formaldehyde total length was also corrected by a factor equal to 1.02, calculated from previous and current survey samples. Total weight of hydrated females was corrected for the increase of weight due to hydration. Data on gonad-free-weight (Wgf) and correspondent total weight (W) of non-hydrated females from the current survey were related by a linear regression model:

$$W = -0.4072 + 1.0965 * Wgf \qquad n=760, R^2=99.6\%$$

Gonad-free-weight of hydrated anchovies was transformed to total weight using the above model. Figure 2.5.3.1 shows the mean female weight per haul. There is a gradient from the coast to offshore, with low weight females near the coast and higher weights offshore.

Sex Ratio (R): Given the large variability of the sex ratio among samples and taking into account that for most of the years when the DEPM has been applied to this population the final sex ratio estimate (in numbers) has come out to be not significantly different from 50%, since 1994 the proportion of (mature) females per sample has been assumed to be equal to 1:1 in numbers. Hence, R was adopted as the average sample ratio between the mean female weight and the sum of the mean female and male weights of the anchovies in each of the samples.

Batch fecundity (F): Following Hunter *et al.* (1985), 111 hydrated females (9 to 49 grams gonad-free weight) were examined and the hydrated oocytes were counted. A linear regression model between gonad-free weight and batch fecundity was fitted to the subset of hydrated females without POFs and used to calculate the batch fecundity of all mature females. Given the spatial structure observed for the mean female weight, two strata were considered and a comparison of regression lines was performed to check for differences between strata in the gonad-free weight and batch fecundity relationship. The first stratum (NE or coastal stratum) was defined from 44°30'N to the North and from the 100 m contour line to the coast. The second stratum (RE or oceanic stratum) was the remaining area. The NE stratum had 11 adult samples and the RE had 24 samples, from which two samples (40 females) and five samples (62 females) respectively were selected for the analysis. After removing five outliers, the analysis showed that there were no significant differences between the two strata (ANCOVA, probability of equal slopes 0.5328, probability of equal intercepts 0.3433, Figure 2.5.3.2), thus a unique area was considered for the final estimation of anchovy batch fecundity in 2002. The resulting linear regression model (Figure 2.5.3.3) was:

$$F = -1984.74 + 563.42* Wgf \qquad n = 80, R^2 = 0.70$$

The batch fecundity estimate was computed as the average of the batch fecundity estimates for the females of each sample as derived from the gonad free weight – batch fecundity relationship.

Spawning Fraction (S): Spawning of Bay of Biscay anchovy usually takes place at about midnight (Motos, 1994), so a daily cycle of spawning is defined from 7 p.m. to 7 a.m., and the stages of gonads according to the oocytes and the follicles (pre and postovulatory) are defined as follows (Motos, 1996):

- Day-M: Females caught in the period going from 20:00 to 7:00 hours showing gonads with oocytes in the nuclear migration stage, which evidences that spawning will take place the following night. This corresponds to pre-spawning females.

- Day-0: Females that will spawn, are spawning or have spawned the day of capture (from 7:00 to 7:00 of the next day), which typically show at the beginning oocytes with early or advanced nuclear migration, later on hydration and finish with young POFs.

- Day-1: follicles of females that spawned the night before capture (7 to 30 hours old).

- Day-2: follicles of females that spawned 2 nights before capture (from 31 to 54 hours old).

- Day-3+: follicles of females that spawned 3 nights or more before capture (> 55 hours old).

Specific criteria to classify Bay of Biscay anchovy ovaries into the above categories were developed (Motos, 1994, Sanz and Santiago, pers. comm.).

Histological slides of 872 ovaries of mature females were obtained from the 35 adult samples. Ovaries of mature females were weighted, stored in formaldehyde and, subsequently, processed histologically. After embedding small ovary sections in resin, 3 μm slides were cut and stained with haematoxylin-eosin. Slides were screened under the microscope to classify them according to the above criteria. Once the ovaries of female anchovies were classified the estimate of spawning fraction per sample was made according to the incidence of postovulatory follicles 1 and 2 days old among mature females. The method described by Picquelle and Stauffer (1985) was applied to estimate thee incidence of spawning 1 and 2 days before and the adopted value per sample was the average between those two estimates. Females showing Day M and Day 0 follicles were corrected for over-sampling.

Biomass estimation: Population at age estimates were derived from the mean weight, the length distribution and the age composition of the anchovies per sample; the latter being obtained by independent otolith sampling per sample or by applying an ALK to the sample length distribution (when no otoliths were available). For the 24 samples arising from the acoustic survey, the ALK provided by Poisson and Massé (2002) was applied, whereas for the 11 samples coming from the purse seine fleet, the ALK made at AZTI from the routine sampling of the landings of this fleet in May and June 2002 (350 otoliths) was used. Initially, spawning-stock biomass and population at age were estimated considering a unique stratum

and no particular differential weighting was applied to the samples for the adult parameter estimates (Table 2.5.3.1). Afterwards, two strata (coastal and oceanic, as defined for batch fecundity) were considered to check whether the adult parameters to estimate the daily fecundity (DF) were different between strata (Table 2.5.3.2). As no differences were found in the reproductive parameters of both strata, a single pooled area was adopted for the estimation of the DF. The final estimate of spawning-stock biomass (SSB) was 30 700 tonnes with a CV of 13% (Table 2.5.3.1).

Table 2.5.3.2 also allows an inspection on the spatial distribution of biomass and the age classes by spatial strata. Biomass was higher in the Oceanic stratum and Age 2 dominated the population in all regions. Age 1 was more abundant in the North-eastern coastal stratum than in the rest. Table 2.5.3.3 gives the mean weight and length at age by region and overall. For the estimation of population at age the assumption whether the sampling was balanced or not was also checked. Derivation of the weighting factors considered per sample is shown in Table 2.5.3.4. Table 2.5.3.5 shows the sensitivity on this assumption of the biomass and the population at age estimates. The biomass remains almost unchanged whether equal (un-weighted) or differential adult weighting factors (weighted) are used in the adult parameter estimates (balanced or unbalanced assumptions), while the population at age estimates are far more sensitive to the procedure adopted. This suggests that SSB estimates are robust to the assumptions about the type of adult sampling available, but not the population estimates. This is due to the fact that Daily Fecundity is rather insensitive to the weighting factors since the assumption of constant DF regardless of area or size of the fishes seems to be correct (Table 2.5.3.2), whereas the population at age estimates are heavily dependent on the size of the fishes and hence on the balance of the weighting factors among samples. No differential weighting the adult samples would have overestimated the overall mean weight of samples by 3%, leading to a symmetrical underestimate of the population in numbers, at the expenses of a reduction of about 11% of the population of one-year-old anchovies. Hence, sampling was considered to be unbalanced for the purposes of number at age estimation.

2.5.4 Comparison with previous estimates and general considerations

The traditional procedures in the DEPM for estimating P_0 comprises the use of non linear regression for fitting the egg mortality curve under the assumption of Gaussian errors on the egg abundance for the different cohorts observed per sample. In addition, staged eggs are converted into daily cohort densities through Lo's ageing method. SGSBSA (ICES, 2002) recommended the use of GLMs for fitting the egg mortality curve for the estimation of P_0 and Z and the use of the Bayesian procedure for assigning ages to stages. Table 2.5.4.1 shows that the traditional biomass estimate is rather robust to the implementation of those improvements in the estimation procedure. Using GLMs reduces the biomass estimate about a 9% with respect to the traditional and about a 6% for the Bayesian ageing method with GLMs. However, moving

the spawning peak time from 24:00 hours to 23:00 hours GMT makes null those differences (0.1% reduction). In addition, following the methodology developed in the GAM EU project, GAMs were essayed for the modelling of P_0 and SSB in space (Sections 3.3.2 and 3.4.2 respectively). This exercise led to an SSB estimate of about 30000 tonnes for a constant mortality rate of eggs in space, which is very consistent with the current traditional and new estimates.

In September 2002, preliminary estimates of biomass for the 2002 anchovy DEPM survey were provided based on two log-lineal models making use of the egg production and spawning area relationships with biomass (the first using temperature and the second Julian day as additional auxiliary covariates). They both indicated a biomass estimate of about 51 000 tonnes for 2002, with a (adopted) CV of around 17%, although the model with Julian day suggested a CV of about 13%. The current estimate based on the full application of the DEPM produces an estimate of 30 700 tonnes, 40% lower than the provisional estimate and just outside its 95% confidence interval. In the past, provisional estimates based on the use of the above relationships and final estimates were closer and therefore supported the use of such models as shortcuts for the provision of biomass estimates immediately after the survey (as first proposed by Uriarte et al., 1999b). In the current case, the discrepancy arises on one hand from a 19% reduction in the egg production estimate (due to a revision of the weighting procedures by stations), and on the other hand from the higher than average anchovy daily fecundity in 2002 (higher by 13.3%). Lower egg production and higher daily fecundity both contribute to a reduction in the SSB estimate, thus explaining the 40% discrepancy between the provisional and the final SSB estimate in the 2002 anchovy survey.

The final estimate of 30 700 tones appoints to a strong decrease regarding the 2001 DEPM estimate (124 000 tonnes, Figure 2.5.4.1 and Table 2.5.4.2). The reason of this decrease arises from the weak recruitment in 2001 which has led to low age 1 spawners in 2002, as pointed out by the age composition estimates. The population at age estimates indicate that about 60% of the population were two-year-old anchovies and only 27% was one-year-old. This is the first time in the whole series of DEPM estimates since 1987 that two-year-olds are more abundant than one-year-old anchovies. The population at age 1 of about 283 million fishes is the lowest ever estimated; with the sole exception of the 1989 one, which was similar (248 millions) but in that case the estimate was considered negatively biased and it was subsequently corrected upward for the purposes of assessment inputs (up to 347).

The percentages at age provided by the DEPM are in close agreement with those arising from the acoustic survey in May 2002, both showing the predominance of the two-year-old anchovies (Poisson and Massé, 2002). This was expected since they both share the age composition of the acoustic fishing hauls entering the DEPM estimates, but it becomes also evident in the ALK of routine samples from AZTI where age two also predominates. The age composition and the catches of the Spanish purse seine fleet landing in the Basque Country in spring

2002 support the above observations (Uriarte pers. comm.); these catches were only about 25% those in 2001 and were largely sustained by big and old anchovies (68% were two-year-old anchovies). However, the acoustic and DEPM surveys diverge in the biomass estimates for 2002. The acoustic estimate of anchovy biomass is about 97 000 t (Poisson and Massé, 2002), much larger than

the DEPM estimate of about 30 000 t. The major difference arises from the amount of biomass estimated at the Gironde area, where the acoustic survey detected a lot of pure anchovy schools (Massé, pers. comm.). However a detailed comparison of these estimates has not been made yet and is outside the scope of this document.

Table 2.2.1.1. Summary of plankton and adult sampling in Portuguese DEPM surveys for sardine.

Variable	March 1988	March 1997	January 1999	January 2002
Survey area	Portugal	Portugal+Cadiz	Portugal+Cadiz	Portugal+Cadiz
Plankton stations	309	373	417	484
Gear (mesh size, µm)	CalVET (200)	CalVET (150)	CalVET (150)	CalVET (150)
Sardine eggs sampled (total)	1 307	1 454	5 110	2 585
Fishing stations (total)	16	28	40	74
Fishing stations (commercial)	5	-	33	43
Gear	MT, BT	MT	MT, BT, PS	MT, BT, PS
Fish sampled (total)	446	1 300	3 824	5 302
Histology stations	9	26	35	67
Fish histology (mature)	186	420	660	1 350
Fish fecundity	37	31	75	113

Table 2.2.1.2. Summary of adult sampling in Spanish DEPM surveys for sardine. The 2002 survey includes four adult samples from the French coast.

Variable	April 1988	March 1990	March 1997	March 1999	April 2002
Survey area	N. Spain	N. Spain	N. Spain	N. Spain	N. Spain
Plankton stations	524	475	462	290	313
Gear (mesh size, µm)	CalVET (100)	CalVET (100)	CalVET (100)	CalVET (100)	CalVET (100)
Sardine eggs sampled (total)	3 922	1 492	1 465	2 340	1 939
Fishing stations (total)	30	14	9	10	32
Fishing stations (commercial)	-	-	-	3	13
Gear	MT	MT	MT	MT, PS	MT, PS
Fish sampled (total)	1 119	587	491	524	2 222
Histology stations	30		9	3	21
Fish histology (mature)	611	333	314	60	389
Fish fecundity	89	68	155	116	73

Table 2.2.1.3. Summary of coverage, sampling and estimation characteristics of the DEPM surveys for the Atlanto-Iberian stock of sardine.

Year	Coverage	Sampling	Estimation
1988	Gulf of Cadiz not sampled	Adequate adult sampling in Spain, limited in Portugal (few samples south of Lisbon).	Post-stratification only in Spanish survey (3 strata).
1990	No Portuguese survey; Egg distribution and production also available for Bay of Biscay (anchovy survey).	Adult sampling more limited than in 1988; No adult data for the Bay of Biscay.	Post-stratification in Spanish sardine survey (3 strata); No adult and SSB estimation for Bay of Biscay.
1997	Adequate; In Spanish survey fish were only caught in eastern Cantabria.	Limited adult sampling in Spain and relatively limited in Portugal.	No post-stratification; Revision of S in Portugal led to extremely low estimate.
1999	Adequate; Sardine egg data also available from Bay of Biscay (SARDYN project).	Limited adult sampling in Spain, adequate in Portugal.	No post-stratification; Uncertainty on estimate of egg production in Cantabria
2002	Adequate; Sardine egg data also available from Bay of Biscay (SARDYN project).	Adequate adult sampling in Spain, good in Portugal.	Post-stratification in both surveys. Low precision due to very low S in Portugal

Table 2.2.1.4. Estimates of sardine daily egg production (coefficient of variation in brackets) for Portugal, Spain and Iberia, based on the traditional estimator. All estimates refer to trillion eggs ($\times 10^{12}$).

Year	Portugal	Spain	Iberia
1988	2.87 (22)	2.97 (33)	5.84 (20)
1990	NA	1.78 (58)	NA
1997	4.41 (49)	0.72 (82)	5.13 (43)
1999	5.24 (30)	0.34 (44)	5.58 (28)
2002	2.07 (33)	0.52 (33)	2.59 (27)

Table 2.2.1.5. Mean female weight (gr) estimates for Portugal and Spanish strata in all DEPM surveys (values in brackets indicate CV).

Year	Portugal	GAL	CANW	CANE
1988	40.7 (7)	64.9 (6)	79.3 (8)	86.3 (3)
1990	-	68.1 (12)	83.7 (2)	83.6 (1)
1997	46.7 (5)	-	-	70.1 (6)
1999	44.4 (5)	-	-	66.3 (41)
2002	44.3 (5)	67.6 (11)	78.6 (8)	77.7 (6)

Table 2.2.1.6. Batch fecundity (10^3 eggs) estimates for Portugal and Spanish strata in all DEPM surveys (values in brackets indicate CV).

Year	Portugal	GAL	CANW	CANE
1988	14.3 (8)	27.3 (6)	33.8 (9)	33.9 (3)
1990	-	26.9 (26)	33.0 (19)	33.0 (20)
1997	17.4 (6)	-	-	26.6 (5)
1999	18.4 (5)	-	-	21.8 (12)
2002	14.3 (6)	23.6 (13)	27.7 (8)	26.9 (6)

Table 2.2.1.7. Sex ratio estimates for Portugal and Spanish strata in all DEPM surveys (values in brackets indicate CV).

Year	Portugal	GAL	CANW	CANE
1988	0.45 (11)	0.35 (12)	0.65 (11)	0.66 (33)
1990	-	0.56 (8)	0.53 (38)	0.45 (28)
1997	0.61 (4)	-	-	0.52 (11)
1999	0.61 (5)	-	-	0.55 (45)
2002	0.61 (3)	0.52 (7)	0.60 (14)	0.49 (22)

Table 2.2.1.8. Spawning fraction estimates for Portugal and Spanish strata in all DEPM surveys (values in brackets indicate CV).

Year	Portugal	GAL	CANW	CANE
1988	0.14 (19)	0.08 (20)	0.13 (11)	0.21 (13)
1990	-	0.10 (32)	0.11 (91)	0.20 (20)
1997	0.03 (26)	-	-	0.18 (15)
1999	0.10 (15)	-	-	0.14 (26)
2002	0.03 (21)	0.24 (38)	0.08 (14)	0.13 (20)

Table 2.2.1.9. Spawning biomass estimates for Portugal and Spanish strata in all DEPM surveys (values in brackets indicate CV).

Year	Portugal	GAL	CANW	CANE	Spain
1988	129.1 (35)	134.2 (66)	33.5 (30)	12.5 (56)	180.2 (50)
1990	-	24.2 (40)	46.1 (72)	7.4 (27)	77.7 (45)
1997	590.3 (56)	-	-	-	20.7 (84)
1999	205.1 (35)	-	-	-	13.4 (77)
2002	350.8 (40)	0	41.3 (39)	9.4 (44)	50.7 (33)

Table 2.2.2.1. DEPM parameter and spawning biomass estimates for Mediterranean sardine in the central Aegean and Ionian Seas (CVs in parentheses). Data from Somarakis *et al.*, 2001.

Parameter	Aegean Sea		Ionian Sea	
Daily egg production, P_I (day^{-1}/m^2)	27.52	(0.518)	7.81	(0.258)
Survey area, A (km^2)	8 702		8 724	
Average weight of mature females, W (g)	19.01	(0.034)	15.87	(0.038)
Sex ratio, R	0.458	(0.095)	0.661	(0.053)
Batch fecundity, F (mean number of eggs per mature female)	6 469	(0.051)	5 149	(0.041)
Spawning fraction, S	0.095	(0.048)	0.087	(0.116)
Spawning-stock biomass, B (MT)	16 174	(0.521)	3 652	(0.282)

Table 2.3.1.1. Acronyms, description of parameters and units for the values presented in Table 2.2.1.2.

Acronyms	Estimates of...	Units
Po	Daily Egg Production per surface unit	Eggs/0.05 m²/day
Z	Daly mortality of eggs	
SA	Positive Spawning Area	Km²
Ptot	Total Daily Egg Production of the Population	Eggs/day *10E-12
SST	Sea Surface Temperature	°C
SSB	SPAWNING-STOCK BIOMASS	tonnes
DF	Daily Fecundity of the Population	eggs/gramme
ABtot	Total Egg Abundance in the area surveyed	eggs *10E-12
AB mean	Average Egg abundance per surface unit	Eggs/0.1 m²

Table 2.3.1.2. DEPM estimates of SSB and associated parameters available for the Bay of Biscay anchovy (see acronyms in Table 2.3.1.1). For the establishment of the lineal regression between Biomass (SSB) and Spawning area (SA) (equation 1) the data of June 1989 and 1990 were deleted.

YEAR	SURVEY DATES	SSB	P tot	Po	Z	SA	DF	Ab tot	Ab mean	SST
1987	02–07 June	29 365	2.199	4.61	0.26	23 850	81.3	3.411	14.3035	16.4
1988	21–28 May	63 500	5.01	5.52	0.18	45 384	81.4	10.41	22.9302	16.5
1989	10–21 May	11 861	0.73	2.08	0.18	17 546	62.3	0.896	5.10858	16.6
1989	14–24 June	10 058	0.826	1.5	0.94	27 917	54.8	0.79	2.825	20.8
1990	04–15 May	97 237	4.518	3.78	0.34	59 757	52.2	7.842	13.1238	16.9
1990	29 May – 15 June	77 254	7.239	5.21	0.62	69 471	90.1	8.052	11.5901	17.7
1991	06 May – 07 June	19 276	1.238	2.55	0.22	24 264	67.5	3.179	13.101	15.6
1992	16 May – 13 June	90 720	5.789	4.27	0.22	67 796	71.6	13.09	19.3072	17.7
1994	07 May – 03 June	60 062	3.829	3.93	0.11	48 735	62.9	11.33	23.246	15.8
1995	11–25 May	54 701	3.094	4.96	0.19	31 189	56.7	8.751	28.0579	14.5
1996	18–30 May		2.771	4.87	0.31	28 448	-	5.953	20.9244	15.2
1997	09–21 May	51 176	2.697	2.69	0.19	50 133	53.2	7.123	14.2084	15.3
1998	18 May – 08 June	101 976	5.595	3.83	0.28	73 131	56.5	11.96	16.3487	15.9
1999	22 May – 05 June		3.593	3.52	0.12	51 019	-	9.061	17.7214	16.8
1999	Area-radial added		3.865	3.42	0.12	55 946	-	9.745	17.2126	16.8
2000	02–20 May		2.612	3.45	0.18	37 883	-	7.949	20.983	16.7

Table 2.3.2.1. Spawning Biomass and DEPM parameters estimates for anchovy in the Mediterranean. (CVs in parentheses).

			Egg Parameters						Adult Parameters							
		Tª	A	A₁	P₁	P	Z	Pₜ	F	S	W	R	RF	DSF	SF	B
SICILIAN CHANNEL	Jun-Jul 1998	18.5 22.5	13295	5329	65.55 (0.21)	26.27 (0.33)	1.63 (0.33)	0.14 (0.33)	4835 (0.16)	0.14 (0.12)	15.18 (0.07)	0.59 (0.12)	319	26	7	13224 (0.22)
	Jun 1999	18.4 22.7	5878	2692	45.86 (0.22)	21.00 (0.32)	1.25 (0.33)	0.05 (0.32)	5871 (0.11)	0.17 (0.10)	14.08 (0.08)	0.55 (0.10)	417	39	6	3138 (0.31)
	Jun-Jul 2000	16.3 25.8	11812	4505	34.98 (0.15)	13.34 (0.24)	2.07 (0.20)	0.06 (0.24)	8379 (0.06)	0.20 (0.28)	18.90 (0.04)	0.62 (0.08)	443	55	5	2850 (0.46)
CATALAN SEA	May 1990	17.6 19.6	17081	8095	120.61 (0.15)	57.16 (0.29)	0.56 (0.44)	0.46 (0.22)	8006 (0.02)	0.36 (0.10)	14.25 (0.04)	0.54 (0.09)	562	110	3	4199 (0.26)
	Jul 1990								7283 (0.12)	0.31 (0.16)	12.79 (0.10)	0.56 (0.10)	569	99	3	
CATALAN SEA & GULF OF LIONS	July 1993	13.3 22.5	44554	33012	86.67 (0.15)	64.22 (0.17)	1.09 (0.26)	2.12 (0.17)	4958 (0.11)	0.31 (0.13)	14.31 (0.07)	0.64 (0.05)	346	69	3	30849 (0.30)
	May-Jun 1994	15 22.0	42085	31692	81.71 (0.18)	61.53 (0.21)	0.47 (0.26)	1.95 (0.21)	7039 (0.02)	0.21 (0.20)	22.92 (0.06)	0.59 (0.19)	307	38	5	52557 (0.36)
LIGURIAN & TYRRHENIAN	July 1993	18.9 22.5	15424	8221	93.57 (0.28)	49.87 (0.32)	0.86 (0.34)	0.41 (0.32)	4894 (0.10)	0.32 (0.11)	14.17 (0.07)	0.63 (0.05)	345	70	3	5829 (0.36)
AEGEAN SEA	Jun 1993	16.7 25.0	17396	17396	259.49 (0.32)	259.49 (0.32)	1.04 (0.46)	4.51 (0.32)	11542 (0.04)	0.28 (0.15)	22.73 (0.02)	0.55 (0.04)	508	78	4	58988 (0.35)
	Jun 1999	18.0 25.0	8604	8604	13.29 (0.39)	13.29 (0.39)	0.53 (0.48)	0.11 (0.39)	4725 (0.05)	0.13 (0.21)	15.77 (0.03)	0.47 (0.09)	300	18	8	6273 (0.43)
IONIAN SEA	May-Jun 1999	18.0 25.0	12362	12362	8.88 (0.24)	8.88 (0.24)	0.52 (0.36)	0.10 (0.24)	9428 (0.08)	0.06 (0.26)	15.60 (0.05)	0.53 (0.07)	604	19	17	5588 (0.33)
SW ADRIATIC	Jul-Aug 1994		14790	9244	50.11 (0.16)	31.32 (0.10)	0.55 (0.12)	0.29 (0.10)	11866 (0.03)	0.16 (0.08)	18.57 (0.03)	0.55 (0.05)	639	56	6	8129 (0.24)

Tª	Temperature range (°C)	F	Batch fecundity
A	Total survey area (km²)	S	Spawning fraction
A₁	Positive stratum area (km²)	W	Mean females weight
P₁	Daily egg production per m² registered in the positive stratum	R	Sex ratio
P	Daily egg production per m² registered in the whole sampled area	RF	Relative fecundity, ratio between F and the W
Z	Daily rate of instantaneous mortality	SF	Spawning frequency
Pₜ	Daily egg production for the whole sampled area (eggs/day $*10^{-12}$)	DSF	Daily specific fecundity (DSF = FSR/W)
		B	Spawning biomass

Table 2.4.3.1. Alternative models considered for batch fecundity in the Portuguese 2002 survey (n=113). Generalised linear models (GLMs) were fit with identity link and R^2 in these cases refers to percentage of explained deviance. Values in brackets indicate standard error for parameter estimates.

Model	Intercept	Slope	R^2	Residual plots
LM (unweighted)	-4208.5 (787)	462.4 (25)	76	Unsatisfactory
LM (weighted)	-3884.2 (995)	452.8 (28)	70	Improved
GLM (Gamma)	-4286.2 (456)	464.2 (21)	81	Much Improved
GLM (NB)	-4285.7 (458)	464.3 (22)	81	Much Improved

Table 2.4.3.2. DEPM parameter and SSB estimates (with CV is brackets) for the 2002 sardine DEPM surveys in Portugal and Spain (unstratified estimates).

Parameter	Portuguese survey	Spanish survey
Egg production (Po, 10^{12} eggs)	2.07 (33)	0.76 (23)
Female weight (gr)	44.3 (5)	75.0 (5)
Batch Fecundity (10^3 eggs)	14.26 (6)	26.09 (6)
Spawning fraction	0.030 (21)	0.128 (21)
Sex ratio	0.611 (3)	0.542 (9)
Daily fecundity (eggs/gr)	5.9 (23)	24.1 (24)
Spawning biomass (10^3 tonnes)	**350.8 (40)**	**31.5 (33)**

Table 2.4.4.1. GLM estimation of egg density, mortality and production for each stratum in the 2002 sardine DEPM surveys in Spain and Portugal. Area refers to the positive stratum.

Stratum	Area (km2)	P01 (eggs /0.05 m2) [cv]	Z (eggs per day) significance	Egg production
Spain, CANE	4 621	2.22 [31]	-0.36 **	0.20 1012
Spain, CANW	5 348	6.12 [32]	0.14 **	0.66 1012
Total Stratified (Spain)	9 970	4.46 [32]	-0.09 --	0.86 1012
Portugal, N	12 280	5.36 [24]	-0.13 --	1.32 1012
Portugal, S	4 343	10.28 [36]	-0.83 **	0.89 1012
Total Stratified (Portugal)	16 623	7.97 [33]	-0.32 --	2.21 1012
Unstratified (Spain)	9 970	3.84 [23]	-0.11--	0.76 1012
Unstratified (Portugal)	16 640	6.23 [33]	-0.16 --	2.07 1012
TOTAL Stratified				3.07 1012
TOTAL Unstratified				2.83 1012

Table 2.4.4.2. Significance of post-stratification for each DEPM adult parameter in the 2002 sardine survey in Portugal and Spain. Vales indicate probability of difference between strata estimated from linear (mean weight, batch fecundity and daily fecundity) and generalised linear model (spawning fraction and sex ratio). Statistically significant differences are highlighted in bold.

Variable	Portuguese survey	Spanish survey	
	West vs South	GAL vs CANW	GAL vs CANE
Mean female weight	**0.036**	0.657	0.786
Batch fecundity	**0.042**	0.725	0.841
Spawning fraction	0.115	**<0.001**	**0.033**
Sex ratio	0.276	**0.002**	0.921
Daily fecundity	0.844	0.054	0.167

Table 2.4.4.3. DEPM parameter and SSB estimates (with the corresponding CV in brackets) for the 2002 sardine DEPM surveys in Portugal and Spain, separately for each stratum.

Parameter	Portuguese survey		Spanish survey		
	West	South	GAL	CANW	CANE
Egg production (Po, 10^{12} eggs)	1.32 (24)	0.89 (36)	-	0.66 (32)	0.20 (31)
Female weight (gr)	48.4 (8)	40.4 (5)	67.6 (11)	78.6 (8)	77.7 (6)
Batch Fecundity (10^3 eggs)	16.0 (10)	12.6 (6)	23.6 (13)	27.7 (8)	26.9 (6)
Spawning fraction	0.024 (28)	0.039 (29)	0.243 (38)	0.075 (14)	0.125 (20)
Sex ratio	0.611 (3)	0.612 (5)	0.519 (7)	0.604 (14)	0.494 (22)
Daily fecundity (eggs/gr)	4.8 (31)	7.4 (31)	44.0	16.0 (23)	21.4 (31)
Spawning biomass (10^3 t)	**272.3 (39)**	**119.6 (47)**	**0**	**41.3 (39)**	**9.4 (44)**

Table 2.4.4.4. Sardine spawning biomass estimates (thousand tonnes) for acoustic (November 2001 and March 2002) and DEPM (January 2002) Portuguese surveys.

Estimate	Portugal	West	South
November 2001 (acoustics)	637.5	350.0	323.5
January 2002 (DEPM, unstratified)	350.8	-	-
January 2002 (DEPM, GAM)	421.2	-	-
January 2002 (DEPM, stratified)	391.9	272.3	119.6
March 2002 (acoustics)	475.8	228.4	229.4

Table 2.5.2.1. Total daily egg production estimates for Bay of Biscay anchovy in 2002 (with the corresponding CV in brackets) using different ageing methods (Lo or Bayesian) and egg mortality curve models (non linear regression or GLM).

1- Lo + Non linear reg	2- Lo + GLM	3- Bayesian + GLM
2.34 * E12 (0.127)	2.13 * E12 (0.125)	2.20 * E12 (0.133)

Table 2.5.3.1. Estimates of the adult parameters, SSB and population at age in the total area, with the DEP estimated using Lo's ageing method and the egg mortality curve fitted as a non linear regression model.

Parameter	Estimate	S.e.	CV
DEP	2.3E+12	3E+11	0.1273
R'	0.5388	0.0039	0.0072
S	0.3023	0.0088	0.0292
F	16825.0	772.1	0.0459
Wf	35.86	1.3522	0.0377
Daily Fec.	76.41	2.7314	0.0357
Biomass	30,697	4058.94	0.1322
Wt	30.5341	1.64333	0.0538
POPULATION	1008.6	146.3	0.1451
Pa 1	0.2485	0.0494	0.1988
Pa 2	0.6169	0.0399	0.0647
Pa 3	0.1346	0.0123	0.0910
Nage 1	253.6	73.5	0.2897
Nage 2	619.9	83.2	0.1343
Nage 3	135.1	18.7	0.1387

Table 2.5.3.2. Estimates of the adult parameters, SSB and population at age by strata, with the DEP estimated using Lo's ageing method and the egg mortality curve fitted as a non linear regression model. The DEP by strata are approximate estimates so that the sum of the DEP by strata equals the pool area estimate. This was done by subtracting from the total DEP estimate the NE coastal strata DEP estimate.

Parameter	COASTAL Estimate	CV	OCEANIC Estimate	CV
DEP	9.5426E+11	0.1974	1.3878E+12	0.1666
R'	0.5415	0.0106	0.5377	0.0093
S	0.2925	0.0632	0.3060	0.0327
F	13572.1	0.0738	18088.9	0.0450
Wf	29.49	0.0622	38.34	0.0379
Daily Fec.	72.88	0.0668	77.64	0.0379
Biomass	13,160	0.2084	17,904	0.1709
Wt	24.68	0.0986	33.64	0.0514
POPULATION	540.1	0.2439	533.4	0.1771
Pa 1	0.3900	0.2374	0.1734	0.2654
Pa 2	0.5088	0.1477	0.6743	0.0558
Pa 3	0.1012	0.1788	0.1523	0.0898
Nage 1	217.0	0.4212	93.6	0.3548
Nage 2	269.6	0.2078	358.9	0.1733
Nage 3	53.4	0.2140	80.9	0.1779

Table 2.5.3.4. Estimation of weighting factors per sample according to the DEP by strata (The DEP by strata are approximate estimates so that the sum of the DEP by strata equals the pool area estimate). Weighting factors for numbers (M_i) are calculated by dividing the weighting factors for biomass (M'_i) with the mean weight of anchovies per sample (w_i).

STRATA	COAST	OCEANIC	TOTAL
Egg Production (DEP) (Aprox)	954,263	1,387,838	2,342,101
%DEP	41%	59%	100%
Number of adult samples	11	24	35
DEP/sample	0.037	0.025	
M'i biomass referred to Oceanic	1.50	1	
Mi for numbers	1.5/Wi	1/Wi	

Table 2.5.3.5. Sensitivity of the SSB and population at age estimates to the assumptions of balanced or unbalanced sampling. The first column contains the adopted estimates with balanced sampling for the biomass estimate and unbalanced sampling for the population at age estimates. The second and third columns show the results of the alternative processing according to the assumptions of the unbalanced and balanced adult sampling for biomass and population at age respectively. Change % refers to the change in the estimates with respect to the first column (adopted estimate).

| Biomass Proc | Unweighted | | Weighted | | | Unweighted | | |
| Popage Proc | Weighted | | Weighted | | | Unweighted (only by inverse of Wi) | | |
Parameter	Estimate	CV	Estimate	CV	Change %	Estimate	CV	Change %
DEP	2.3421E+12	0.127	2.3421E+12	0.127	0.0%	2.3421E+12	0.127	0.0%
R'	0.5388	0.007	0.5391	0.007	0.1%	0.5388	0.007	0.0%
S	0.3023	0.029	0.3011	0.031	-0.4%	0.3023	0.029	0.0%
F	16825.0	0.046	16425.6	0.048	-2.4%	16825.0	0.046	0.0%
Wf	35.86	0.038	35.08	0.040	-2.2%	35.86	0.038	0.0%
Daily Fec.	76.41	0.036	76.00	0.037	-0.5%	76.41	0.036	0.0%
Biomass	30,697	0.132	30,864	0.133	0.5%	30,697	0.132	0.0%
Wt	29.67	0.059	29.67	0.059	0.0%	30.53	0.054	2.9%
POPULATION	1038.7	0.148	1044.5	0.149	0.6%	1008.6	0.145	-2.9%
Pa 1	0.2695	0.204	0.2695	0.204	0.0%	0.2485	0.199	-7.8%
Pa 2	0.6009	0.074	0.6009	0.074	0.0%	0.6169	0.065	2.7%
Pa 3	0.1297	0.098	0.1297	0.098	0.0%	0.1346	0.091	3.8%
Nage 1	283.6	0.300	285.3	0.302	0.6%	253.6	0.290	-10.6%
Nage 2	621.3	0.134	624.7	0.134	0.5%	619.9	0.134	-0.2%
Nage 3	133.8	0.138	134.6	0.139	0.5%	135.1	0.139	0.9%

Table 2.5.4.1. Differences in the DEP, SSB and population at age estimates when incorporating the recommended procedures for the DEP estimation (Bayesian ageing method and GLMs).

Biomass Proc Popage Proc Parameter	Traditional Lo's Unweighted Weighted Estimate	CV	GLM on Lo's Unweighted Weighted Estimate	CV	Change %	GLM on Bayesian 24:00 spawning Unweighted Weighted Estimate	CV	Change %	GLM on Bayesian 23:00 spawning Unweighted Weighted Estimate	CV	Change %
DEP	2.34E+12	0.127	2.13E+12	0.125	-9.0%	2.20E+12	0.133	-5.9%	2.34E+12	0.134	0.1%
R'	0.5388	0.007	0.5388	0.007	0.0%	0.5388	0.007	0.0%	0.5388	0.007	0.0%
S	0.3023	0.029	0.3023	0.029	0.0%	0.3023	0.029	0.0%	0.3023	0.029	0.0%
F	16825.0	0.046	16825.0	0.046	0.0%	16825.0	0.046	0.0%	16825.0	0.046	0.0%
Wf	35.86	0.038	35.86	0.038	0.0%	35.86	0.038	0.0%	35.86	0.038	0.0%
Daily Fec.	76.41	0.036	76.41	0.036	0.0%	76.41	0.036	0.0%	76.41	0.036	0.0%
Biomass	30,697	0.132	27,937	0.130	-9.0%	28,882	0.138	-5.9%	30,734	0.139	0.1%
Wt	29.67	0.059	29.45	0.060	-0.7%	29.45	0.060	-0.7%	29.45	0.060	-0.7%
POPULATION	1038.7	0.148	952.6	0.147	-8.3%	984.8	0.154	-5.2%	1048.0	0.155	0.9%
Pa 1	0.2695	0.204	0.2748	0.205	2.0%	0.2748	0.205	2.0%	0.2748	0.205	2.0%
Pa 2	0.6009	0.074	0.5968	0.076	-0.7%	0.5968	0.076	-0.7%	0.5968	0.076	-0.7%
Pa 3	0.1297	0.098	0.1284	0.101	-1.0%	0.1284	0.101	-1.0%	0.1284	0.101	-1.0%
Nage 1	283.6	0.300	265.3	0.302	-6.4%	274.3	0.305	-3.3%	291.9	0.306	2.9%
Nage 2	621.3	0.134	565.7	0.132	-8.9%	584.9	0.140	-5.9%	622.4	0.141	0.2%
Nage 3	133.8	0.138	121.5	0.137	-9.2%	125.6	0.144	-6.1%	133.7	0.145	-0.1%

Table 2.5.4.2. Comparison of the DEPM 2001 and 2002 biomass and population at age estimates.

Variable	BIOMAN 2001	BIOMAN 2002
SSB (tons)	120 400	30 697
Nage 1 (numbers)	4 114	284
Nage 2 (numbers)	1 638	621
Nage 3 (numbers)	145	134
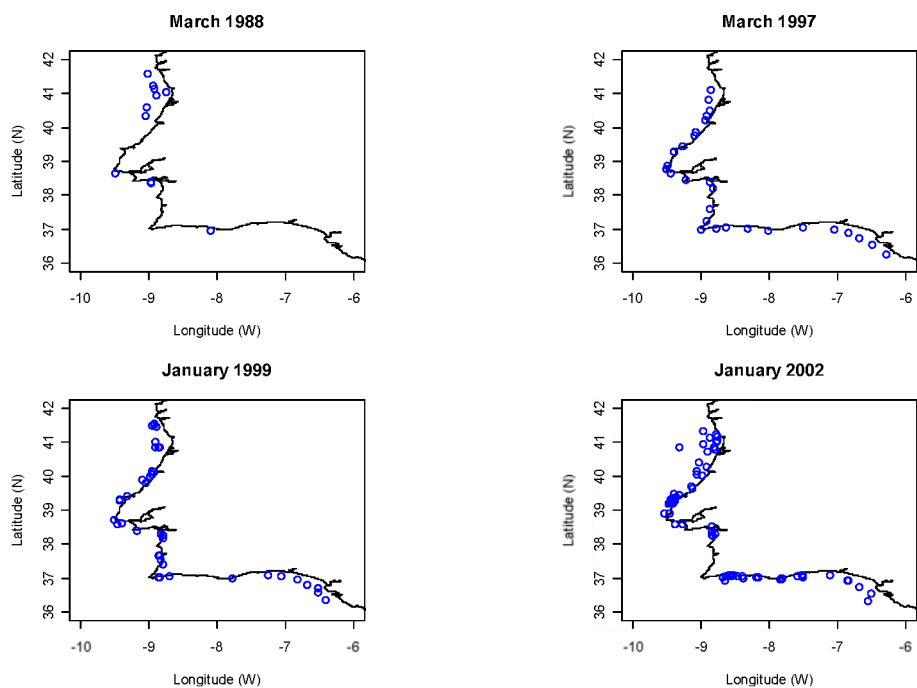
Figure 2.2.1.1. Location of adult sardine sampling during the Portuguese DEPM surveys.
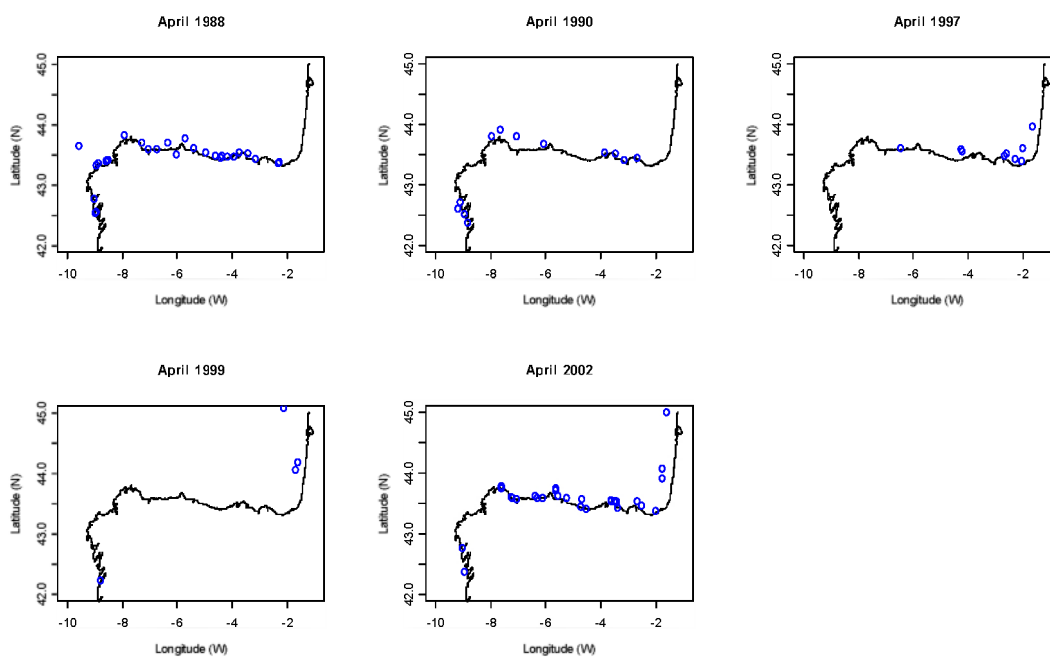
Figure 2.2.1.2. Location of adult sardine sampling during the Spanish DEPM surveys.
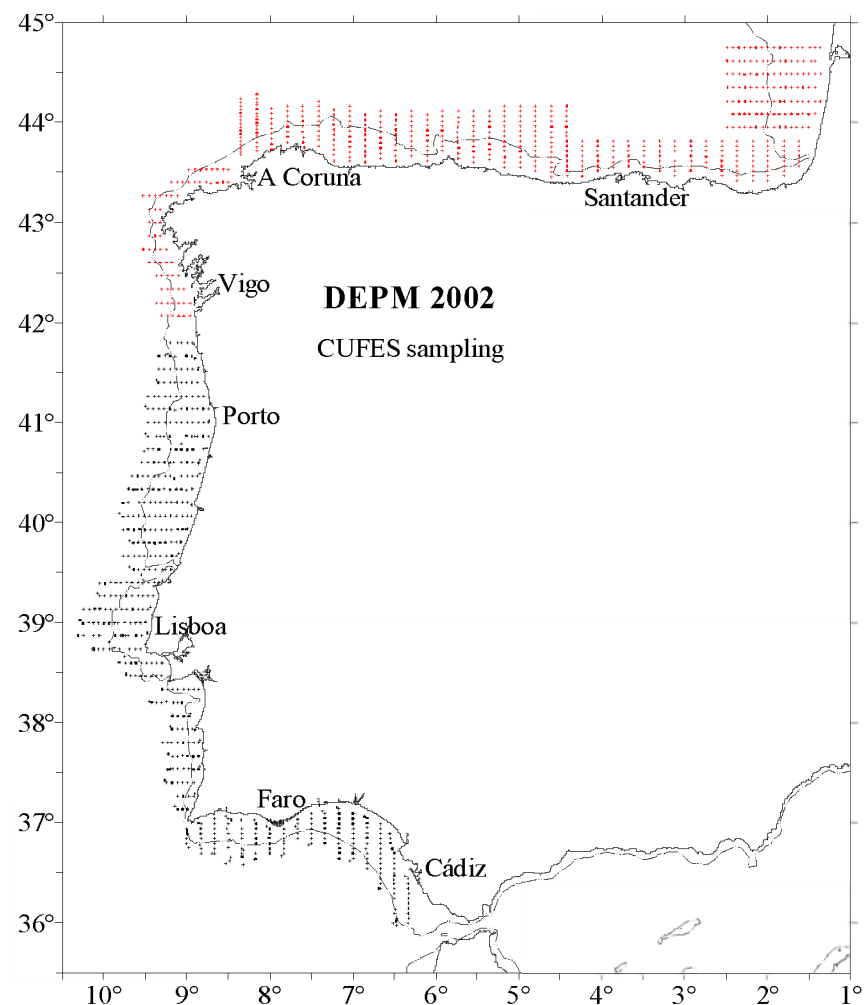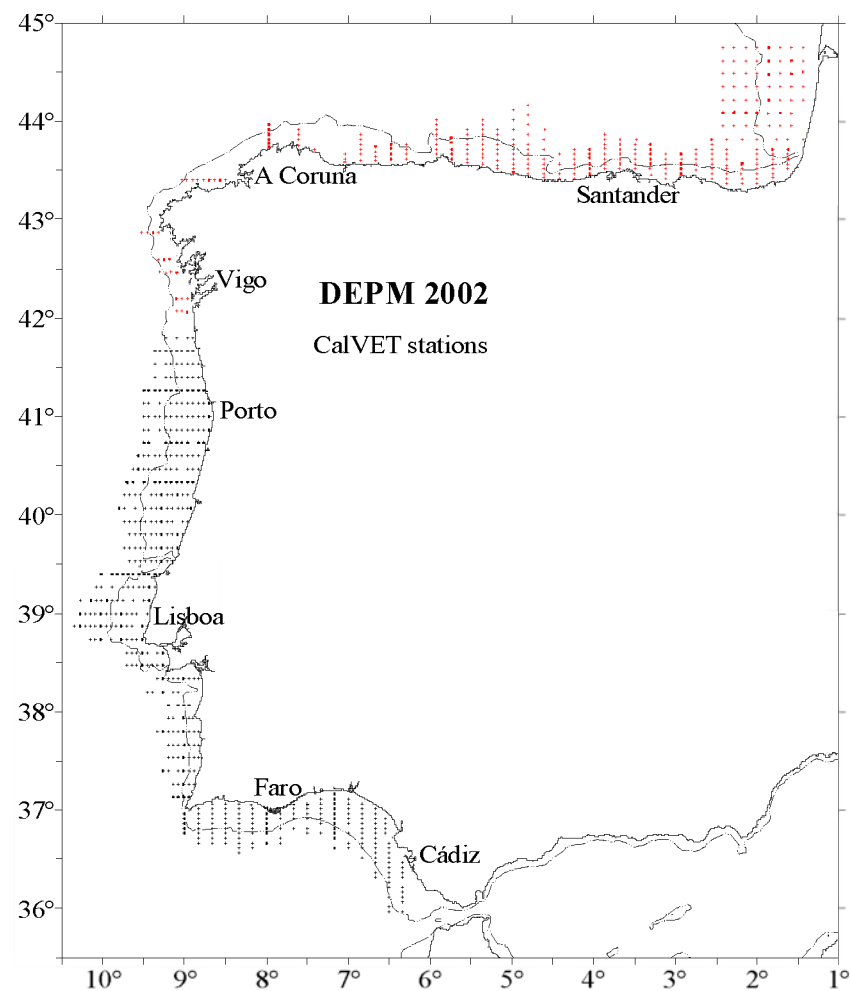
Figure 2.4.1.1. Location of CalVET (left) and CUFES stations (right) during the 2002 DEPM survey for the Atlanto-Iberian stock of sardine (black and red points correspond to the Portuguese and Spanish surveys respectively).
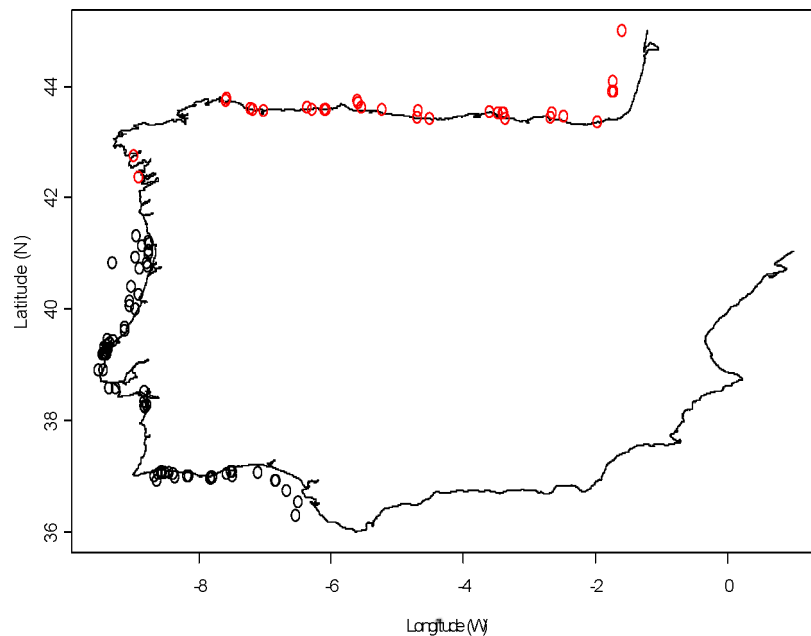
Figure 2.4.1.2. Location of fishing stations during the 2002 DEPM survey for the Atlanto-Iberian stock of sardine (black and red circles correspond to the Portuguese and Spanish surveys respectively).
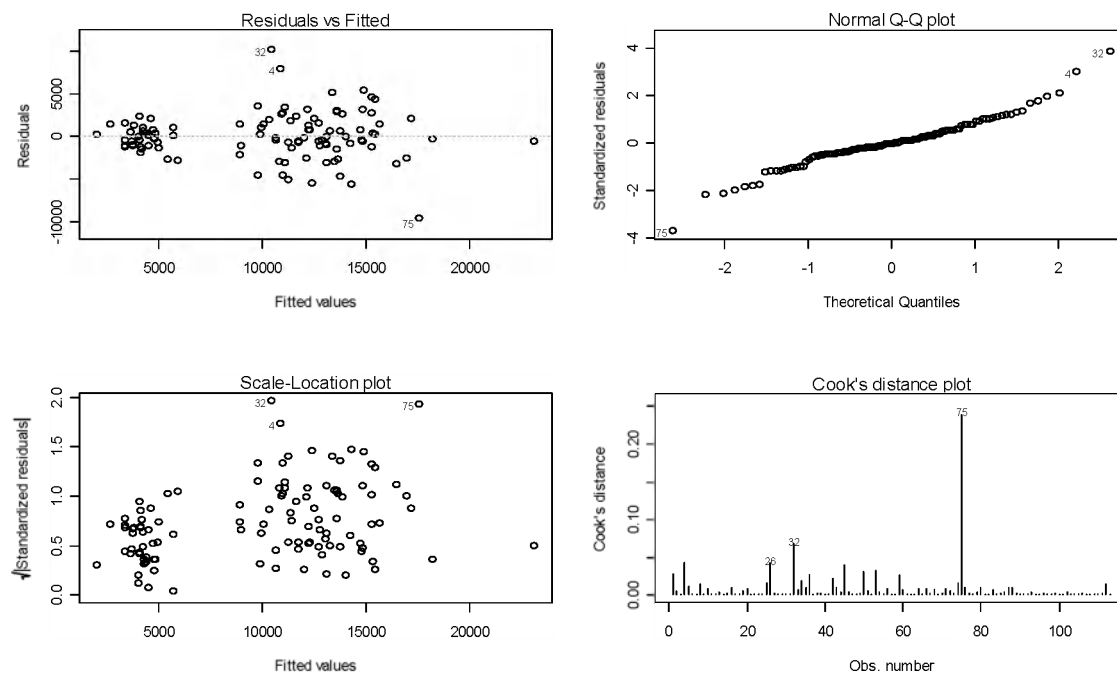
Figure 2.4.3.1. Residual inspection plots for unweighted linear model fitted to sardine batch fecundity data from the 2002 Portuguese survey (n = 113).
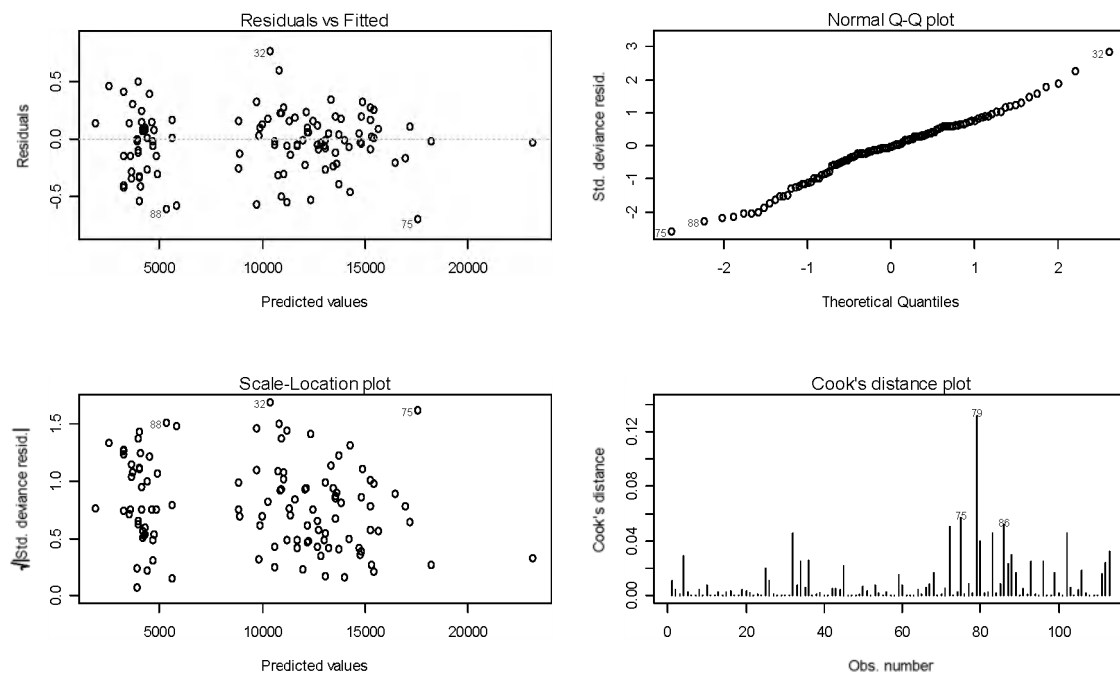
Figure 2.4.3.2. Residual inspection plots for generalized linear model (Gamma distribution with identity link) fitted to sardine batch fecundity data from the 2002 Portuguese survey (n = 113).
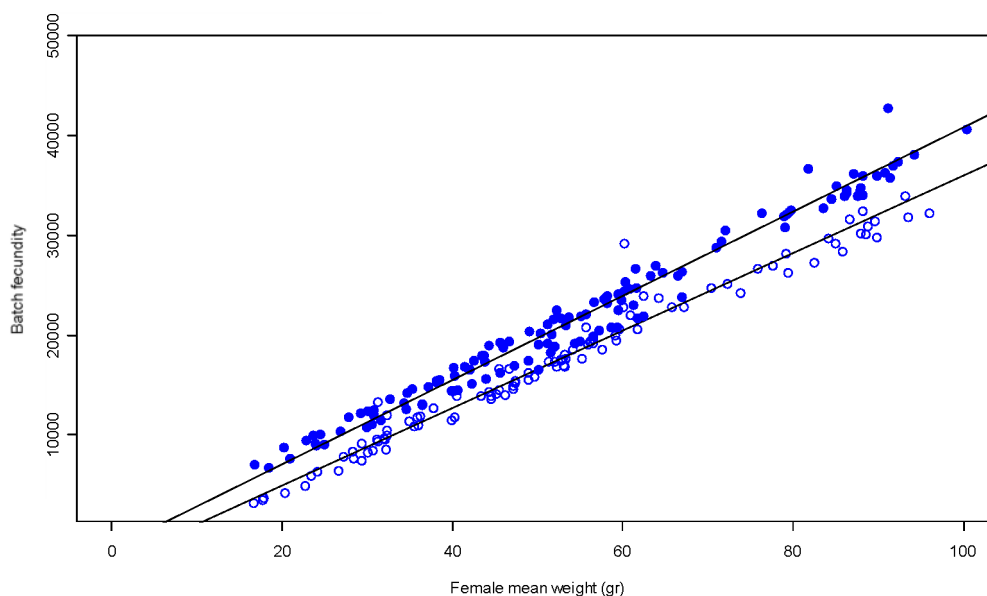
Figure 2.4.3.3. Relationship between mean female weight and batch fecundity in the 2002 (open circles) and all previous (closed circles) sardine DEPM surveys. Lines correspond to linear regression fits.
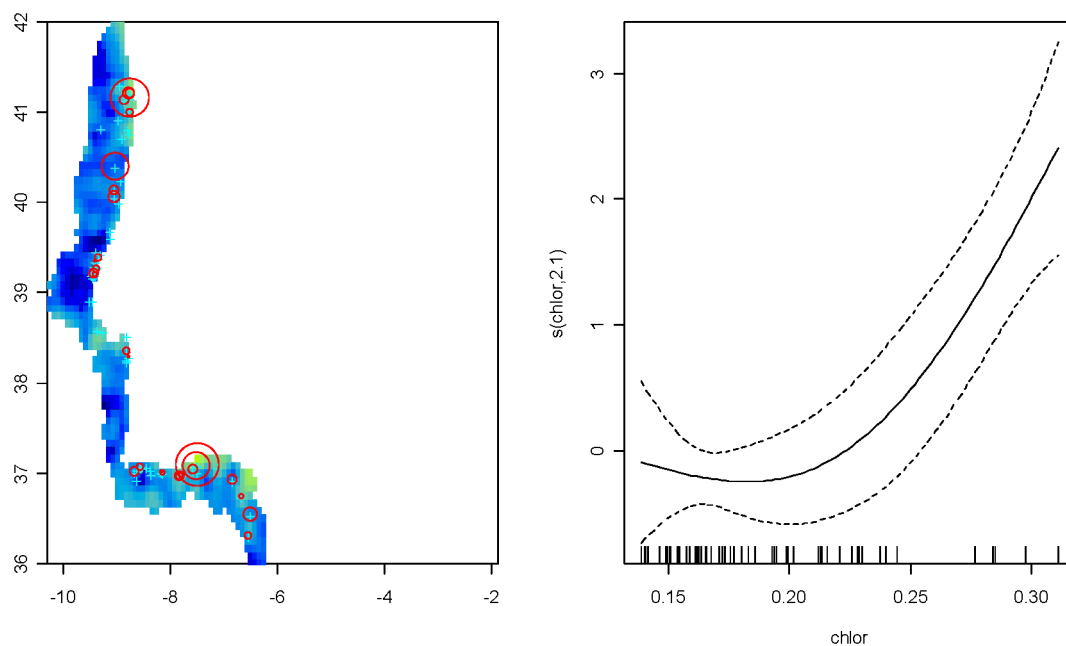
Figure 2.4.3.4. Distribution of modelled chlorophyll fluorescence (image plot: green-high, blue-low) and observed spawning fraction (red circles - non-zero S estimates, light blue crosses - location of adult sampling) during the 2002 Portuguese DEPM survey (left). Partial smooth effect of chlorophyll fluorescence to sardine spawning fraction from a GAM fitted to the same data (right).
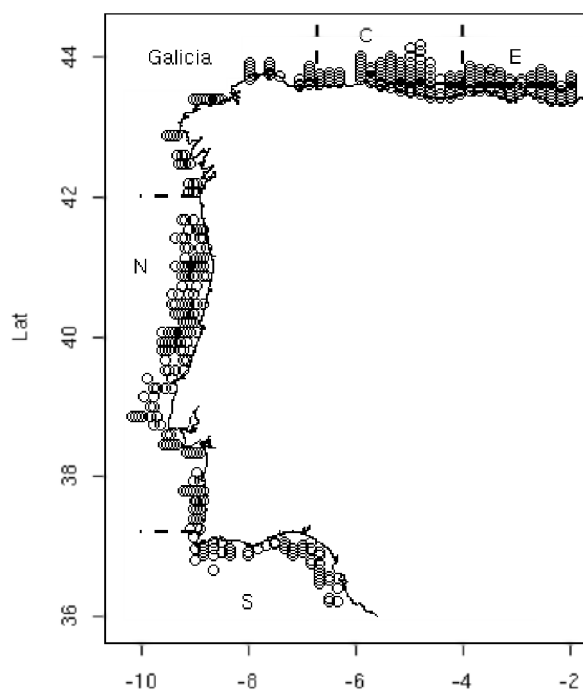
Figure 2.4.4.1. Ichthyoplankton observations and area stratification used for post-stratified estimates of the 2002 sardine DEPM survey.
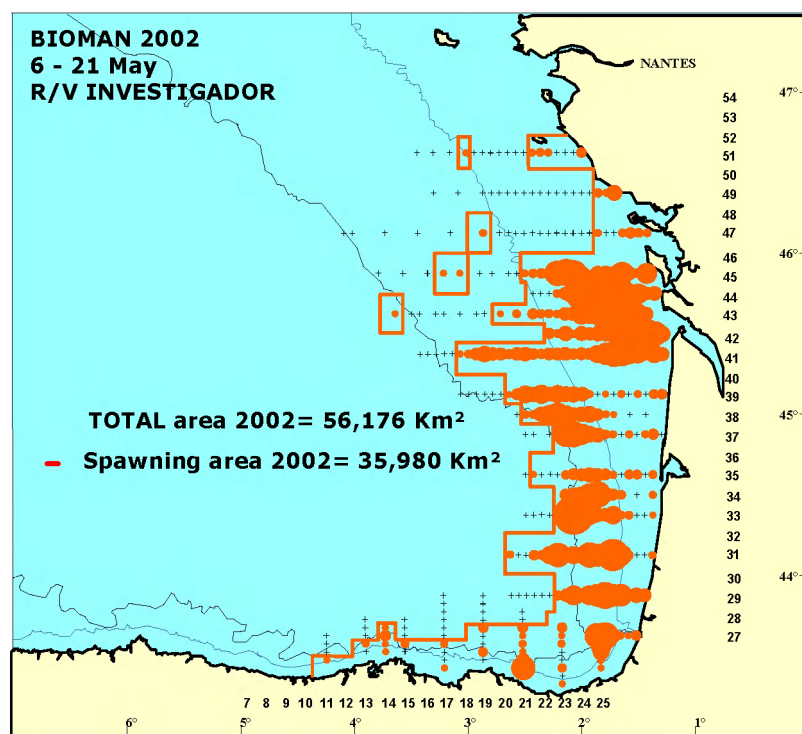
Figure 2.5.1.1. Anchovy egg/0.1m² distribution found during BIOMAN 2002. Solid line encloses the positive spawning area.
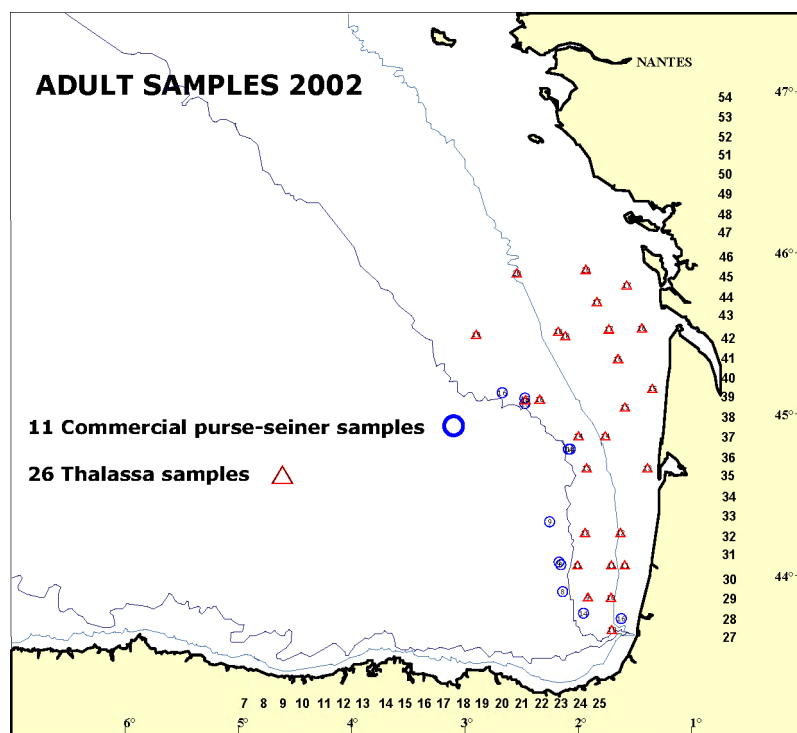
Figure 2.5.1.2. Adult samples obtained by commercial purse-seiners (in blue) and by RV "Thalassa" (in red) for the estimation of anchovy adult parameters in 2002.
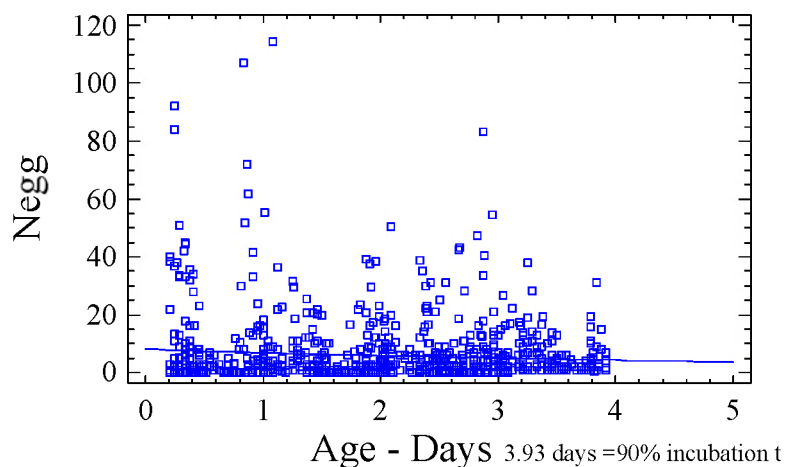
Figure 2.5.2.1. Egg mortality exponential curve fitted to daily cohort egg abundances and mean ages derived from Lo's ageing method using weighted non linear regression (as traditionally has been done).
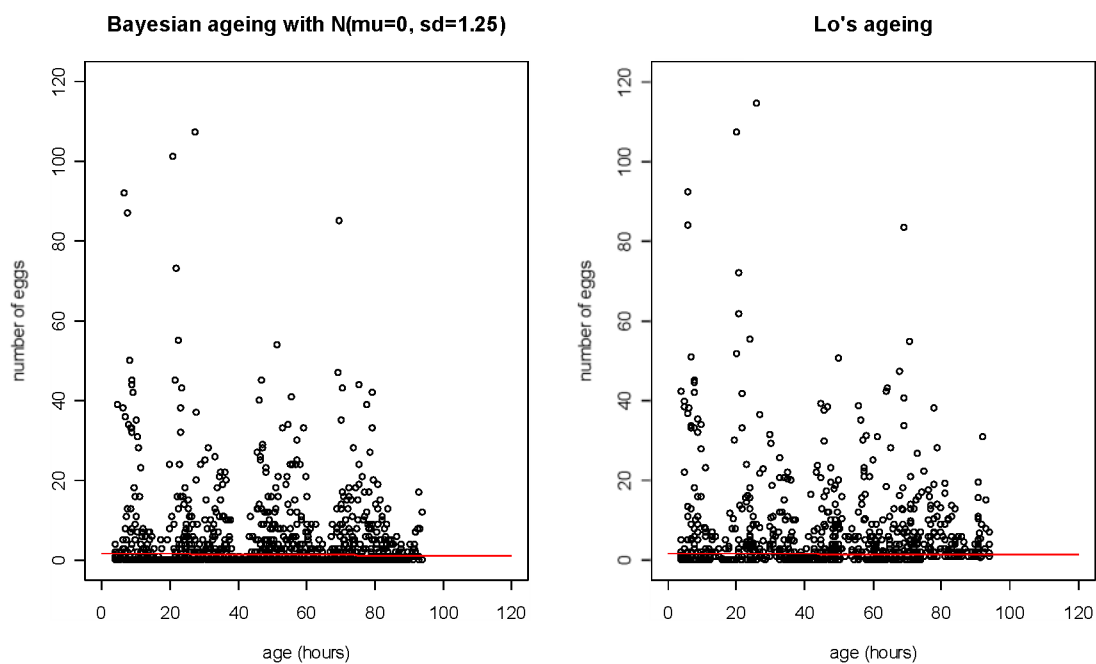
Figure 2.5.2.2. Egg mortality exponential curve fitted to the daily cohort egg abundances and mean ages derived from the new Bayesian ageing method (left panel) and from the Lo's ageing method (right panel) using GLMs with negative binomial error distribution and log link.
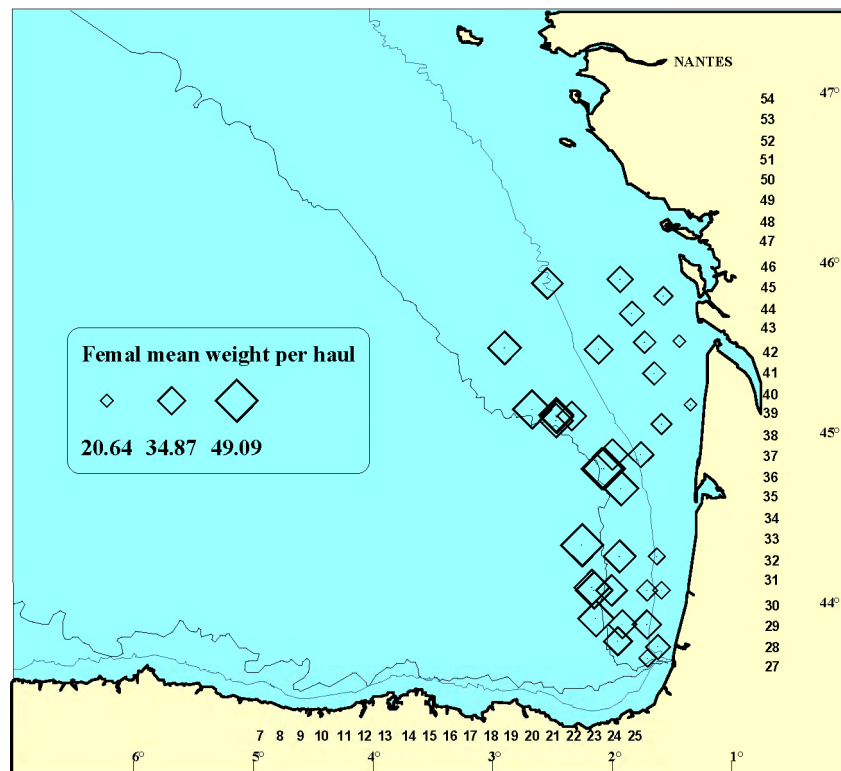
Figure 2.5.3.1. Mean female weight distribution in space for Bay of Biscay anchovy in 2002.
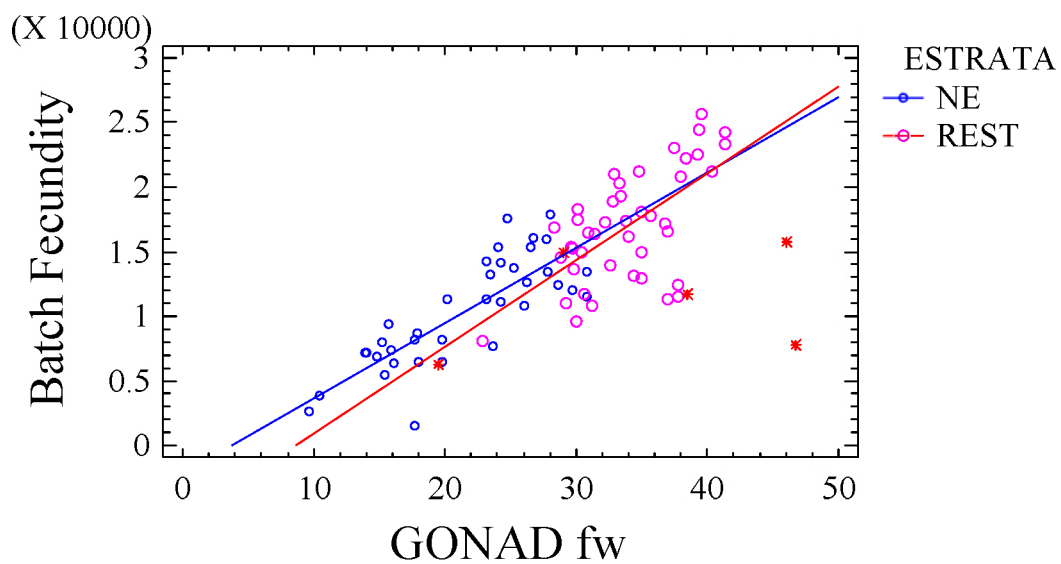
Figure 2.5.3.2. Batch fecundity *vs.* gonad free weight for the hydrated anchovy females. A regression line was fitted separately to each stratum (blue and red lines represent the model fitted to the NE and RE strata respectively). Red points represent the outliers, which were removed for the model fitting.

Plot of Fitted Model
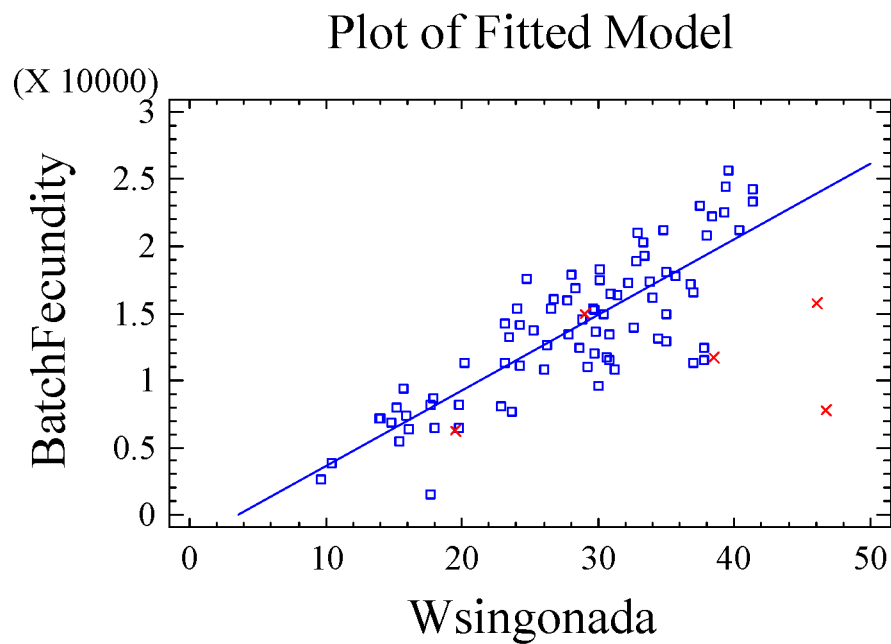
Figure 2.5.3.3. Batch fecundity *vs.* gonad free weight for the hydrated anchovy females. Regression line fitted to all the data, assuming that there was no difference between strata. Red points represent the outliers, which were removed for the model fitting.
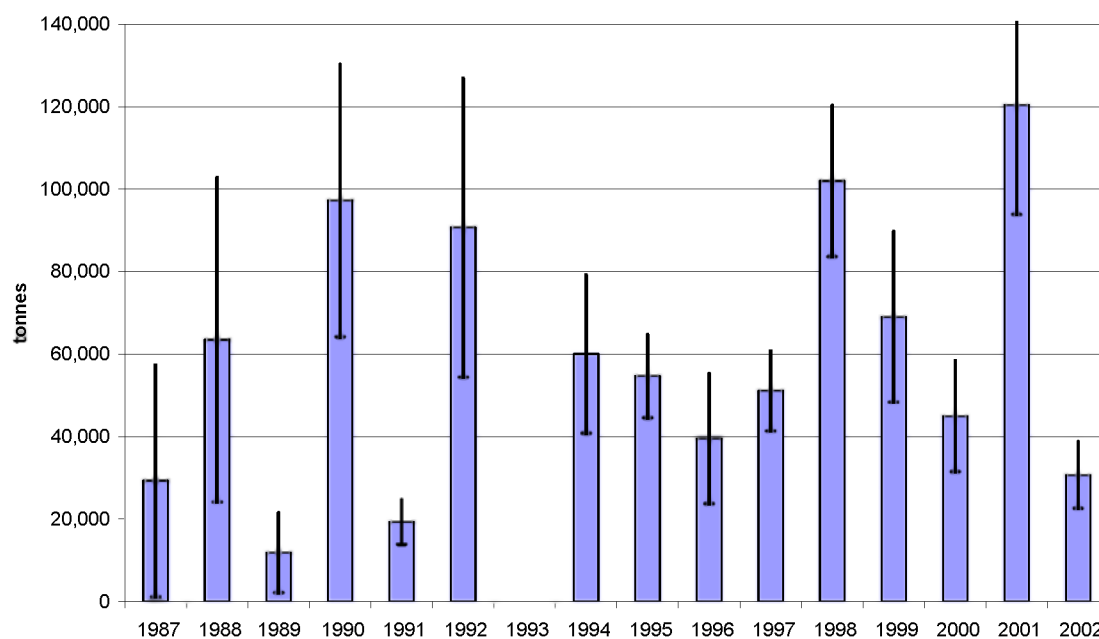
Figure 2.5.4.1. Series of biomass estimates (tonnes) for the Bay of Biscay anchovy since 1987. Most of them are full DEPM estimates, except in 1996, 1999 and 2000, which were deduced indirectly from the relationship of biomass with the spawning area and P_0.

3   GAMS in DEPM estimation

This section is dedicated to the use of generalized additive models (GAMs) in DEPM estimation. Section 3.1 summarizes aspects of modelling that have received particular attention during the recent EU project on GAMs and Section 3.2 briefly describes the most important methodological innovations resulting from this project. Section 3.3 demonstrates the use of GAMs in the estimation of egg production and compares GAM-based with traditional estimates for sardine and anchovy. Section 3.4 extends, for the first time, the application of GAMs to the estimation of adult DEPM parameters and spawning-stock biomass, demonstrating the potential of this method to provide more precise and informative estimates. Finally, Section 3.5 describes the current position of the group in relation to the use of GAMs in DEPM estimation and identifies the additional work necessary to permit a final evaluation on whether GAMs can safely substitute the traditional estimators in routine DEPM applications for sardine and anchovy in the future.

3.1   Introduction

GAMs have been shown to be a promising tool for modelling egg distribution and estimating egg abundance (e.g. Borchers *et al.*, 1997; Stratoudakis *et al.*, 2003), but several methodological problems remained to be solved prior to the use of the method in the estimation of daily egg production (Bernal, 1999; ICES, 2002). Most of these problems, particularly for sardine and anchovy, have been recently addressed as part of the EU project "Using environmental variables with improved DEPM methods to consolidate the series of sardine and anchovy estimates" (EU Study 99/080). The issues improved during this project were:

- performing model selection using GAMs – previously this was done on a relatively *ad hoc* basis with elements of subjectivity;

- modelling in a co-ordinate independent way in a GAM (the model should be insensitive to changes in co-ordinate system);

- modelling interactions in a GAM framework (the crucial issue here is how to scale covariates relative to each other);

- performing inference with GAM models;

- dealing with the over dispersion commonly encountered in egg survey data;

- modelling simultaneously spawning and mortality rates within a GAM framework;

- ensuring stability of models with "difficult" data (many zeros, etc.);

- making the developments easily available to users.

Several members of the SGSBSA participated in the above project, thus the methodological improvements, as well as the necessary software and training in the use of the new tools have been immediately incorporated to the Study Group. Most of the above issues required a change in the basic statistical methodology applied in GAM estimation, and the associated improvements are readily applicable to any situation in which GAMs are used. On the other hand, modelling simultaneously egg production and mortality is a problem specific to DEPM and for that several statistical improvements, new data acquisition and new analysis of existing data were performed, both during the above-mentioned EU project and afterwards by the Study Group members.

A simplified general formula of the GAM to be applied for egg production estimation can be expressed as:

$$E\,[\,N_i\,] = g^{-1}\,(\text{offset} + s\,(\,x_1\,,\,by = P_0\,) + s\,(\,x_2\,,\,by = \text{age}\,))\,,$$

where:

N_i = number of eggs in a daily cohort i;
g^{-1} = the inverse of the link function, i.e., the function that makes the relationship between the response and the predictor linear (e.g., logarithm in the case of Poisson);
P_0 = daily egg production;
age = mean age of eggs in daily cohort i;
$s\,(\,x_1\,,\,by = P_0\,)$ = the smooth function that describes the relationship between egg production (on a log scale) and the set of covariates x_1 (for example s (Lat,Long, by = P_0));
$s\,(\,x_2\,,\,by = \text{age}\,)$ = the smooth function that describes the relationship between egg mortality and the set of covariates x_2 (for example s (Depth, by = age)).

Estimates of *age* and N_i can be obtained directly from the samples, using an ageing procedure (Section 3.2.1). The offset is a fixed parameter that accounts for differences in sampling size (i.e., volume of water filtered or effective surface sampled). Also, an error structure (which can differ from normal distribution) has to be chosen. The "wiggliness" of the smooth functions used is automatically chosen by the new GAM software using General Cross-validation (Section 3.2.3). Nevertheless, the set of covariates used for explaining egg production and mortality rates is to be chosen by the scientist, based on the knowledge of the species and the characteristics of the spawning area. Also, whether covariates should enter the equation as univariate (for example, s (Lat, by= P_0) + s (Long, by = P_0)) or higher dimension smoothers (for example, s (Lat , Long, by = P_0)) has to be chosen by the scientist. A brief description of the methods used to estimate egg production using GAMs is detailed in Sections 3.2.1 to 3.2.5 below.

3.2   Methodology

3.2.1   Egg ageing

Estimating egg production in fish species that show fast egg development rates, such as sardines and anchovies, requires estimates of egg mortality in order to use information from all egg stages. To do so, traditionally eggs are aggregated into cohorts taking into account their ages, and an egg mortality curve is fitted to the abun-

dance of daily cohorts given their mean age (e.g., Piquelle and Stauffer, 1985). As only egg stages, and not ages, are observed in the samples, a number of ageing procedures for staged eggs is available in the literature (e.g., Lo, 1985, Bernal et al., 2001). Within the GAM project, and building from the work of Bernal et al. (2001), an innovative and statistically sound ageing procedure was developed and integrated within the GAM analysis of DEPM. The new ageing method is based on the same information traditionally used to age sardine and anchovy eggs, i.e.:

- Information from the survey: egg abundance by stage, sampling time, and environmental conditions affecting the development rate of the egg (usually temperature);

- Information from incubation experiments: the evolution of egg stages as their age increases for a given temperature;

- Information from observations on the reproductive biology of the species: distribution of spawning activity throughout the day.

The evolution of development stages mainly depends on temperature (e.g., review by Pépin, 1991), and to a lower degree on other parameters (see Section 4.2.5). Information on the rate of egg development as a function of stage and temperature is acquired from incubation experiments, in which eggs are reared in a number of tubes or tanks, maintained at a know and constant temperature, and from which samples are drawn periodically. The output from an incubation experiment is treated in the new ageing procedure as a multinomial process in which the probability of staying at a given stage or moving to the next depends both on the (known) age of the egg and on the incubation temperature. Thus, from the output of the incubation experiment we obtain $p(s|a,T)$, the probability of being at stage given age and temperature.

When a fish species shows daily spawning synchronicity (the case of sardine and anchovy, see Section 4.2.4), the information on the distribution of spawning activity throughout the day affects the ageing process. If spawning only occurs at certain times, eggs can only have certain ages given the elapsed time between spawning and observation (sampling). In an extreme case, if spawning is perfectly synchronous (eggs are spawned at say, midnight), and development up to hatching lasts only for a day, the observed eggs can only have exactly the elapsed time between midnight and the time they are sampled. If egg development lasts for more than one days, then the approximate mean age observed in the incubation experiment can be used to decide at which midnight it was released, and then just use the elapsed time between the spawning event and the survey time to attribute the exact age. In reality, spawning synchronicity information can be formalised as a probability density function (pdf) of age given survey time; f_t (a | τ). An additional, recent, discovery is that mortality should be used to properly age eggs, due to the fact that older eggs are less probable to find, and so, when different daily cohorts can be allocated to an egg, it is more probable that it is younger than older.

Using the information described above and Bayes' Theorem, it is possible to obtain the distribution of ages given the information obtained in the survey (egg stage, temperature and sampling time):

$$\hat{p}(a \mid s, \tau, T) \propto f_t(a \mid \tau) p(s \mid a, T)$$

and, thus, assign an age to every staged egg by sampling from the posterior distribution $\hat{p}(a \mid s, \tau, T)$ or classify eggs in cohorts using the posterior distribution directly. The new ageing procedure has a number of advantages in relation to the previous methods, both in relation to the way that incubation data are modelled, as well as in the actual assignment of ages. In relation to the incubation model:

- The new model is clearly described and has a sound statistical justification. This is a difference with respect to the different implementations of Lo's method, which:

  - although easy to implement, allocates randomness to the wrong variable (age, which in reality is fixed and decided by the researcher);

  - it is composed of two different models, one of mean age and another of stage duration. Estimation of mean age is not well documented in the different implementations (e.g., Miranda et al., 1990, Motos, 1994), and sometimes is either wrongly described or implemented. The assumed model for stage duration also varies in the different implementations of Lo's method, and again assumptions are not clearly described (i.e., in Motos, 1994 or Miranda et al., 1990);

- The new model has the properties of any other likelihood method, including asymptotic unbiasedness and efficiency, as well as making easy the comparison with other likelihood models.

- The uncertainty of the data from the incubation experiment is incorporated through the multinomial model.

In relation to the assignment of ages, the advantages of the new ageing model are:

- It allows for the inclusion of knowledge on the daily spawning synchronicity in as complex a way as the data at hand permit/justify;

- It has the potential of including prior distributions of other variables that can affect egg development, and thus the age of an egg at a given stage of development;

- The posterior distribution of ages has the uncertainty of both the incubation experiment and the assumed spawning synchronicity built in, and these can be easily accounted for in any subsequent estimation that includes age (e.g., the egg mortality curve). Also, the posterior distribution of ages can be used directly to allocate eggs into daily cohorts.

Differences between the multinomial and Lo's model of incubation data, and between the Bayesian and Lo's ageing method, and how these affect estimation of egg production are further investigated in Sections 4.2.2 and 4.2.5 respectively.

3.2.2 Sea area and survey limits estimation

Ideally, for design-based inference (which is the form of inference traditionally used in DEPM applications), the survey region should be defined before applying the design. In practice, the design of such surveys is somewhat adaptive, with sampling effort continuing offshore (on transects perpendicular to the coast) until eggs are sufficiently sparse to meet some cut-off criterion (see Sections 2.4.1 and 2.5.1 for decisions in recent sardine and anchovy surveys respectively). Design-based analysis of these surveys allocates non-overlapping areas to each sample point (plankton haul) and the survey region is considered to be the region defined by the sum of these areas. This constitutes something like a systematic design without a random start point, which is not an ideal design, but with the intense coverage of points that these surveys achieve it approximates satisfactorily simple random sampling (Smith and Hewitt, 1985).

For GAM-based analysis, a survey region whose boundary is clearly and unambiguously defined is needed in order to predict from the model only on points within the survey area and to avoid extrapolations. Several issues on how best to define the inner (inshore) and outer (offshore) limits of the survey area were discussed by the Study Group members and within the EU GAMs project. Assumptions and protocols to define the survey area were unified and a software to semi-automatically define survey areas and limits was produced within the project. The main characteristics of this software are described in Section 3.2.5, while the main decisions on how to define the survey area are summarized below:

- Inner (inshore) limits are defined by a smooth version of the coastline. This is to avoid extrapolation to unsampled inlets like estuaries or Rias (e.g., in the North-West corner of the Iberian Peninsula);

- Outer (offshore) limits are defined by the outermost survey points of each transect, but adding an extra distance equal to half the distance between survey points in that transect;

- Limits on the start and end of the survey (west and east limits of the survey if the transect are vertical or north and south limits if transects are horizontal) are defined using the position of the outer transects, but adding an extra distance equal to half the distance between transects.

Knowing the exact area represented by a sampling point (or any other point on which prediction takes place) is also important, both for traditional and GAM-based estimation of egg production. Egg production is measured in units of number of eggs per m^2 per day, thus the surface sea area represented by each point is needed to obtain the estimate of total daily egg production within the survey area. Once more, it is crucial not to extrapolate outside the survey area and to estimate as precisely as possible the exact area associated to each point, particularly in points near the coastline where imprecise estimation could lead to extrapolation on land. Estimation of the area represented by each point within the survey area should consider the distance from the point to any of the surrounding points and to the survey limits, so the area represented by each point are never overlapping, and the sum of area represented by all points within the survey area is equal to the total surveyed area. Specific software that automatically calculates the area represented by each point on a regular or an irregular grid along the survey area was created within the GAM project, and its characteristics are defined in Section 3.2.5.

3.2.3 Model fitting and selection

Within the GAM project, an integrated flexible framework for GAM fitting using penalized regression splines (e.g., Wahba, 1980; Parker and Rice, 1985; Eilers and Marx, 1996) was developed. The main aspects of the new framework are:

1) Integrated smoothness selection via GCV (General Cross-Validation) or unbiased risk estimation (effectively AIC);

2) Optimal low rank representation of model component smooth functions of arbitrary numbers of covariates;

3) A rigorous approach to interval estimation based on a Bayesian model of the smoothing process;

4) Extension of the generalized additive model to include "multiplicative offsets" for smooth terms;

5) An efficient approach for using a negative binomial error model with this GAM framework;

6) Development of optimally stable, numerically efficient methods for fitting and GCV/AIC smoothness selection;

7) The development of a complete modelling package implementing this framework freely available within the R software.

Points 1, 2, 3 and 7 aimed at improving general GAM fitting and providing a widely available framework for applications. Point 4 was developed to deal with particular a aspect of the GAM-based estimation of egg production (dealing properly with egg mortality), while points 5 and 6 were developed based on the feedback from the original applications to the DEPM data, but which are also relevant to the modelling of other data sets. Although Wood (2000 and 2003) describe in detail most of the above points, a brief summary of the main innovations in GAM fitting and model selection are given below:

- *GAMs using penalized regression splines:* GAMs can be represented by choosing a basis and a "wiggliness" penalty for each component of the model, using the bases to set up a model matrix and then fitting the model by penalized likelihood maximisation. When choosing the dimension of each basis there is a trade-off between computational effi-

ciency and potential flexibility of the smooth term. In addition, construction of a basis for a multi-dimensional smooth terms is non-trivial if the region covered by the data has a complex shape (e.g., coastal waters of the Iberian Peninsula). During the GAM project, a method for constructing optimal low rank smoothers, termed thin plate regression splines, was developed. The technical work in improving the statistical background of GAMs is reported in detail by Wood (2003), and is used to represent GAMs in the freely available R package *mgcv*.

• *Integrated smoothness selection by GCV/-UBRE/AIC:* Given the representation of the GAM fitting problem as a penalized generalized linear modelling problem, the estimation of the degree of smoothness appropriate for each model term becomes the problem of choosing the appropriate smoothing parameter by which to weight the wiggliness penalty for each smooth term in the model fit. Gu and Wahba (1991) pioneered the efficient estimation of multiple smoothing parameters using GCV for a class of rather computationally costly spline models, and in the work preceding the GAM project (Wood, 2000) this approach was extended to problems including GAMs represented using penalized regression splines. The software described in Section 2.2.5 implements this approach with particular emphasis to GAMs, and resolves a number of practical problems associated with ensuring that the methods work on real data sets. Special attention to solve specific problems in relation to treat egg survey data in the GAM framework, like the existence of extensive areas of zeros when a log link is used in the model were dealt with and mostly solved in the associated software. Effectively, the software detailed in Section 2.2.5 allows automatic selection of smoothness, although selection of the dimension of the smoothers and which covariates should be included together in a multidimensional smoother has still to be chosen by the scientist.

3.2.4 Model prediction and variance estimation

Predictions from a fitted GAM can be readily obtained using the software described in Section 3.2.5 in either the sampled grid or in any regular grid (see Section 3.2.2). Extrapolation outside the range of the covariates used for the fitting is strongly inadvisable in the GAM framework, due to flexible nature of the relationships between the response and the covariates. Thus, special care to avoid extrapolation should be taken (see for example how to solve geographical extrapolation in Section 3.2.2).

Different methods to obtain confidence intervals and variance estimates can be used in the GAM context. On one hand, theoretically well-founded Bayesian confidence intervals were developed for the general GAM framework included in the *mgcv* software. Nevertheless, these methods are not applicable when combining GAM methods for modelling egg production from DEPM surveys with simultaneous estimation of egg mortality,

which involves estimates of age. In this case, confidence intervals are estimated by nonparametric bootstrap, using haul as the sampling unit. The bootstrap procedure was modified to include errors in the fitting, as well as errors in the ageing estimation procedure. Also, when spawning biomass estimates from DEPM are obtained using GAMs both for egg production estimate and for adult parameter estimates, the bootstrap procedure can be expanded to include errors in all the parameters that affect the DEPM estimate of SSB (this component of variance estimation is still needed to be incorporated in the *depmodel* software). Thus, reliable estimates of variance and confidence intervals for spawning biomass can in principle be obtained from a full GAM-based DEPM analysis (see Section 3.4 and 3.5), although this is not currently available.

3.2.5 Software

Three packages that allow fitting GAMs in general (*mgcv*), implement a GAM-based DEPM estimation (*depmodel*), and solve geographical problems associated to the analysis of DEPM data (*geofun*) were developed within the EU project on GAMs. The software was developed in the form of add-on packages for the freely available statistical software R (Ihaka and Gentleman, 1996, CRAN: http://cran.r-project.org/). In general, installation and use of these packages is relatively straightforward and well documented in the CRAN site and the documentation within each package. The packages (which are available at http://ruwpa-st.andrews.ac.uk/depmodel) are briefly described below:

mgcv
This is the main package to fit GAMs. The main statistical and computational methods used in the package are already published (Wood, 2003) or are in preparation. The package represents a large methodological improvement in comparison with other available software to fit GAMs, especially in relation to:

• Automatic model selection using General Cross-validation (GCV) or Unbiased Risk Estimation (UBRE)

• Multidimensional smoothers using the thing plate approach (Wood, 2003)

• Efficient and robust model fitting procedure. It allows also fitting over-dispersed data using the negative binomial distribution.

depmodel
This package allows GAM-based DEPM analysis and estimation, including functions for:

• Fitting egg incubation models using a flexible extended continuation-ratio multinomial model (see Section 3.2.1);

• Assigning ages to staged eggs using a new Bayesian ageing method, that relies on the multinomial incubation model and a probability density function of

spawning activity throughout the day (see Section 3.2.1);

- Classifying eggs in cohorts and estimating cohort abundance and mean cohort age;

- Fitting GAM-based models of egg production, using the general formula shown in Section 3.1.

- Obtaining non-parametric bootstrap estimates of egg production, with uncertainty due to ageing incorporated.

Functions to obtain summary statistics for the models described above, as well as functions to plot the results are also included in the package. Some additional improvements of the GAM-based DEPM analysis, like including adult parameter variability in the variance estimation using non-parametric bootstrap, or performing a full GAM-based SSB estimation using DEPM are still not included in the actual version of *depmodel*.

geofun

The *geofun* package is designed to modify spatial data for appropriate use in spatial modelling (e.g., using GAMs) and provides some useful functions to represent/map model outputs. Its main functions are to:

- Transform pairs of spatial variables (usually Latitude and Longitude) into distances along a given reference line (e.g., the coast line or a selected isobath) and perpendicular to it;

- Find the limits of a survey and detect points of any given grid that are within the survey limits;

- Estimate the area represented by each point of a grid (regular or irregular) within the survey limits using Dirichlet tessellation;

- Estimate the area represented by the points located on the edge of the survey, according to the criteria described in Section 3.2.2;

- Provide functions for plotting the results of a spatial model.

The package has been designed specifically to work with marine coastal surveys, where distance from the coast and distance along the coast may be more informative geographic covariates than latitude and longitude. Also, extrapolation to areas on the coast should be avoided, and thus if predictions in points near the coast should be made, point area estimates should be corrected to avoid extrapolation on land. It should be noted that point area is computed by calls to external packages (*spatstat* and *deldir* packages available at CRAN) that use Dirichlet tessellation.

3.3 Application to sardine and anchovy DEPM surveys

This section summarizes the methodology and main results from the fitting of GAMs to sardine (Section 3.3.1) and anchovy (Section 3.3.2) eggs from DEPM surveys (see survey details in Section 2). GAM-based estimates

of egg production (and their CV) are compared to those obtained under the traditional estimator (Picquelle and Stauffer, 1985) to evaluate the performance of GAMs in terms of bias and precision.

3.3.1 Sardine

The four Iberian DEPM ichthyoplankton surveys for sardine (1988, 1997, 1999 and 2002) and a Spanish ichthyoplankton survey (1990) were used to estimate the daily egg production and mortality in the Atlanto-Iberian stock of sardine using GAMs. Eggs at stage of development were transformed into daily cohort densities according to the procedure described in Section 3.2.1 for age assignment that relies on data from an incubation experiment (see Section 4.2.2.2) and an assumed daily probability density function of spawning (~N(19,1), but also see Section 4.2.4). Daily egg cohort abundance was modelled as a function of spatial and environmental variables, using a GAM with an over-dispersed Poisson error distribution and a log link. The following variables (abbreviation used in the remainder appears in brackets) were considered in the model fitted to each survey:

- Latitude (*Lat*): Observed latitude (North, in hundredths of a degree) in a haul (range 35.97 – 44.32);

- Longitude (*Long*): Observed longitude (west, in hundredths of a degree) in a haul (range 1.88 to 10.34);

- Distance along the coast (*Along*): from a fixed point on the coast (42° N, close to the northern Portuguese/Spanish border): The distance is estimated along the coastline (range -1007.9 to 815.8 km, with positive values in Spanish surveys);

- Perpendicular distance (*Perp*): from the coast: Estimated distance of survey stations from closest point to the coast (range 0.5 to 105.6 km);

- Depth (*Depth*): Fitted bottom depth (in metres) in a haul (range 8 to 7041 m). Fitted values (very flexible spatial GAM to log-transformed depth observations from all surveys; 600 fitted degrees of freedom, 96.4% of total deviance explained) were used to avoid problems with incorrect/unknown survey observations and to obtain estimates on a regular grid of points within the survey area;

- Temperature (*Temp*): Fitted water temperature (in decimal degrees C) at near surface waters (5–10 m) in each haul (range 10.9 to 19.3° C). Fitted values (very flexible spatial GAM to temperature observations from each survey; 400 fitted degrees of freedom, more than 97% of deviance explained in each survey) were used for the reasons explained above;

- Effective area (*Efarea*): The effective area of the sampler (in m^2). This is 0.05 m^2 when the CalVET is towed vertically, but variable when towing deviates from the vertical due to currents, bad weather, etc. (see ICES, 2002 for method of estimation in these cases).

The first four variables provide (in pairs) unique information for the location of each haul (spatial variables), while depth and temperature provide information about the associated habitat (environmental variables). The two pairs of spatial variables and the pair of environmental variables were used as bivariate smooth functions in the original model fitting. When necessary, variables within a pair were re-scaled to approximate anisotropic interaction effects (in the case of the *Along:Perp* pair, *Along* was divided by the ratio of the range of the two variables). Further, depth observations were highly right skewed, so log-transformed values were used instead, while the natural logarithm of *Efarea* was used as an offset variable to standardise density observations per unit area.

Original models for each DEPM survey were fitted according to the following rules:

- Do not allow for spatial variation in mortality;

- Do not include in the same model the two pairs of spatial variables (start with Lat:Long);

- Include a bivariate smooth function of environmental variables;

- Do not allow for more than 60 df in bivariate spatial smooths;

- Include the logarithm of Efarea as an offset variable.

The best model was chosen based on the GCV score (Wood, 2000) among competing models that converged, according to the following procedure:

- Switch pair of spatial variables;

- Test for significance of environmental pair interaction;

- Test for significance of univariate environmental effects (if applicable).

Final predictions were performed on a regular grid, as it facilitated image plots and estimation within sub-areas of the stock that are more comparable across years. Variance and confidence intervals for egg production were calculated using a non-parametric bootstrap procedure with haul as the sampling unit. Estimation was based on re-sampling with replacement from the original set of incubation and survey data, refitting the original model and repeating the whole process a large number of times (>1000 for each model). Estimates were separated for models fitted to resamples of both survey and incubation data or of survey data only (the difference between the two giving an indication of the variation due to ageing).

Table 3.3.1.1 summarises the model selected for the estimation of sardine daily egg production and mortality in each Iberian DEPM survey. Given that the flexibility of the fitted GAMs was restricted to maintain comparability with the traditional estimation, the resulting models led to relatively low percentages of explained deviance. Overall, the explained deviance ranged from 43–60%, with indication of a large over-dispersion (scale parameter estimates around or beyond 10). It is also worth not-

ing that in the first three surveys estimates of the mortality parameter are highly significant and consistent among them in indicating hourly mortality rates in the order of 1.5–2%. This is not the case in the latter surveys (when a two months gap is introduced between the onset of the two national surveys), where estimates of mortality are non-significant (1999) or plainly unrealistic (2002). Finally, inspection of standard diagnostic plots indicated the presence of some extreme outliers and a generally poor relation between fitted and observed values.

Figure 3.3.1.1 shows the distribution of bootstrap estimates of sardine egg production after the removal of some large outliers (estimates more than 3 standard deviations larger than the bootstrap mean). Inspection of the data sets and models that led to these large outliers (that correspond to 0.4% – 1% of the bootstrap sample in each year) suggest that these can occur when observations with influential values of explanatory variables are omitted in a bootstrap replicate, causing the density surface to assume unrealistic shapes. Final estimates of coefficients of variation (CVs) were based on the bootstrap datasets after the elimination of these large outliers, although estimates using all bootstrap re-samples are also tabulated to facilitate comparisons (Table 3.3.1.2). Table 3.3.1.2 also shows some evidence of positive bootstrap bias (in the order of 5–10%), but the estimated CVs are robust to this bias and provide a reasonable basis for estimating 95% confidence intervals (CIs) assuming a log-normal distribution. Although Figure 3.3.1.1 suggests that there is little difference between assuming a normal or a log-normal distribution for the estimation of CIs, the latter distribution was preferred given that it led to CIs that were generally closer to those based on the bootstrap percentiles.

Table 3.3.1.3 shows the traditional and GAM-based estimates of egg production together with the estimated CVs. Figure 3.3.1.2 provides the same information but with 95% confidence intervals (CIs) plotted instead. CIs are calculated from the tabulated CVs, assuming a normal distribution for the traditional estimates and a log-normal for the GAM-based ones. Overall, point estimates of the two methods are satisfactorily close, with the largest relative discrepancy being 18% of the traditional estimate (1997 survey). A 15% difference is also observed in 1999, but this is justified by the fact that in the GAM method were also used additional data (a second leg of the Spanish survey in the Cantabrian Sea and an extremely high observation from southern Portugal) that were excluded from the traditional estimation (ICES 2002). Based on these results, it seems reasonable to suggest that, despite the large methodological differences between the two estimators (in ageing staged eggs, calculating the sea area associated to a survey point, etc.), point estimates obtained by the two methods are comparable and describe a very similar temporal pattern. Also, as demonstrated by the 1990 survey, the two methods perform similarly irrespectively whether the entire Iberian peninsula or just national waters are considered in estimation.

In relation to the precision of the egg production estimates, the results presented in Table 3.3.1.3 and Figure 3.3.1.2 show that in the three years that identical data sets were used for traditional and GAM-based estima-

tion, the latter led to a reduction between 5 and 12% in the estimated CV. This improvement is obtained despite the fact that the GAM-based method also incorporates variation due to the ageing procedure (which in these surveys contributes up to 1% in the tabulated CV) and using simultaneously the data from the two national surveys (it is likely that fitting separate models to the two national surveys could lead to an additional improvement in precision, but boundary effects could make distribution plots less useful). In 1999, where the data used by the two methods differ, the GAM method provided an estimate of CV higher by 5% than the traditional. Although it is possible that model choice was inadequate for that year, there is no doubt that the inclusion of the highest density ever observed for sardine during DEPM surveys in a CalVET tow (>700 eggs) contributed to this lower (but more realistic given the data) precision.

Figure 3.3.1.3 shows the fitted egg production in comparison with the observed egg abundance in each of the four Iberian DEPM surveys for sardine. These graphs show that the fitted models capture adequately the main features of sardine egg distribution in each DEPM survey. They also permit a synthetic view of the changes in sardine egg distribution over the Iberian Peninsula along the study period, which complements earlier work on sardine spawning areas from GAMs with a binomial error distribution (Bernal, 1999; Stratoudakis et al., 2003); in 1988, more than half of the total production occurred in the northwestern and northern Spanish coast. Ten years later (1997) the situation had dramatically changed, with Galicia and western Cantabrian being practically void of sardine spawning activity. This coincided with a retraction of spawning in the northern Portuguese coast, although intense inshore spawning was observed in the southern Iberia (Algarve and Gulf of Cadiz) during that year. By that time, almost 70% of the total spawning activity had shifted to the Portuguese waters and the Gulf of Cadiz. The same general pattern was observed in 1999, when more than 90% of the total spawning activity took place in Portuguese waters and the Gulf of Cadiz. In that survey it should be noted the moderate recuperation of spawning in the northern Portuguese coast and the appearance of spectacularly high egg densities in the mid-shelf of the Gulf of Cadiz. The situation seems to be reverted in 2002 (with a more notable recuperation in northern Portugal), but the low egg production in southern Iberia lead to the lowest estimate within the existing series.

3.3.2 Anchovy

For the Bay of Biscay anchovy, GAMs for estimating daily egg production and mortality rate were applied to the 1996–1999 egg surveys. The explanatory variables available, with their correspondent abbreviations in brackets, were the following:

- Latitude (*Lat*): Observed latitude (North, in decimal degrees) in survey stations, ranging from 43°N to 47°N.

- Longitude (*Long*): Observed longitude (west, in decimal degrees) in survey stations, ranging from 1°W to 5°W.

- Distance along the coast (*Alongdist*): Distance (in km) along the coast from a fixed coast point (8.48°W, 43.37°N) to the coastline point closest to the survey station.

- Perpendicular distance from the coast (*Perpdist*): Distance (in km) of the survey station from the closest point in the coast.

- Depth (*Depth*): Fitted bottom depth (in metres) ranging from 15 to 3800 m.

- Temperature (*Temp*): Fitted water temperature (in degrees C) at 10 meters depth in survey stations, ranging from 13 to 18.6. Assuming that the spawning mainly occurs at 10 m depth, this temperature was considered as the incubation temperature and was used for assigning ages to staged eggs.

- Sst (*Sst*): Fitted sea surface temperature (in degrees C) in survey stations, ranging from 13.7°C to 19.6°C.

- Sss (*Sss*): Fitted sea surface salinity (in PSU – practical salinity units) in survey stations, ranging from 30 to 36.56 psu.

- Effective area (*Efarea*): Sea surface area corresponding to the volume of water filtered by the vertical Pairovet net in each station. This is equivalent to the top area required for a cylinder with a height equal to the actual depth of sampling of the Pairovet in the station to have a volume equal to that actually filtered by the vertical net in the station (according to flow meters). This variable enters the model as an offset in the fitting, to produce egg abundance per surface unit.

Depth, Sst and Sss were not known at locations apart from the survey points, and even in some stations they were not available or reliable due to sampling errors. In order to predict on both the survey grid and on a regular grid within the survey limit avoiding unknown or incorrect values, different models were fitted to the observed values of these variables each year and fitted values were used instead of the original ones. Depth surface was obtained by distance inverse weighted interpolation with a power of 2 and a search ratio of 10 nautical miles, applied to a combination of data from ship echo-sounder readouts during the surveys (1994–1999), data from ETOPO2 global bathymetric model and coastline from GEBCO Global digital atlas. Alternatively, Sst and Sss surfaces for each year were fitted as a function of latitude and longitude using very flexible GAMs with normal errors and identity link. Table 3.3.2.1 shows the number of observations (N), the number of knots (k) and the percentage deviance explained by each of the models for Sst and Sss. The response residuals were less than 0.6°C and 1 psu for the Sst and Sss models respectively.

The short life span of anchovy makes the level of biomass strongly dependent on the recruitment occurring each year, which at the same time is very variable and

dependent on the environmental conditions. Figure 3.3.2.1 shows the egg abundance found in 1996–1999 surveys, together with the environmental situation as described by sea surface salinity and sea surface temperature. 1997 presented very high salinities over the whole area. Lower salinities (mainly in the area in front of the Gironde River) were found in 1996 and 1998. Especially in 1998 the lower salinity areas were more spread along the French coast and higher temperatures were also encountered. On the contrary, 1999 showed low salinities and high temperatures across the whole area. Hence, environmental conditions appeared to be indispensable for modelling adequately the egg spatial distribution.

The smooth terms considered for modelling the daily spawning rate were:

- s(Long, Lat) for describing the spatial location. Two bivariate smooths, s(Long, Lat) and s(Alongdist, Perpdist), were available for representing the geographical location of the points. However, it was decided to use only one pair in a given model. Given the "rectangular form" of the Bay of Biscay the differences between both options were minor, but s(Long, Lat) was selected as the more natural one.

- s(Sst.fit, Sss.fit) for describing the environmental situation. Given that Sst and Sss were highly correlated, a bivariate smooth was considered.

- s(Log(Depth)). Depth was log transformed due to its skewed distribution.

- s(Log(Depth), Alongdist/87) in order to analyse whether the effect of Depth on the anchovy daily spawning rate distribution changed in space. For example in the northern Spanish coast the depth gradient is very steep, being the 200 m depth contour line very close to the coast, whereas in the French coast the gradient is smoother, and this could affect the egg distribution. Alongdist was re-scaled (divided by the mean of the ranges ratio) in order to avoid anisotropy problems.

And for the daily mortality rate:

- Constant over the whole area, as assumed in the traditional DEPM.

- s(Long, Lat) for describing daily mortality rate varying very smoothly in space

Models considered and correspondent names are shown in Table 3.3.2.2. All models were fitted using the package *depmodel* (Section 3.2.5). GAMs with over-dispersed Poisson error distribution and log link were considered. Staged eggs were transformed into daily cohorts using the procedure described in Section 3.2.1, with the multinomial model for the distribution of stage given age and temperature as described in Section 4.2.2.1 and spawning time distribution assumed to be normal with mean at 23 and standard deviation of 1.25 (see Section 4.2.4).

Models m4 and z4 had the lowest GCV score and the largest % deviance explained in all years. Models m4

have a GCV between 8 and 14 and explained between 50 and 60% of the deviance, with large values of the scale parameters indicating over dispersion. The significance and degrees of freedom of the smooth terms were rather consistent from year to year. Same models but allowing z to vary in space (z4), presented very similar GCV but a slightly higher % of deviance explained (between 54 and 65%). Estimates of the daily mortality rate parametric coefficient decreased, although the smoothed mortality term was significant in all the years. Diagnostic plots for these models did not show any important trend, though some outliers were detected. By the time being, and similarly to the traditional DEPM, invariant mortality in space was assumed and m4 was selected as the best model for anchovy. Summary statistics for this model are presented in Table 3.3.2.3.

The GAM-based total egg production estimate was computed as the sum of the predicted values on a grid of points within the survey area. In order to avoid the effect of using different sea areas when comparing the GAM based and the traditional estimates, the GAM based estimates were computed using three different approaches:

a) predicting on the survey grid with the sea area represented by each station being the same as used in the traditional method.

b) predicting on the survey grid with the sea area represented by each station computed by *geofun* (Section 3.2.5).

c) predicting on a regular grid (denser than the survey grid) using the sea area computed by *geofun*.

Table 3.3.2.4 shows the GAM-based total egg production estimates from model m4, together with the traditional DEPM estimates, while Figure 3.3.2.2 provides image and contour plots for the fitted egg production models. Estimates of the variance associated to each of the total egg production estimates in (a) were obtained by non-parametric bootstrap from the original incubation experiment and survey data. Estimates for re-sampling only the survey data and of re-sampling both survey and incubation data were stored separately, so that the variance associated to the incubation experiment could also be evaluated.

Figure 3.3.2.3 shows distributions of the bootstrapped egg production values after removing the outliers (estimates larger than twice the first GAM-based egg production estimate). Added line shows the density function of a normal distribution with mean and standard deviation taken from the bootstrap sample. The distributions of the bootstrap estimates are quite close to a normal distribution. Table 3.3.2.5 shows % bootstrap bias, computed as the % difference between GAM estimate of egg production and bootstrap mean, and CV estimates, considering firstly only re-sampling on the survey data and secondly re-sampling on the incubation and survey data. Some evidence of positive bias is observed. CV estimates are similar for including or not re-sampling on the incubation data, suggesting that the incubation experiment did not add much variation to the final egg production estimate. Figure 3.3.2.4 shows the daily egg production estimates given by the traditional DEPM and by the se-

lected GAMs, with their correspondent confidence intervals computed from the estimated CVs assuming normal distribution. In this case, confidence intervals for GAM-based estimates are larger than those of the traditional method. However, note that the traditional CV estimates seem surprisingly small (as low as 5% in one case), suggesting that they might be negatively biased. The GAM method CVs are very plausible, between 13% and 21%, and are more reliable.

3.4 GAMS in adult parameter and biomass estimation: a first example

One of the main advantages from the application of GAMs to DEPM estimation is that, given an adequate number of fish samples, its use can be extended to the estimation of adult parameters and hence the direct model-based estimation of spawning-stock biomass (Borchers *et al.*, 1997). However, unlike eggs (where, for example, around 800 observations are available in each sardine survey in the Iberian Peninsula), adult sampling is always considerably more limited (35–40 adult stations in most years) and in some cases parts of the survey area are not sampled. Here, is presented an exploratory attempt to provide GAM-based estimation of spawning biomass for sardine and anchovy using the 2002 survey data.

3.4.1 Sardine

Adult sampling in the 2002 Portuguese and Spanish surveys was considerably intensified, providing adult parameter estimates from 102 fishing stations (74 in Portugal and the Gulf of Cadiz and 28 in Spain). These data (described in Section 2.4) are used here to explore for the first time the application of GAMs to sardine adult parameter and spawning-stock biomass (SSB) estimation. Models were fitted using the explanatory variables listed in 3.3.1 (apart from *Efarea*). *Alongdist* and *Perpdist* were estimated by *geofun* (Section 3.2.5), while temperature was obtained from the same model that provided fitted sea surface temperatures for the egg data set. *Depth* in this case refers to observed sampled depth, while additional information (fishing gear, sampling time and sample size) was also available.

A GAM with a binomial error distribution and a logit link was fitted to spawning fraction and sex ratio (with the binomial denominator being the number of histologically observed gonads and the total number of sampled fish respectively). A GAM with a normal error distribution and an identity link was fitted to mean female weight and batch fecundity. In the case of batch fecundity, it is natural that the fitted model will be very similar to that for mean weight given that fecundity is a variable derived from female weight. In all cases, only one bivariate set of explanatory variables was considered, aiming to capture the observed spatial structure. Table 3.4.1.1 provides a summary of the model selected for each variable and Figure 3.4.1.1 shows the residual inspection plots for the mean weight and spawning fraction models respectively. Overall, relatively simple bivariate models seem to have captured adequately the main structure in the 4 adult parameters estimated along Iberia.

To obtain an estimate of SSB, each adult parameter was predicted on the regular grid used for GAM-based estimation of egg production, and the traditional DEPM equation was applied to each grid point. SSB was estimated by summing across the grid, leading to an estimate of 466.2 thousand tonnes, of which only 45 (10%) in northern Spain. Figure 3.4.1.2 shows the spatial distribution of the predicted values of sardine spawning fraction and mean weight during the 2002 DEPM survey, both exhibiting strong spatial patterns. Spawning fraction is very low in Portugal, with patches of spawning activity in Cadiz and northern Portugal. In northern Spain, spawning activity is more intense and homogeneous, but with an increasing trend from central to eastern Cantabria. In mean weight there is a clear latitudinal trend, with fish in the Cantabrian Sea being considerably heavier than in western and southern Iberia. There is also evidence that first time spawners are concentrated predominantly in the Gulf of Cadiz and in a restricted zone in northern Portugal, which is in good agreement with existing knowledge on sardine recruitment zones.

The use of GAMs for adult parameter and SSB estimation also permits to visualise the distribution of spawning biomass in space. Figure 3.4.1.2 shows the estimated SSB along Iberia simultaneously with the distribution of observed total eggs (left) and the distribution of acoustic energy allocated to sardine (right). Overall, the two surveys seem to provide a reasonably similar regional distribution, although in the case of the Portuguese acoustics fish densities are observed closer to the shore. This most likely reflects the processes of passive offshore egg transport through diffusion and advection, which is often observed in the northern Portuguese coast. Finally, GAMs could also be used in relatively large data sets (probably when merging information from various years) to explore issues related to sampling (e.g., impact of sampling gear and time).

3.4.2 Anchovy

For anchovy, adult sampling in 2002 supplied a total of 35 samples (see Section 2.5), allowing a first exploration of GAM fitting to adult anchovy DEPM parameters. The two key adult parameters for DEPM are female weight (W) and spawning fraction (S), since batch fecundity is directly dependent on W (by a linear model) and expected sex ratio in weight can also be inferred from W under the hypothesis of 1:1 sex ratio in numbers. The latter is a hypothesis validated for the Bay of Biscay anchovy in 1997 and 1998 (Uriarte *et al.*, 1999a). Spawning fraction can be either dependent on W (if age dependent) and/or on the environmental conditions (temperature, plankton productivity, etc.) in the different spawning areas. Hence, spatial trends in these two parameters can be fitted by a GAM, provided that a sufficient number of adult samples is available within the survey area.

Modelling mean female weight (W): GAM fitting to W was based on a simple bivariate smooth of Lat and Long coordinates, assuming a Gaussian error distribution and an identity link function. No environmental covariate was explored. Two GAM models were tested; one based on the default maximum number of knots allowed auto-

matically for smoothing by the GAM function in *mgcv* (max of 30 knots) and a second restricting the maximum number of knots to 10. This comparison was deemed necessary since the former model required 26 degrees of freedom (df) for the fitting of 35 observations. The summary statistics (Table 3.4.2.1) show that the default model explained 99% of the observed spatial variability in mean female weight, while the second (which required 7.4 df) explained 89% of the variability. The potential of bias due to overfitting is evident in the first model, and the second model was considered good enough. Figure 3.4.2.1 shows the standard checking for this later model whereas Figure 3.4.2.2 presents the fitted surface with the observed female mean weight.

Modelling spawning fraction (S): GAM fitting to S was based on a simple bivariate smooth of Lat and Long coordinates, assuming a binomial error distribution, a logit link function and weights (binomial denominator) equal to the number of mature females examined histologically per haul. No environmental covariate was explored. Figure 3.4.2.3 shows the standard checking figures of the fitted model for S. This parameter could not be successfully fitted by GAM, with only 2% of the spatial variability being explained and the fitting was not significant (Table 3.4.2.2). Potential relationships with female weight or Depth were explored but also found non-significant, hence S was taken as a random Gaussian variable represented in space by its mean and CV.

Modelling sex ratio (R'): Given that the sex ratio for anchovy is since 1994 assumed to be 1:1 in numbers, the expected sex ratio in weight (R') can be inferred from W_f and the W_m (mean weight of females and males respectively) as follows:

$$R' = \frac{W_f}{\left(W_f + W_m\right)}$$

Males have a slightly lower weight than females according to a fitted linear model (Figure 3.4.2.4).

Modelling batch fecundity (F): Batch fecundity can be directly estimated from the gonad free weight of females by the standard linear relationship fitted in traditional DEPM analysis. However, given the very good fitting of GAM to mean weight of females we decided simply to repeat the fitting for F attaining similar good levels of fit.

Modelling egg production (P₀): Two different GAMs with Poisson error distribution and log link were fitted to the egg abundances by ages obtained from the egg survey and following the method and software described in Sections 2.1 to 2.3. First model, model 0, considers solely a spatial component for the daily egg production rate, P_0, through a bivariate smooth of Latitude and Longitude. Second model, model 1, also includes an environmental term, a bivariate smooth for Sss and Sst. As in the traditional DEPM, both models assumed constant mortality over the whole survey area. The improvement obtained by including the environmental covariates was noticeable and significant, attaining in total an explanation of 64.6 of the original deviance. Summary statistics of the fitted GAMs for P0 estimations are presented in Table 3.4.2.3.

Egg production surface (Figure 3.4.2.5) was obtained by predicting over the regular grid and total egg production was computed as the sum of the predicted values across the regular grid. In order to obtain an estimate of SSB, each adult parameter was predicted on the regular grid used for the GAM-based estimation of egg production. Female weight and batch fecundity were obtained by predicting from the correspondent fitted GAMs on the regular grid. Male weight was derived from the female weight predicted surface using the linear model shown in Figure 3.4.2.4. So that sex ratio surface was computed as the proportion of female weight over the total weight in each of the regular grid points. As no spatial structure was found for the spawning fraction, a constant mean surface over the whole area was considered. Then, the total predicted SSB was obtained by first applying the traditional DEPM equation to each grid point, and then summing across the whole area. Table 3.4.2.4 presents the final total egg production and SSB estimates for Bay of Biscay anchovy in 2002 and Figure 3.4.2.6 shows the spatial distribution of SSB.

3.5 Discussions and recommendations

Overall, the methods and software developed in the EU project on GAMs provide effective tools for estimating daily egg production, analysing data from incubation experiments and estimating the total area of plankton surveys and the corresponding repartition among fixed stations in a statistically rigorous manner. They have been shown to yield estimates of egg production that have higher precision than traditional methods, without any evidence of bias. Despite the fact that GAM-based estimation of precision is penalized by the consideration of variation due to ageing, reductions in the estimated coefficient of variation (CV) by 5–15% were observed in the sardine data to which it was applied. No reductions were observed in the anchovy application, but there is reason to suspect that the traditional CV estimates are negatively biased; the new CV estimates are considered more reliable. GAMs can also be an effective means of estimating the distribution of adult parameters and spawning biomass within the stock region, avoiding ad-hoc decisions on post-stratifications and providing the potential for additional improvements in the precision of the biomass estimates. Further, in the case of sardine, the estimates of SSB and the spatial distribution of adult parameters and SSB are in good agreement with other sources of information (acoustic surveys) and with existing knowledge on the species dynamics in the Iberian Peninsula.

GAM-based estimation of egg production also has the potential to relax the assumption that egg mortality is constant throughout the survey region and can model egg mortality surfaces and empirical relationships between environmental/oceanographic variables and mortality. Apart from improving estimation, this has the potential to provide useful biological insight to the processes that control pelagic egg mortality, which, together with the improved understanding of the spatial distribution of reproductive parameters, open new perspectives in the study of sardine and anchovy dynamics and its relation to environmental conditions. However, the introduction of

very flexible mortality surfaces is not yet recommended for the estimation of egg production, as there is a potential for positive bias that is not yet fully understood. In addition, the experience from the application of GAMs to adult parameter estimation has demonstrated that model selection is a critically important phase of estimation, requiring familiarity with model fitting and inspection processes but also a good understanding of the biological properties and the spatial distribution of all DEPM variables.

The conclusion of the GAMs project and the application of the *depmodel* software within SGSBSA have also brought to light a series of methodological developments and new research needed to improve further DEPM estimation. In the case of egg production, these include additional exploration of negative binomial fitting and anisotropic smoothing, novel and more detailed information on the most likely daily distribution of spawning activity for small pelagic fish species and simulation exercises to understand the impact of small-scale spatial variation in egg distribution to the estimation of cohort abundance and mortality. In the case of adults, these include the preparation of appropriate software for the estimation of the CV and confidence limits of SSB estimates, as well as a more rigorous methodological procedure for avoiding model overfitting when using relatively sparse data sets.

Based on the above, the Group believes that GAMs are in a position to eventually substitute the traditional estimator as the recommended method for routine DEPM estimation. However, this can only be achieved when adult sampling is sufficiently dense in space (to permit model fitting), covers the entire survey area (to avoid model extrapolations) and takes place sufficiently close in time to the plankton sampling (to avoid discrepancies between local adult and egg estimates that turn more important when SSB is estimated locally by GAMs). In addition, it requires particularly refined skills for the definition of model selection criteria, based simultaneously on a good understanding of the underlying fitting methodology, the software outputs and the dynamics of the species under study. The Group also believes that traditional estimation should be maintained in the future for comparative purposes or for situations where GAMs cannot be applied (very sparse data sets, lack of spatial structure, etc.). However, it recommends some modifications in the estimation procedure: it seems sensible to suggest that sea area estimation is only performed with the new software, while post-stratification is always considered when strong spatial patterns are detected in the survey data.

Table 3.3.1.1. Summary statistics and selected models for the estimation of sardine egg production in the four Iberian DEPM surveys.

Year	Model description	n	GCV	Scale	% Deviance	Z (se)
1988	s(Lat, Long, 56) + s(log(Depth), 6) + s(Temp, 8) + age	2 298	9.892	9.585	47.6	0.0138 (0.0024)
1990	s(Lat, Long, 23) + s(log(Depth),Temp, 28) + age	1 136	4.975	4.746	60.3	0.0191 (0.0042)
1997	s(Along, Perp, 18) + s(log(Depth), 4) + s(Temp, 8) + age	2 049	11.580	11.404	49.9	0.0131 (0.0045)
1999	s(Along, Perp, 43) + s(log(Depth), Temp, 14) + age	2 270	24.447	23.816	57.5	0.0057 (0.0035)
2002	s(Along, Perp, 54) + s(log(Depth), Temp, 18)	1 994	11.778	11.347	43.2	0

Table 3.3.1.2. Bootstrap bias (% difference between GAM estimate of egg production and bootstrap mean) and estimated coefficient of variation (CV) using all resamples (CV-all) and after eliminating large outliers (CV-final), separately for runs considering variation in both survey and incubation data (Boot – all) or only in the survey data (Boot – survey).

Year	Boot - all			Boot - survey		
	% Bias	CV-all	CV-final	% Bias	CV-all	CV-final
1988	9.2	0.152	0.150	10.4	0.151	0.147
1997	1.1	0.317	0.305	0.9	0.312	0.303
1999	4.2	0.338	0.328	3.2	0.332	0.322
2002	3.3	0.221	0.162	1.5	0.189	0.151

Table 3.3.1.3. Estimates of sardine daily egg production (coefficient of variation in brackets) for Portugal, Spain and Iberia, based on the traditional and GAM-based estimator. All estimates refer to 10^{12} eggs day^{-1}.

Year	Traditional			GAM-based		
	Portugal	**Spain**	**Iberia**	**Portugal**	**Spain**	**Iberia**
1988	2.87 (22)	2.97 (33)	5.84 (20)	2.32 (22)	3.48 (17)	5.80 (15)
1990	NA	1.78 (58)	NA	NA	1.86	NA
1997	4.41 (49)	0.72 (82)	5.13 (43)	2.96 (37)	1.27 (33)	4.23 (31)
1999	5.24 (30)	0.34 (44)	5.58 (28)	5.82 (39)	0.58 (33)	6.40 (33)
2002	2.07 (33)	0.52 (33)	2.59 (27)	1.73 (21)	0.82 (40)	2.55 (16)

Table 3.3.2.1. Number of observations, number of knots and % deviance explained for the Sst and Sss models used in anchovy GAMs.

	Sea Surface Temperature			Sea Surface Salinity		
Year	**N**	**K**	**% dev**	**N**	**k**	**% dev**
1996	315	300	98.7	117	100	99.2
1997	510	500	99.9	146	120	96.2
1998	591	500	98.8	197	160	98.5
1999	344	300	99.5	130	110	97.8

Table 3.3.2.2. Models considered and correspondent abbreviated names in GAMs of anchovy egg production.

	Mortality	
Daily egg production	**Constant**	**s(Long,Lat)**
s(Long,Lat)	m1	z1
s(Long,Lat)+s(Sst.fit, Sss.fit)	m2	z2
s(Long,Lat)+s(Sst.fit, Sss.fit)+s(log(Depth.fit))	m3	z3
s(Long,Lat)+s(Sst.fit, Sss.fit)+s(log(Depth.fit), Alongdist/87)	m4	z4

Table 3.3.2.3. Summary statistics of selected model (m4) for the estimation of anchovy egg production from 1996 to 1999.

Year	n	GCV	Scale	% Deviance	Z
1996	1 118	9.562	8.923	8.923	0.014
1997	1 736	8.569	8.173	8.173	0.010
1998	1 899	14.563	14.001	14.001	0.007
1999	899	7.924	7.283	7.283	0.007

Table 3.3.2.4. Total daily egg production estimates from the traditional DEPM and from the sum of the predicted GAM m4 surface using different sea areas in anchovy.

Year	DEPM estimate		GAM based estimate	
	survey grid	survey grid	survey grid	regular grid
	traditional	traditional	geofun	geofun
1996	2.77E+12	2.74E+12	2.82E+12	3.06E+12
1997	2.70E+12	2.83E+12	3.03E+12	3.05E+12
1998	5.59E+12	5.42E+12	5.61E+12	5.73E+12
1999	3.59E+12	4.08E+12	4.17E+12	3.92E+12

Table 3.3.2.5. Bootstrap bias (%) and CV estimates for re-sampling only on the survey data and for re-sampling on both the incubation and survey data in anchovy GAMs.

Year	Only survey		All	
	% bias	cv	% bias	cv
1996	8.48%	0.2149	9.72%	0.2148
1997	4.20%	0.1306	4.55%	0.1388
1998	16.72%	0.173	2.42%	0.1237
1999	5.39%	0.1528	5.52%	0.1424

Table 3.4.1.1. Description of selected GAM for each adult sardine DEPM variable (data from 2002 survey).

Variable	Model	n	Fitted df	GCV	% Dev
W	s(Lat, Long)	100	24	165.3	79.7
F	s(Lat, Long)	98	19	-	73.9
R	s(Along/34, Perp)	89	14	5.93	39.2
S	s(Along/42.5, Temp)	86	17	1.04	60.4

Table 3.4.2.1. Summary statistics of the fitted GAM for anchovy mean weight.

Model	n	Fitted df	GCV	% Deviance
s(Long,Lat)	35	25.98	7.6996	99.3
s(Long,Lat, k=10)	35	7.422	11.372	88.6

Table 3.4.2.2. Summary statistics of the fitted GAM for anchovy spawning fraction.

Model	n	Fitted df	GCV	% Dev
s(Long,Lat)	35	2	-0.52153	3.02

Table 3.4.2.3. Summary statistics of the fitted GAMs for anchovy daily egg production.

	Model	n	GCV	Scale	% Deviance	Z
model 0	s(Long,Lat)	1 494	8.8712	8.7069	57.7	0.0077
model 1	s(Long,Lat) + s(Sst, Sss)	1 494	8.2974	8.2974	64.6	0.0080

Table 3.4.2.4. Total egg production and SSB GAM-based estimates for Bay of Biscay anchovy in 2002.

	Total egg production			SSB (k=10)
	Survey grid	Survey grid	Regular grid	Regular grid
	Sea area classical	Sea area geofun	Sea area geofun	Sea area geofun
model 0	2.396E+12	2.382E+12	2.307E+12	29 681 153 393
model 1	2.420E+12	2.418E+12	2.305E+12	29 655 692 907
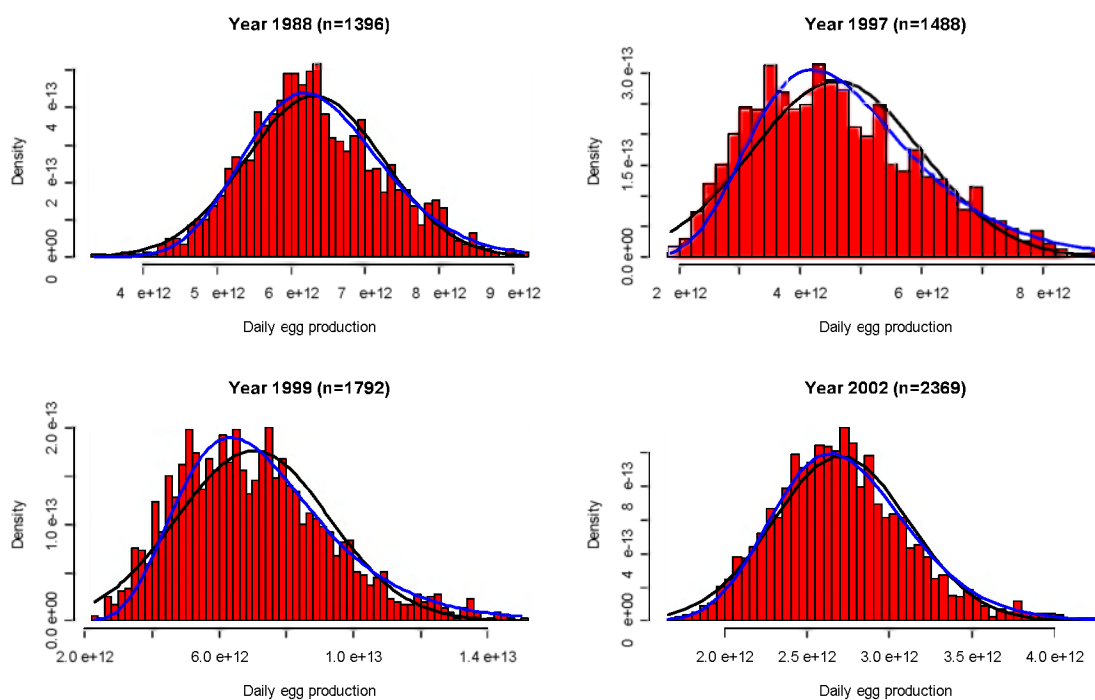
Figure 3.3.1.1. Distribution of bootstrap estimates of sardine daily egg production, after removing observations more than three standard deviations larger than the bootstrap mean. Continuous black/blue line corresponds to normal/log-normal density for a mean/log-mean and standard deviation equal to that of the bootstrap sample.
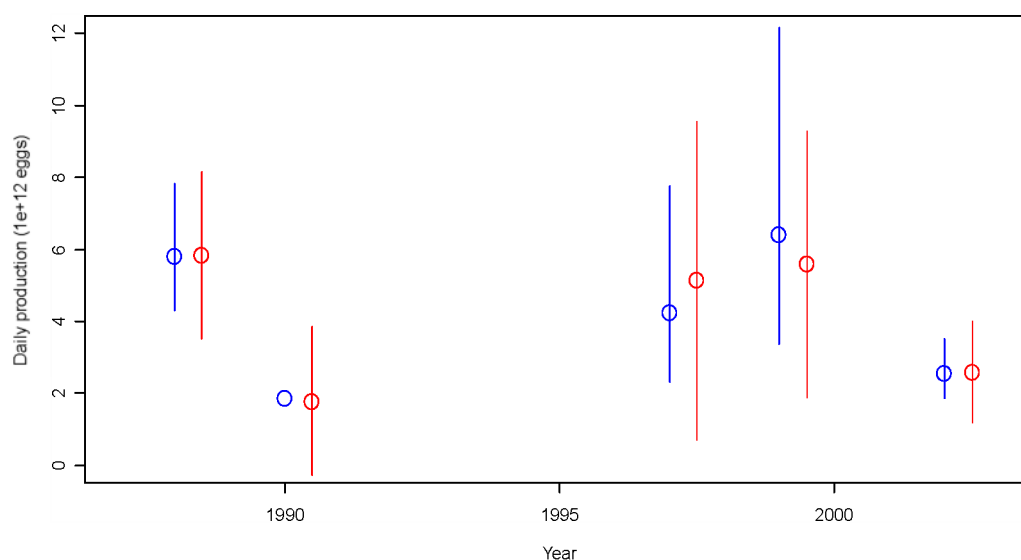
Figure 3.3.1.2. Time series of estimates of sardine daily egg production based on the traditional method (red) and the new GAM method (blue). Lines are estimated 95% confidence intervals (CIs), which for the traditional method are based on an assumed normal distribution and in the GAM-method on a log-normal distribution. Traditional method estimates have been shifted one year to the right for presentation. Estimate for 1990 only refers to Spanish survey and GAM, CI is not available.
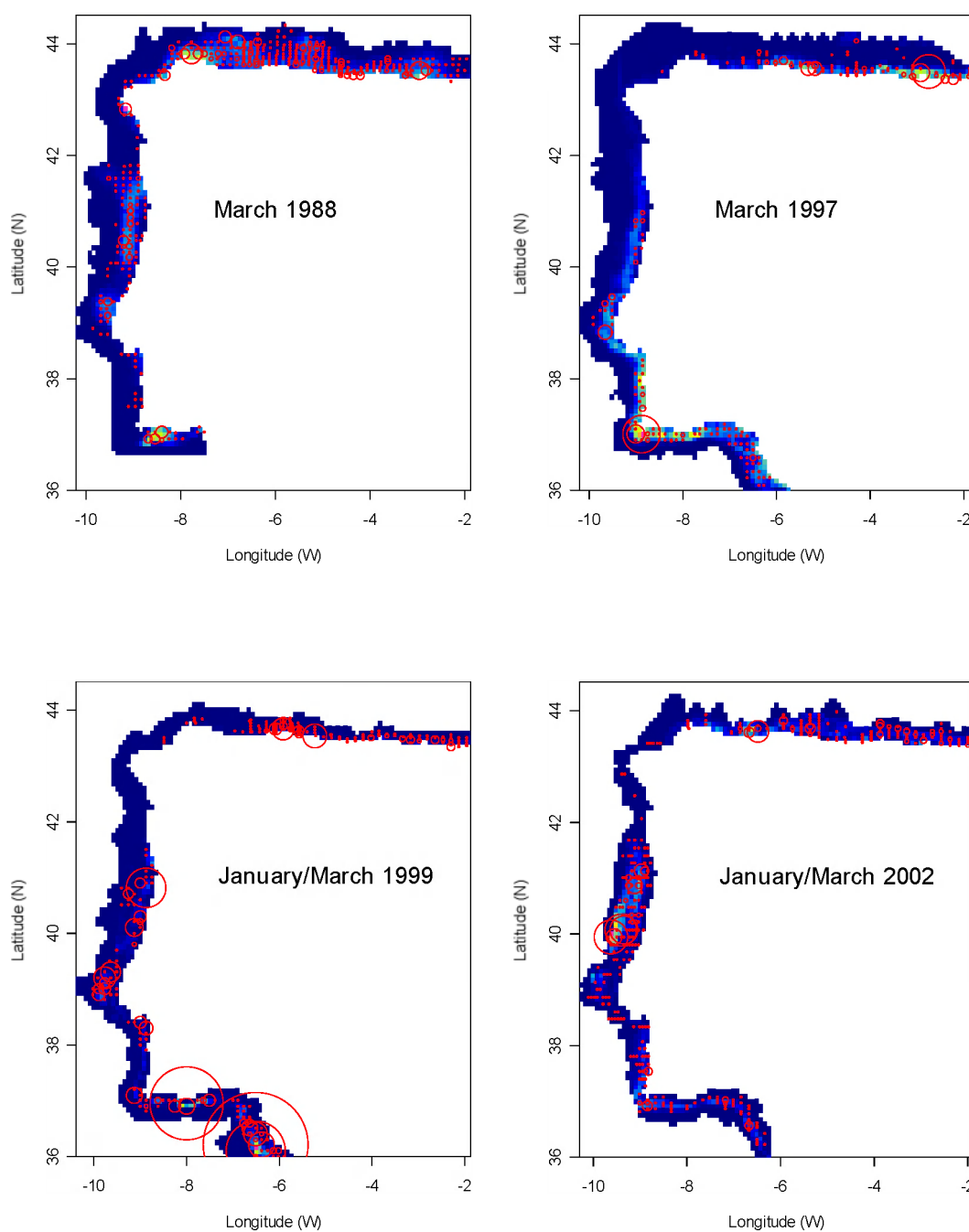
Figure 3.3.1.3. Modelled distribution of daily egg production (colour scale) and observed total number of sardine eggs (red circles) during the 1988 (upper left), 1997 (upper right), 1999 (bottom left) and 2002 (bottom right) Iberian DEPM surveys. Diameter of circles is proportional to the total number of eggs in a station.
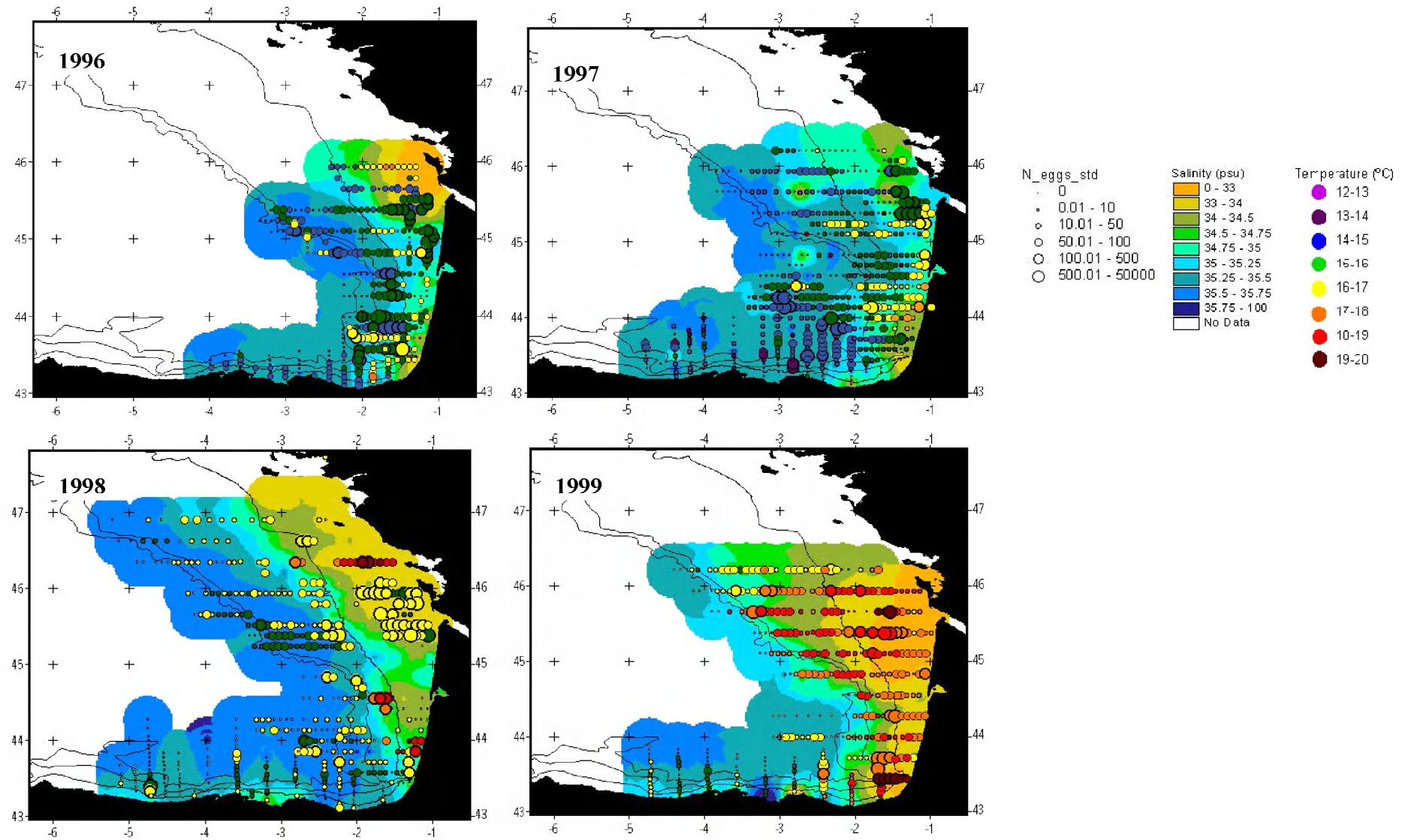
Figure 3.3.2.1. Environmental situation and egg abundance for 1996–1999 surveys. Background colour represents the sea surface salinity. Circles correspond to survey stations. Size of the circles is proportional to egg abundance and colour of the circles is given according to the sea surface temperature at each station.
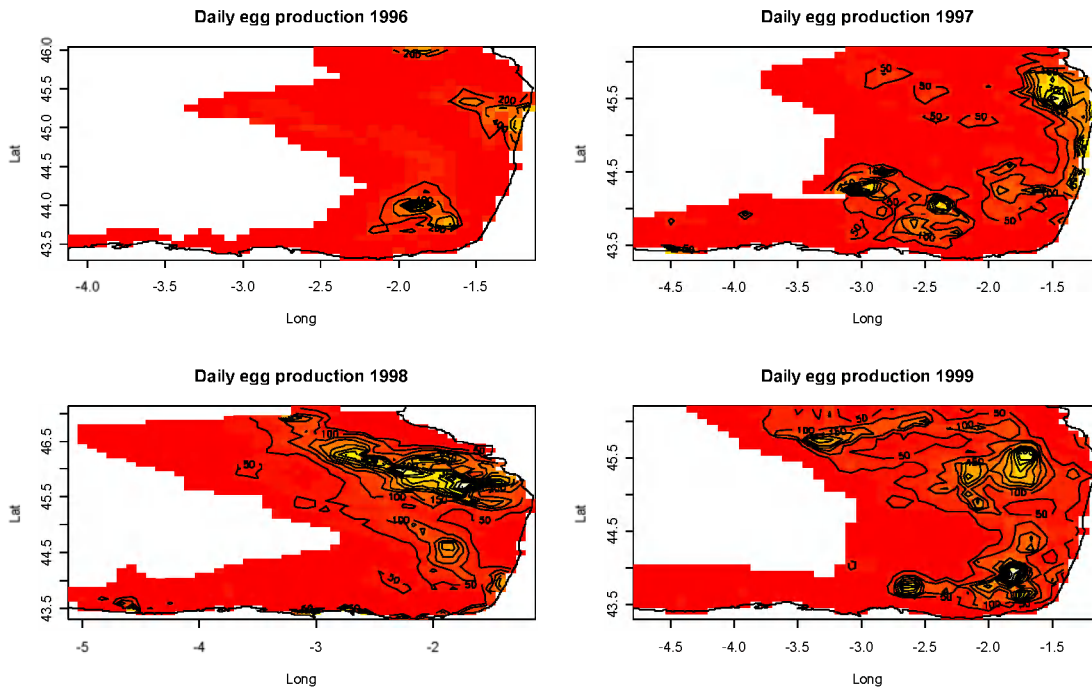
Figure 3.3.2.2. Fitted daily egg production surface image and contour plot for model m4 from 1996 to 1999 fitted to anchovy eggs.
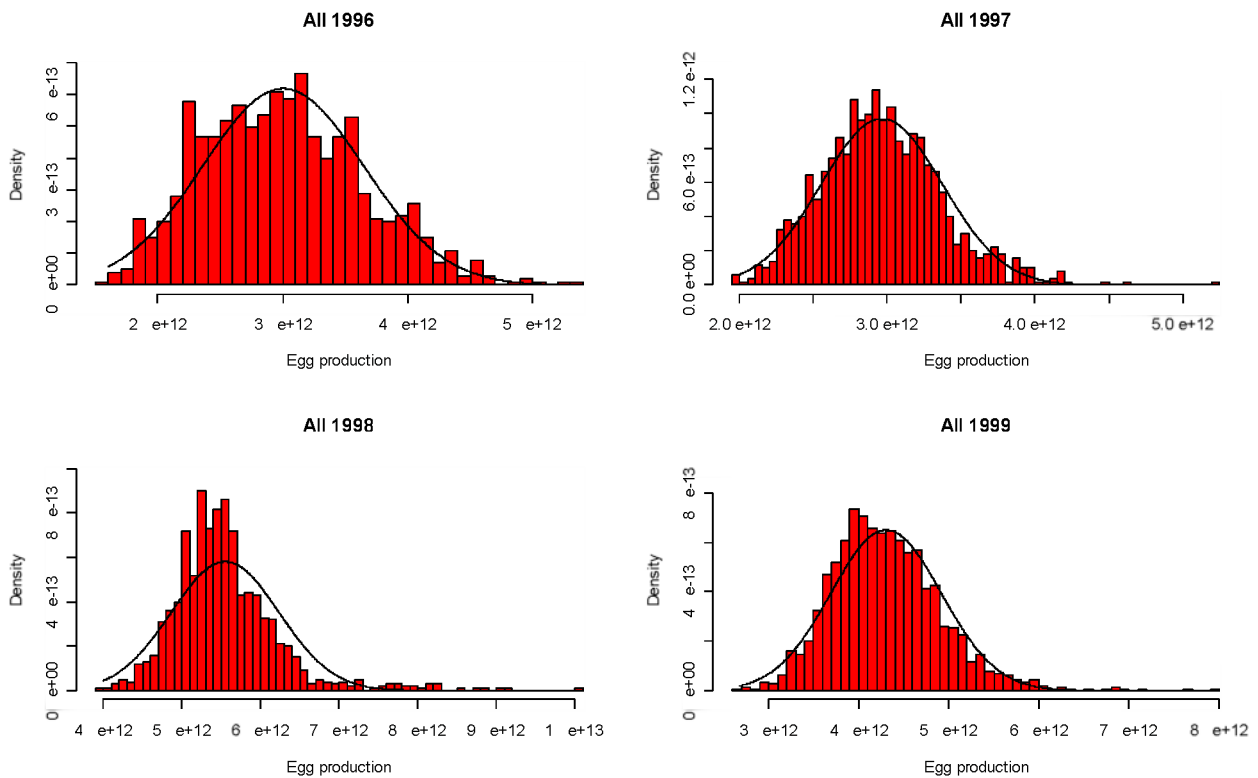
Figure 3.3.2.3. Histogram of the total egg production bootstrap estimates considering re-sampling on both the incubation experiment and survey data. Line represents the density function of a normal distribution with the observed bootstrap estimate mean and standard deviation.
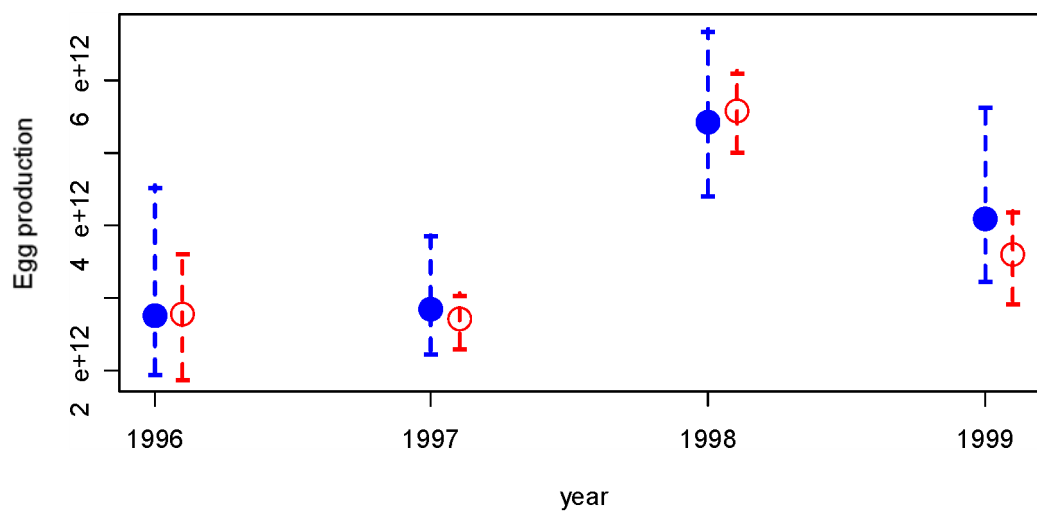
Figure 3.3.2.4. Time series of estimates of anchovy daily egg production with estimated 95% confidence intervals (dashed line) based on the traditional method (red; assuming normality), the new GAM method (blue; assuming normality).
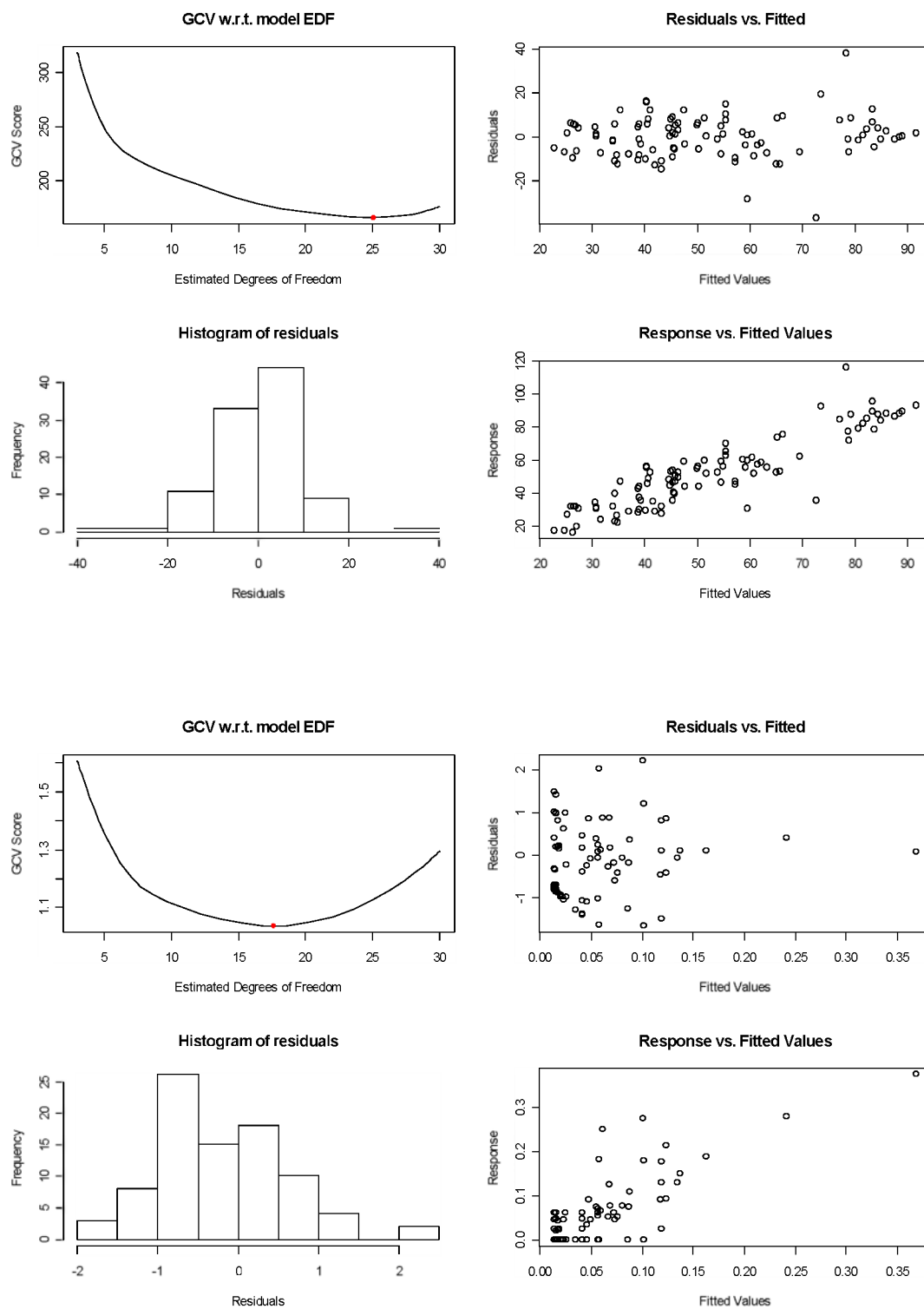
Figure 3.4.1.1. Inspection of residual plots testing the adequacy of the GAMs fitted to mean female weight (top) and spawning fraction (bottom) of sardine from the 2002 Iberian DEPM survey.

Sardine spawning fraction Distribution in 2002 **Sardine female mean weight distribution in 2002**
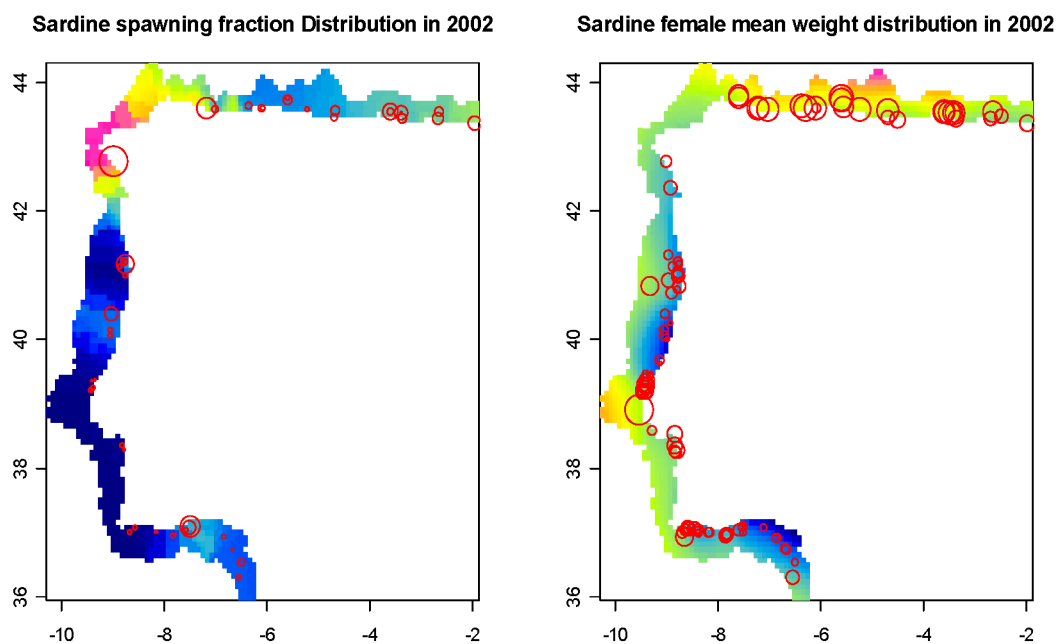
Figure 3.4.1.2. GAM-fitted (colour scale) and observed (circles) sardine spawning fraction (left) and female mean weight (right) from the 2002 Iberian DEPM surveys.

Fitted DEPM SSB and observed egg distribution **Fitted DEPM SSB and acoustic EDSU in March 2002)**
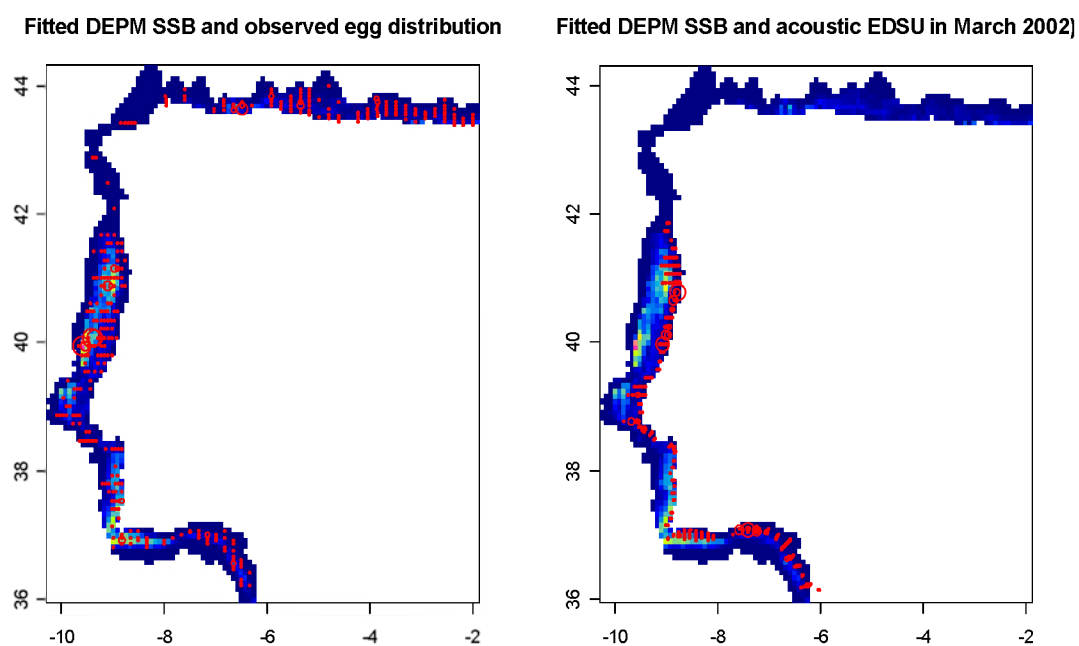
Figure 3.4.1.3. GAM-fitted (colour scale) spawning-stock biomass against observed egg density (red circles, left) and acoustic energy attributed to sardine (red circles, right). SSB and egg densities refer to the 2002 Iberian DEPM survey, acoustic data refer to the March acoustic survey off Portugal.
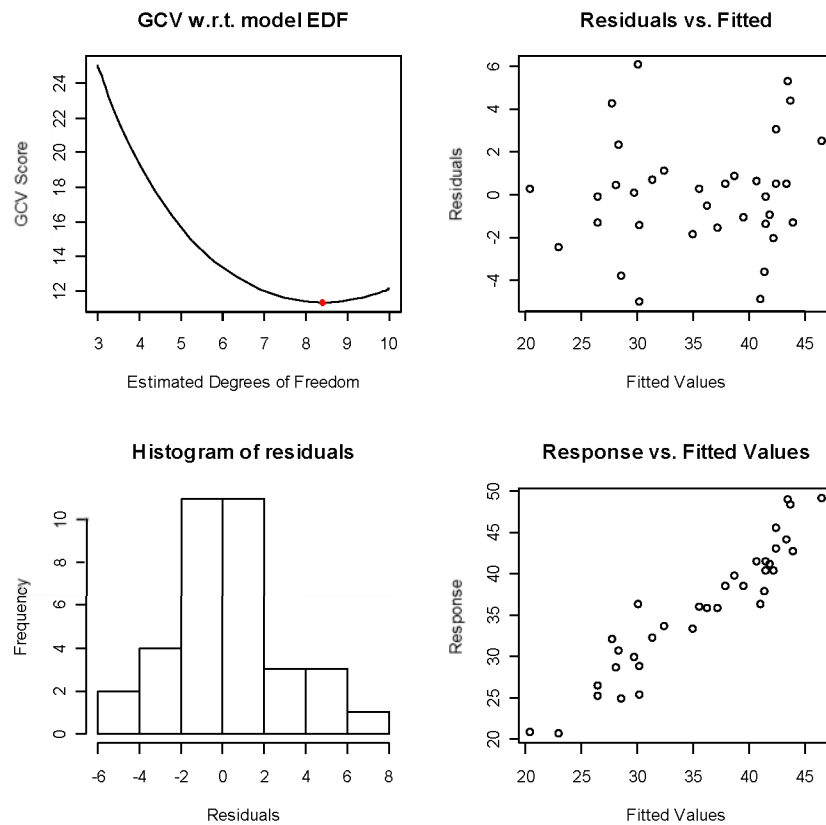
Figure 3.4.2.1. Standard checking plots for the fitted GAM model on anchovy female weight (allowing a maximum of 10 knots).
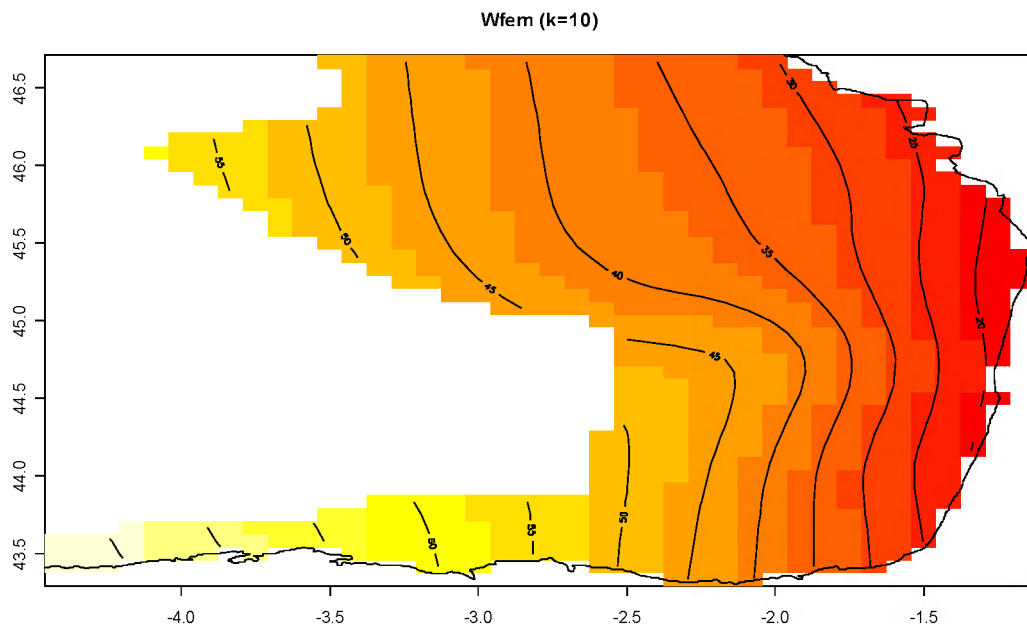
Figure 3.4.2.2. Fitted female mean weight surface for anchovy in 2002 (allowing a maximum of 10 knots).
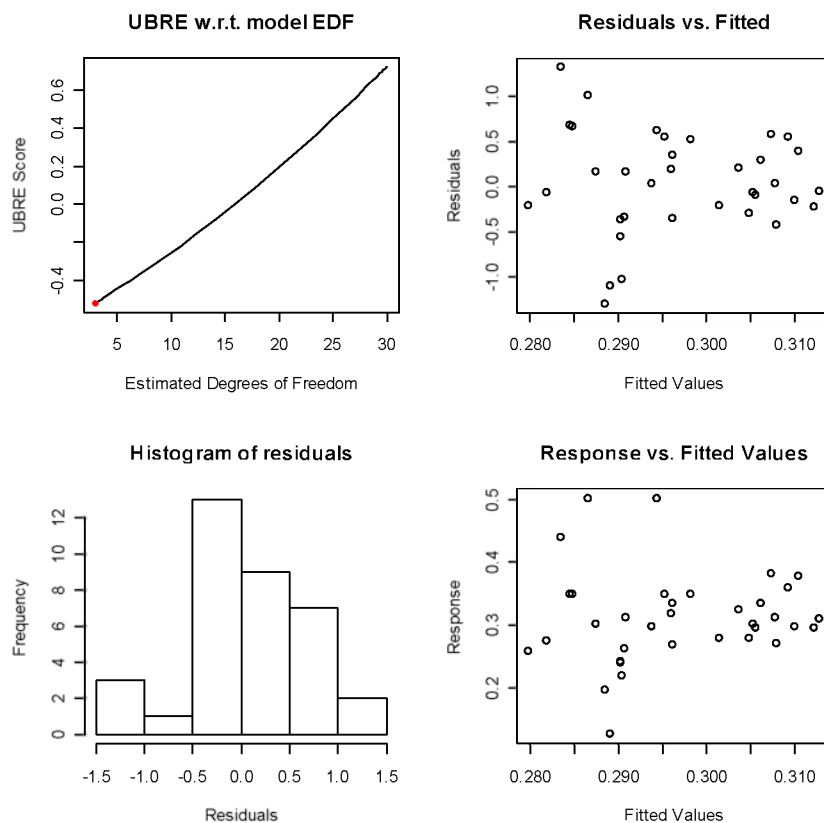
Figure 3.4.2.3. Standard checking figures of the fitted model for anchovy spawning fraction in 2002.
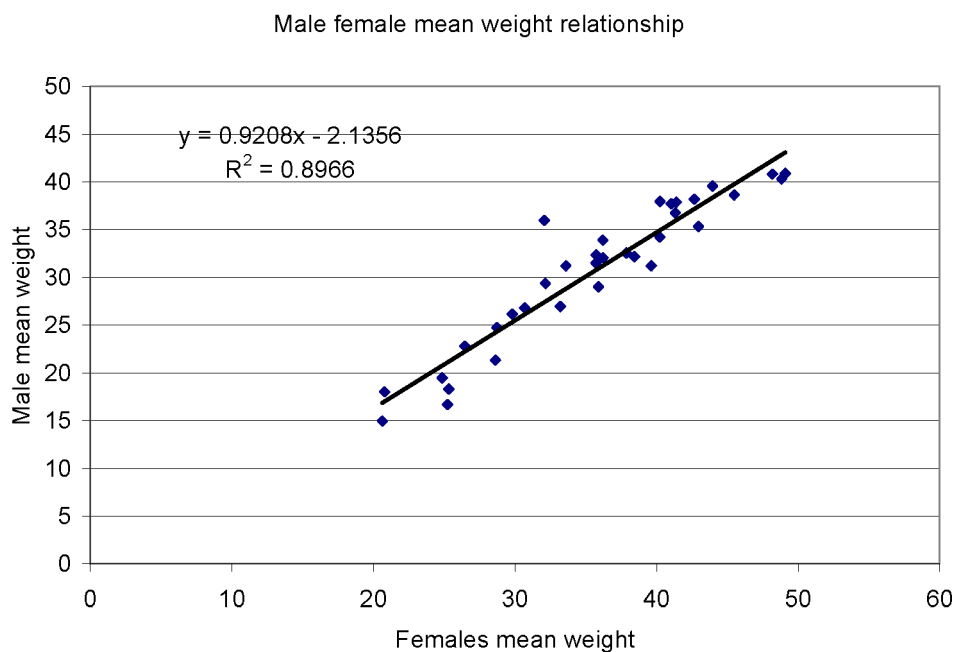
Figure 3.4.2.4. Fitted linear model of anchovy male weight as a function of female mean weight.

spawning rate
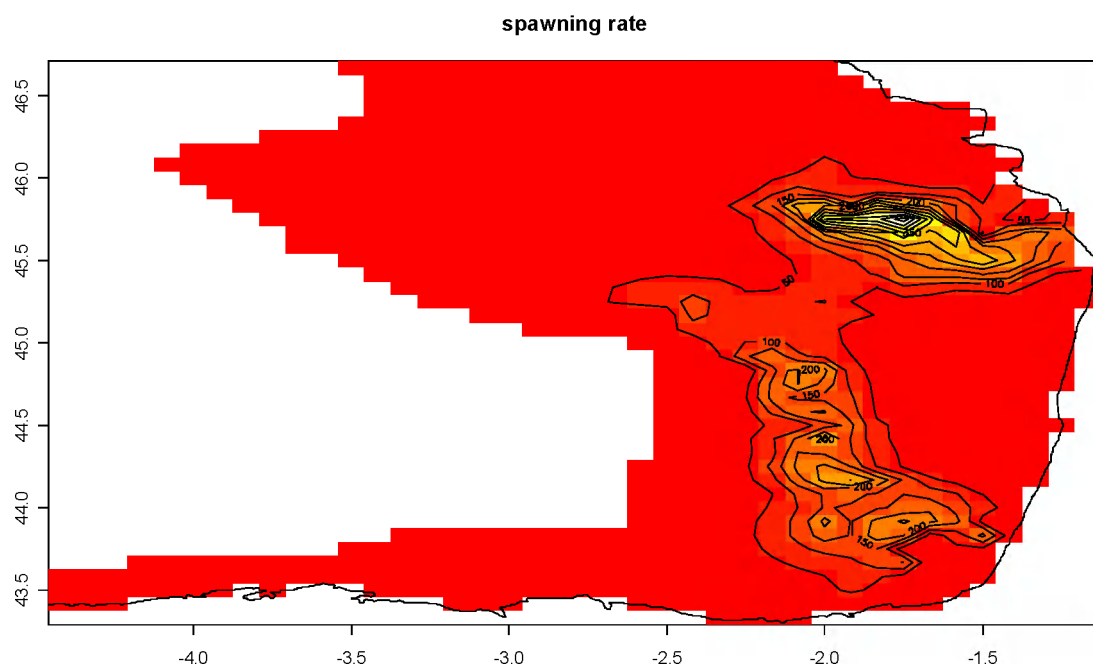
Figure 3.4.2.5. Fitted daily egg production rate for the Bay of Biscay anchovy in 2002.

ssb
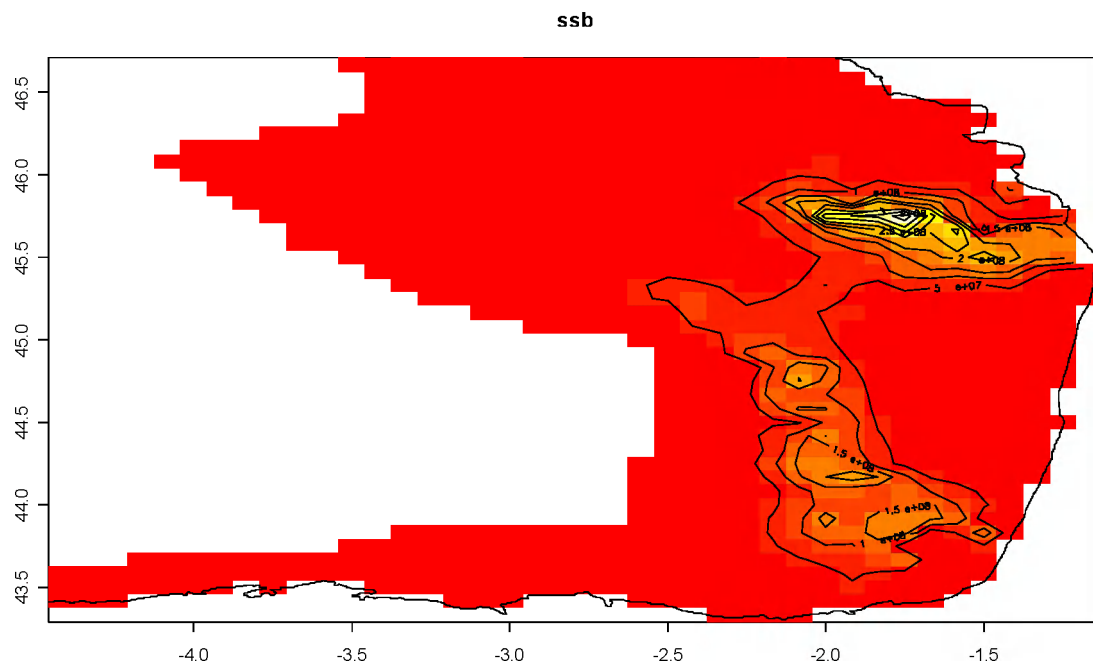
Figure 3.4.2.6. Fitted SSB surface for the Bay of Biscay anchovy in 2002.

4 Other advances in DEPM methodology

This section includes research topics related to DEPM that have advanced in recent years, but for which the developments have not been that far-reaching to warrant entire chapters. Section 4.1 is dedicated to the Continuous Underway Fish Egg Sampler (CUFES), providing a summary of recent advances, its application in DEPM surveys and its potential for the future. Section 4.2 is dedicated to egg staging and aging, where considerable progress has been made in the planning and execution of incubation experiments and the development of statistically robust methods for modelling such data (see Section 3.2.1) and using them in the ageing of staged eggs. Section 4.3 is specific to the application of DEPM to Iberian sardine, revising data on the maturation cycle of the species, comparing macroscopic and microscopic maturity scales and describing the seasonality of sardine spawning off Portugal. Finally, Section 4.4 is dedicated to spawning fraction estimation and the dating of post-ovulatory follicles, based on new data from sardine spawning populations in the north-eastern Atlantic (Iberian Peninsula) and the eastern Mediterranean (Aegean and Ionian Seas).

4.1 The Continuous Underway Fish Egg Sampler (CUFES)

4.1.1 Introduction

The Continuous Underway Fish Egg Sampler (CUFES) was developed in the 1990s (Checkley *et al.*, 1997) and has proven efficient in sampling the highly contagious distribution of pelagic fish eggs and simultaneously collecting a wealth of oceanographic and spatial information. The sampler (which consists of a submersible pump, concentrator, electronics and sample collector) operates continuously at nearly all sea conditions, providing a real-time estimate of the volumetric abundance of pelagic fish eggs at pump depth (usually 3 m). Apart from its obvious application in the characterization of spawning habitats (Van der Lingen *et al.*, 1998; Checkley *et al.*, 1999a; Checkley *et al.*, 1999b), it has also been used to improve the cost/precision ratio in the estimation of egg production in DEPM surveys (Lo *et al.*, 2001; ICES, 2002). On the other hand, CUFES has some disadvantages as an egg sampler than need to be taken into account: many eggs are damaged during the collecting process (which may bias estimates of daily production because younger eggs may be more vulnerable than older eggs), all egg stages may not be fully vulnerable to the pump (due to differences in the vertical distribution), selectivity problems may occur (particularly when 500 μm mesh size is used) and the samples may not independent leading to complicated variance formulations. Overall, accumulating evidence seems to indicate that CUFES performance as a quantitative sampler can be species-specific (depending on egg size, spawning depth and ascending velocity rates) and habitat-specific (local environmental conditions that affect mixing/stratification

along the water column), precluding for the time being a global evaluation of its performance. For CUFES to be used in a DEPM survey, a way must be found to take advantage of the high spatial resolution and rapid monitoring of characteristics of the instrument without giving up either precision or incorporating new biases.

Three possible ways for incorporating CUFES into a DEPM survey have so far been considered (ICES, 2002):

i) Building a CUFES to CalVET conversion coefficient into the survey design, and use CUFES as the primary sampler, with CalVET only being used to convert the CUFES egg density to a full water column value. Direct conversion of CUFES to a full water column tow would be expected to be imprecise since the conversion factor would be a function of the specific gravity of the egg stage and the extent of vertical mixing which may be highly variable within and between surveys. Computation of such conversion coefficients, largely support this view with R^2 as low as 50% in some cases. Thus using the direct conversion method is likely to diminish any gains in precision that a CUFES-based DEPM might afford.

ii) Develop a mixing model with environmental covariates to convert CUFES counts to full water counts, and use CUFES as the basic egg sampler while monitoring the environmental parameter that input the mixing model; a minimal CalVET sampling for validation purposes would only take place. In theory, if the extent of mixing and specific gravity of the eggs were known, one may be able to convert the abundance of eggs taken in CUFES to a full water column tow with reasonable precision and low bias. If this were possible, one would be able to carry out an entire DEPM egg survey without stopping the vessel, although a minimal CalVET sampling would always be desirable for ad-hoc validation purposes. No such model has been developed and tested within the context of the DEPM, although general egg mixing models exist. Thus this option is in the realm of research direction, but it can not be already adopted with existing knowledge. Within EU project PELASSES work has been carried out in this direction (see following section) but additional results are needed before further evaluation of this issue can be made.

iii) Use CalVET as the primary sampler and use CUFES to map the spawning area and to schedule the sampling of CalVET samples. Improvement in the design of CalVET-based plankton surveys can be obtained with CUFES. For example, in the Bay of Biscay anchovy DEPM surveys presence and absence of eggs in CUFES at the outer edges of the expected spawning distribution is being used to decide whether to abandon or not the coverage of radial tracks, while the CUFES abundance serves to identify areas where sampling could be intensified. A more thorough implementation of CUFES in the design and estimation of CalVET-based DEPM surveys for sardine is found in California (Lo *et al.*, 2001). A rule is established to start CalVET sam-

pling along a radial transect conditioned on egg densities in CUFES being above a threshold of 2 eggs per minute. This leads to a spatial stratification of the sample according to abundance, those of low abundance being only covered by CUFES whereas in areas of high egg abundance both CUFES and CalVET are hauled. Daily egg production is primarily calculated for the later area and the total area estimation is based on a raising factor according to the CUFES egg density ratio in the two strata.

Section 4.1.2 describes recent advances in the comparison of CUFES with vertical plankton samplers, Section 4.1.3 summarizes the use of CUFES in the most recent sardine DEPM survey, while Section 4.1.4 highlights other potential uses for this sampler. Finally, Section 4.1.5 synthesizes the opinion of SGSBSA on the potential use of CUFES in the DEPM for the near future.

4.1.2 Summary of results from PELASSES

The spring acoustic surveys of Portugal, Spain and France were internationally coordinated during 2000 and 2001 (PELASSES project), covering the area from the Gulf of Cadiz to the northern Bay of Biscay and following a standard survey design and sampling methodology. As part of this methodology, CUFES was for the first time used simultaneously with acoustics to map the distribution of pelagic fish eggs and thus offer auxiliary information for acoustic estimation. In addition, CUFES was tested for the first time as a quantitative sampler for the estimation of sardine and anchovy egg abundance in the water column. For that, apart from the direct comparisons between with CalVET/PAIROVET (Section 4.1.2.1), the vertical distribution of eggs was described in the inner Bay of Biscay and a model for the estimation of egg abundance in the water column based on CUFES and oceanographic data was developed and tested (Section 4.1.2.2).

4.1.2.1 CUFES and CalVET/PAIROVET experimental sampling

Stratoudakis et al. (2001) presented preliminary results from the comparison between CUFES and CalVET egg samples simultaneously collected during the spring 2000 PELASSES survey off Portugal and the Gulf of Cadiz. Data from vertical plankton tows (CalVET), simultaneous CUFES samples of 4 minutes duration and underway CUFES samples of 1.5 nm fixed length (immediately before and after each fixed station) were collected in 118 locations along the continental shelf of the survey area. Among the 4 sets of samples, stationary CUFES indicated sardine and anchovy egg absence more frequently than CalVET and underway CUFES (the latter generally outperforming CalVET in egg detection), although sardine egg presence/absence agreement among all sets of samples was relatively high (73–81%). Sardine egg density estimates in the 4 sets of samples were generally correlated (log-scale), with a relatively small group of outlying stations showing persistent differences between CalVET and the 3 CUFES samples (before, during and after CalVET). The latter indicated that such pronounced differences in the performance of the two samplers are

probably related to uncharacteristic vertical egg distributions, themselves resulting from local water column mixing conditions.

Additional work on the comparison of the two samplers, as well as on the comparison of CUFES performance between vessels and years, was performed during the PELASSES surveys in the Bay of Biscay (AZTI and IFREMER). Uriarte et al. (2003) present a broad comparison of PAIROVET (two CalVET nets, Smith et al., 1985) and CUFES, based on the analysis of two years of coupled surveys performed in the Bay of Biscay almost simultaneously by RV "Investigador" (rented by AZTI) and RV "Thalassa" (IFREMER). In spring 2000 and 2001, the two vessels performed experimental paired sampling of PAIROVET and CUFES for anchovy and sardine eggs. Paired sampling was performed over a wide area within the Bay of Biscay, aiming to cover a wide range of environmental and oceanographic conditions. The results showed that CUFES is a powerful sampler for egg detection (equal or superior to PAIROVET), particularly for sardine eggs (see also Section 4.1.3). While the probability of detecting sardine eggs in underway CUFES but not in PAIROVET ranged between 44–55%, the probability of detecting sardine eggs with PAIROVET but not with CUFES ranged between 11–22% (Uriarte et al., 2003). This implies a higher power of sardine egg detection for CUFES than for PAIROVET. Van der Linger et al. (1998) also found higher probability of detecting sardine (*Sardinops sagax*) eggs with CUFES than with PAIROVET, but they found the contrary for anchovy (*Engraulis capensis*). Recent evidence (M. Santos, pers. comm.) suggests that selectivity of anchovy eggs by the CUFES may be responsible for this discrepancy, given that anchovy eggs were often found to pass through the mesh and lost from the sample held in the collector.

However, CUFES provided a poor representation of egg abundance in the water column during the PELASSES surveys in the Bay of Biscay, with CVs ranging from 75%–100%. The relationship between PAIROVET and CUFES is affected by the vessel itself (probably due to the type of hull) and changes between years. In addition, in RV "Investigador" this relationship changed with the motion of the vessel (stationary or underway), probably linked to the turbulence and mixing induced by the vessel while moving. RV "Investigador" showed an increase of about 50% for anchovy and 25% for sardine in the efficiency of egg catching with CUFES on station in comparison to underway sampling (Figure 4.1.2.1.1). This is consistent with previous observations made on the same vessel for anchovy (Bez, 2000). This effect was also noted by Van del Linger et al. (1998) for round herring (*Etrumeus whiteheadi*), but was not detected for sardine. The analysis showed statistically significant year and vessel effects for both species (with the exception for anchovy, where non significant differences were found between vessels in 2000, Table 4.1.2.1.1). This is a discouraging result concerning the goal of standardising the CUFES sampling performance among years or vessels.

Stratoudakis et al. (2001) and Uriarte et al. (2003) showed that the fraction of eggs which are partly damaged is far higher in CUFES than in CalVET/-

PAIROVET, in the latter study ranging between 38–57% for anchovy and between 15–21% for sardine. This is probably caused by the increased mechanical pressure exerted on eggs during pumping and concentration. For damaged eggs, only some broad stage grouping is generally possible, such as NE (no embryo), EE (early embryo) and LE (late embryo). In addition, the percentage of damaged eggs depends on egg stage, with the youngest eggs (NE) being considerably more vulnerable than LE ones. For these reasons, any use of egg stages obtained with CUFES should mostly be based on broad staging groups (see Section 4.1.3).

4.1.2.2 CUFES as a quantitative sampler of egg abundance in the water column

The poor precision of CUFES as an estimator of egg abundance in the water column can be partly reduced by modelling the vertical egg distribution (with or without the incorporation of environmental covariates). Specific experimental sampling was carried out by MBA and AZTI with the Longhurst-Hardy Plankton Recorder (LHPR, Williams et al., 1983) along with CUFES and PAIROVET sampling. LHPR was used to describe the vertical distribution of anchovy and sardine eggs according to the physical structure of the water column and wind forcing. In addition, the Density Gradient Column (Coombs et al., 1985) was used to measure egg buoyancy. Stratified vertical sampling was used to tune a model of vertical distribution of epipelagic eggs that use buoyancy, physical conditions of the water column (including turbulence), and spawning depth (Sundby, 1991). The purpose of this study (Boyra et al., 2003) was to model and predict the depth distribution of sardine and anchovy eggs under different oceanographic conditions and to quantify the proportion of eggs sampled by CUFES at 3m depth. Sundby's (1983) model for the vertical egg distribution was improved through modifications of the vertical propagation of wind-induced turbulence. Measurements of egg settling velocity and buoyancy by stages were incorporated, including adaptability of the latter to the environment. The model was generally successful in describing the dependence of the vertical egg distributions on the density profiles of the water column and wind induced turbulence at surface. The coefficient of determination was rather similar for sardine and anchovy vertical egg distributions: around 80% (geometric mean of R^2 at all environmental scenarios), indicating a significant improvement over previous models. However, while in high surface salinity environments R^2 of almost 90% were achieved, these were reduced to about 70% in the low surface salinity cases, suggesting that additional research is required in the latter. Boyra et al. (2003) also compared the modelled vertical egg distributions to those observed during the PELASSES surveys, by considering the goodness of fit of the model prediction to the egg density at 3 m depth (LHPR samples), averaged within the main environmental scenarios (Figure 4.1.2.2.1 and 4.1.2.2.2). For low salinity stations, relative errors of about 30% were common for both species, with no indication of systematic pattern in the deviations. In high salinity areas the errors were smaller, particularly for sardine. However, in individual LHPR stations the re-

lationships were rather noisy and the same was observed in preliminary analysis of the CUFES egg densities in these stations (Boyra, pers. comm.). Individual fitting was worse than that achieved for the average by environmental scenarios and hence the potential application of the model of vertical distribution cannot be, yet, properly assessed. Additional research is currently underway aiming to provide more conclusive results on the applicability of CUFES as a quantitative sampler of egg abundance in the water column (Uriarte, pers. comm.).

4.1.3 The use of CUFES in the 2002 sardine DEPM survey

In the 2002 DEPM surveys for the Atlanto-Iberian stock of sardine, CUFES was used for the first time to delimit the spawning grounds and to allocate CalVET sampling effort (see Section 2.4.1). Table 4.1.3.1 presents the results of the comparison between the performance of CalVET and CUFES in the Portuguese survey. Comparisons were based on presence/absence of eggs in the CUFES sample obtained along the 3 nm leading to a CalVET station and the respective CalVET sample. In the 470 samples considered, the percent of discordance was 24%, with 6% indicating only CalVET presence and 18% indicating only CUFES presence of sardine eggs. The higher probability of egg detection by CUFES is probably due to the larger area covered and amount of water filtered by CUFES, confirming that it consists a powerful sampler to delimit sardine spawning grounds. Figure 4.1.3.1 shows that similar results are obtained when the presence of sardine eggs in CalVET and CUFES is separately compared for broad groups of egg stages (loosely termed Day-1, Day-2 and Day-3 groups). In the Spanish survey, a calibration was carried out in the Bay of Biscay along 7 transects perpendicular to the coast, where both samplers were used simultaneously. A total of 54 samples were obtained with CalVET and 54 x 3 with CUFES (following the sampling design used in PELASSES). Comparison of performance of CUFES at, before and after the CalVET stations (Table 4.1.3.2) show a high degree of within-CUFES consistency, with agreement ranging from 85 – 96%. Comparison between CalVET and CUFES (Table 4.1.3.3) also shows good agreement between samplers, although unlike other observations (Stratoudakis et al., 2001; Uriarte et al., 2003; and the Portuguese 2002 survey), CUFES did not perform CalVET in the detection of sardine eggs. To verify differences between the proportion of different development stages obtained from CUFES and CALVET, the positive stations were analyzed (37 CalVET and 105 CUFES). Samples from CUFES were classified using two staging scales; one considering the four classes described in Section 4.1.2.1 (no embryo, early embryo, late embryo and disintegrated) and another using the standard 11 stages described by Gamulin and Hure (1955), see also Annex 1. Eggs from CalVET were classified only into 11 stages, but for comparison purposes were also grouped in the broader four classes. Overall, there were no large differences between the stage distribution obtained by CUFES and CalVET, although it is again evident that CUFES damages eggs more often than CalVET (Figures 4.1.3.2 and 4.1.3.3).

4.1.4 Using CUFES to test DEPM assumptions

Traditional DEPM estimation of egg production is based on the assumption that daily production is constant over time and along the survey area. GAMs can relax the assumption of spatial stationarity, but still assume that on any given location production is constant over time and all cohorts present in the water column are adequately sampled by the vertical plankton tows. However, these assumptions can be violated by egg transport (it is known that egg distribution is affected by advection and diffusion) and/or spatio-temporal variation in reproduction (very little is known on the fine scale dynamics and behaviour of reproductive schools of small pelagic fish).

CUFES (coupled with acoustics) provides a unique means of obtaining synoptic views of the fine-scale egg and fish distribution at a narrow fine spatio-temporal scale, thus allowing testing of the above assumptions. A first application with this objective was performed during the November 2001 Portuguese acoustic survey (Zwolinski, 2003). Two small areas in the Gulf of Cádiz and in the Algarve (approximately 120 nm² each), were sampled intensively for one night with CUFES. Eleven parallel transects 1 nautical mile (nm) apart and 10 miles long were performed in each zone. Individual CUFES samples were taken at 6 minutes intervals of underway sampling, in order to obtain samples from approximately 1nm. Based on these data, Zwolinski (2003) described the autocorrelation structure within daily egg patches (evidence of diffusion) and the small-scale differences in the distribution of sequential daily egg patches (Figure 4.1.4.1). The latter is useful in showing that small-scale spatial variation (either due to small shifts in the location of spawning schools or due to egg displacement after release) can increase considerably the noise/signal ratio of CalVET samples (which are meant to provide estimates for all daily cohorts locally released in the 2–4 days up to sampling). For example, under a standard DEPM survey the area depicted in Figure 4.1.4.1 would probably be covered by 6–9 CalVET stations, where (depending on the location) eggs from either or both cohorts would have been detected in widely varying relative abundances.

4.1.5 Recommended use of CUFES in future DEPM surveys

The main aim of most of the research presented above is to evaluate the potential of CUFES to substitute CalVET/PAIROVET as the primary quantitative egg sampler in future sardine and anchovy DEPM surveys. The results so far indicate that, although CUFES is a powerful sampler for egg detection (equal or superior to CalVET/PAIROVET), there are still important problems to resolve before it can be considered as a quantitative estimator of sardine and anchovy egg abundance in the water column. Precision in the estimation of egg abundance in the water column by CUFES is poor, while significant effects due to motion, vessel and year provide additional complications. Poor precision can only be partly remedied by modelling the vertical egg distribution that incorporates environmental covariates into the model formulation. However, sufficiently accurate and precise model predictions of aerial egg abundance are still not available for all oceanographic conditions en-

countered in stratified environments, and further work is undergoing. In addition, CUFES shows a marked selectivity of anchovy eggs (ICES, 2003b), but this is less clear for sardine eggs. Based on the above, the group recommends that CUFES continues to be used in the next DEPM surveys for anchovy and sardine as an auxiliary sampler (along the lines defined in ICES, 2002), but this recommendation can be revised when additional results become available.

4.2 Egg staging and ageing

This section reports on significant recent advances in the staging and ageing of sardine and anchovy eggs for DEPM purposes. Section 4.2.1 summarizes the results of a recent staging workshop that took place before the 2002 surveys, as a result of a recommendation from the previous group meeting (ICES, 2002). Section 4.2.2 describe the application of new methods of analysis to old and new egg incubation data for sardine and anchovy, while Section 4.2.3 identifies factors other than temperature that may also affect pelagic egg development. Finally, Section 4.2.4 reviews existing information on the daily spawning activity of sardine and anchovy, highlighting its importance in egg ageing.

4.2.1 Staging workshop

During the first meeting of SGSBSA (Lisbon, October 2001), an informal workshop was performed to obtain reference collections of sardine and anchovy egg stages (see Annexes 1 and 2) and to assess the consistency in egg staging among institutes and readers. Significant differences were reported among institutes, particularly in relation to the criteria used to classify eggs as destroyed (ICES, 2002). As a result, a workshop was proposed to take place within the framework of PELASSES, before the analysis of the 2002 DEPM samples.

The meeting took place in San Sebastian during January 2002. The main objective of the workshop was to identify the main differences between readers and institutes and to unify staging criteria. A first staging of anchovy and sardine eggs obtained with PAIROVET and CUFES samplers was carried out to identify the differences between individuals and groups (scientists from IFREMER, AZTI, IEO and IPIMAR). Anchovy and sardine eggs were staged in 11 stages (Moser and Ahlstrom, 1985; Gamulin and Hure, 1955) for the eggs collected with PAIROVET and in 3 stages for the eggs collected with CUFES. Those 3 stages were No Embryo (NE), Early Embryo (EE) and Late Embryo (LE) that is a grouping of the eleven stages (definition proposed by PELASSES and adopted by this Study Group). Stage NE grouped stages I, II and III, stage EE grouped stages IV, V, VI, and stage LE grouped stages VII to XI.

During the first staging, each participant used the criteria that were normally employed for routine staging. The percentage of agreement was measured with respect to the modal stage weighted by the reader experience (Eltink et al., 2000). After the analysis of the data from the first staging, the identification criteria for each stage were discussed and the group complemented the definitions of each development stage for anchovy and sardine

based on the original ones of Moser and Ahlstrom (1985) and Gamulin and Hure (1955). A second staging subsequently took place, using the revised definitions for each stage. The analysis of the second set of data demonstrated that the percentage of agreement increased for both species (Tables 4.2.1.1 to 4.2.1.3). The improvement was bigger for anchovy (15% for PAIROVET and 10% for CUFES), probably due to the relatively limited previous experience in the staging of anchovy eggs at some institutes. For sardine improvements were also registered (8% for both PAIROVET and CUFES) and this was subsequently reflected in the reduction of discrepancies in the percentage of destroyed eggs in the Portuguese and Spanish 2002 surveys (Table 4.2.1.4).

4.2.2 Analysis of new and published egg incubation data for sardine and anchovy

Two methods were used to analyse the evolution of egg stages through ages for different temperatures, using data from published and new incubation experiments. The new ageing method (developed within the GAMs project and described in Section 3.2.1) and the traditionally used ageing method (Lo 1985) were both applied to analyze data from the anchovy and sardine incubation experiments.

The traditional ageing method is based on fitting the incubation experiment data by the model proposed by Lo (1985):

$$\overline{a}_{i,t} = \theta_0 \, e^{\theta_1 t + \theta_2 i} \, i^{\theta_3} \tag{1}$$

where t denotes temperature, i stage, $\overline{a}_{i,t}$ mean age within stage i incubated at temperature t and θ_0, θ_1, θ_2 and θ_3 are the model parameters. Estimates of $\overline{a}_{i,t}$ were computed for sardine and anchovy as the mean elapsed time from the beginning of an incubation experiment up to the checking time, weighted by the number of observations of each development stage (Miranda *et al.*, 1990; Motos, 1994). Then, model (1) was fitted to the incubation data usually by least squares on the log-transformed equation. The ageing method described in Section 3.2.1 is based on the fitting of a multinomial model to the incubation data. In contrast to Lo's model, this approach considers age and temperature as fixed explanatory variables and stage as a random variable. For each stage i the conditional probability of an egg being at stage i or above given that the egg is in stage i-1 or above is modelled by fitting a binomial generalized linear model (GLM) depending on age and temperature. The probability of an egg being in each stage can therefore be derived as a product of the fitted conditional probabilities. The multinomial model was fitted using the package *dep-model* (Section 3.2.5).

In the case of anchovy (Section 4.2.2.1), the new method was applied to existing incubation data (Motos, 1994), while in the case of sardine both existing (Miranda *et al.*, 1990) and new incubation data (experiment carried out in 2002 by IEO) were used in the analysis (Section 4.2.2.2).

4.2.2.1 Anchovy

The incubation experiment for anchovy (Motos, 1994) was carried out in May-June 1992 during the annual DEPM survey on board RV "Cornide de Saavedra". Two incubations, in two distinct locations, were performed using five completely hydrated females and five mature males selected from the tow. After inciting the females to spawn by an abdominal massage, the eggs were mixed with sperm and kept in a tube. When the first cellular divisions were detected, the eggs were placed in 50 ml glass tubes in an incubator block. Two rows of seven tubes were prepared and positioned across the block covering a temperature range from 8° to 22°C (Table 4.2.2.1.1) Eggs were randomly sampled at regular intervals from each tube. For each sample the elapsed time from the beginning of the experiment, the temperature and the number of eggs in each developmental stage (Moser and Ahlstrom, 1985) were recorded. Given the difficulty of distinguishing stage I eggs from non-fertilized ones, stage I eggs were excluded from the stage classification. At each sampling period, dead eggs were removed from the tubes and seawater from a reserve held at the same temperature was added to maintain a similar water level in the tube during the whole experiment. Sampling from each tube finished when eggs had begun hatching or when there were no more eggs to sample (all the eggs were already sampled or dead).

Given that the temperature gradient was stable and similar in both incubations (Table 4.2.2.1.1), and assuming that there was no difference between incubations, data were gathered and analysed jointly. The observed (age, stage) data are shown in Figure 4.2.2.1.1. Expected values from Lo's model for the observed temperatures and for all stages are shown in Figure 4.2.2.1.2. Two models were considered. The first assumes that the effects of the explanatory variables, age and temperature, were the same across stages, while the second allows for the coefficients of age and temperature to differ among stages. The first model was found to be inadequate for these incubation experiment data. The second, more flexible model, provided a substantially better fit to the data and was selected for assigning ages to anchovy eggs (Figure 4.2.2.1.3).

In general, multinomial models allow a better and more natural way of analysing incubation experiment data (the development of each stage egg is not deterministic), while introducing additional flexibility (variability is better reflected by means of a multinomial model). Furthermore, hatching time can be derived directly from the multinomial model, instead of fitting a second model to the age-assigned eggs. There are alternative models available in R (www.r-project.org) for fitting the multinomial distribution of ordered categorical random variables as stages, with probabilities depending on explanatory variables like age and temperature. For example, proportional odds models (that model cumulative probabilities and assume that the effect of the explanatory variables are identical for all categories) can be fitted using the function polr in the library Mass. The function lrm from the libraries Hmisc and Design allows also fitting the conditional probability of being in one category given that it is in the previous category or above using

the logit link (the so-called continuation-ratio models). These approaches were also investigated as part of the GAMs project, where the model adopted in *depmodel* and used here was found to outperform all others for the incubation data at hand.

4.2.2.2 Sardine

A new incubation experiment for sardine was successfully carried out by IEO in 2002, following the recommendation of the previous Study Group meeting (ICES, 2002). The incubation experiment was performed according to that described for anchovy (Motos, 1994 and Section 4.2.2.1), using the same incubator. Eggs were obtained from a survey onboard RV "Vizconde de Eza" in the Gulf of Cádiz (SW Spain) between 10–17 February 2002. Sardine adults were caught using a commercial trawl, and eggs and sperm were extracted from macroscopically identified spawning sardines. Hydrated eggs were obtained by abdominal pressure and immediately fertilised by sperm collected from males. Once fertilised, viable eggs were placed in small jars filled with filtered seawater within the incubator. Five temperatures within the range of 11 to 17°C were used in the experiment (Table 4.2.2.2.1). Five replicates for each temperature and around 200 eggs for each replicate were used in the experiment. Two additional jars with filtered water were placed in each row corresponding to each temperature used in the analysis, in order to allow for refilling of the egg jars with water at the same temperature.

Samples from each temperature of the incubator were taken randomly from one of the replicates of that temperature in each sampling event. On the 6 first hours of the experiment samples were taken at 1 hour intervals, and, afterwards, the sampling frequency was set to 3 hours up to hatching. Around 30 eggs for each temperature were sampled each time (Table 4.2.2.2.1), and for each sample, elapsed time from the starting of the experiment, temperature, replicate number and stage of each of the sampled eggs were recorded. After being staged (Gamulin and Hure, 1955), eggs were returned to the same jar, and any loss of water was replaced. Additionally, at least twice a day, all dead eggs were siphoned from the bottom of the jar and the water volume was refilled from filtered water at the same temperature. With this procedure, at least 1/3 of the water volume was renewed each day.

The observed (age, stage) data are shown in Figure 4.2.2.2.1. Figure 4.2.2.2.2 shows both the published model from Miranda *et al.* (1990) and the Lo model fitted to the Cádiz incubation experiment (with stage duration estimated using observed standard deviation on stage age). The multinomial model of the evolution of stages through ages for different temperatures is shown in Figure 4.2.2.2.3, together with the observed evolution of percentage of eggs in each stage for different observed times (ages) and temperatures.

The original data from Miranda *et al.* (1990) were not available to refit the models, and thus only the data from the Gulf of Cádiz were used to assign ages to sardine staged eggs using the new ageing methods. The multinomial model was found to perform better than the traditional (Lo's) model with the sardine incubation data. The

multinomial model has two main advantages over the traditional models when applied to these data. First, it has a sound theoretical basis (as explained in Section 3.2). Age (i.e., sampling time) is fixed in most incubation experiments and stage is the random variable observed, and this is the way the data are treated in the multinomial approach, but not the traditional one. Also, stage is a factor (a discrete variable) and fitting a model like Lo's that treats stage as continuous, has the difficulty of not knowing a-priori the stage duration, i.e., the spacing on the x-axis. Second, the traditional way of treating incubation experiments is to fit a model to the mean age of the stages as a function of stage and temperature and then to fit a separate model to stage duration. Two models of stage duration as a function of stage and temperature have been used in the literature:

- Constant stage duration for a given stage for any temperature (Lo, 1985; Motos, 1994), or

- Continuous model of stage duration as a function of stage and temperature (Miranda *et al.*, 1990).

As shown by the data (Figure 4.2.2.2.1), stage duration changes both with stage and temperature. Nevertheless, stage duration cannot be represented by a monotonous function of stage, as stages III and IV consistently show a shorter duration than other stages. The multinomial models pick the lower probability of stages III and IV adequately from the data, and provide a natural way to interpolate the probability of being at any stage for a given age and temperatures not observed on the incubation experiment but within the observed range. There are different ways and available software packages to fit a multinomial model to data like the one obtained from an incubation experiment. In general the fitting procedure included within *depmodel* provides more flexibility to represent the transitions between the stages and models the data from the Cádiz experiment adequately. However, one disadvantage of this extra flexibility is that it produces longer tails (non-zero probabilities of being in any given stage, at ages far greater than the mean age at that stage), increasing (maybe artificially) the range of possible ages for a given stage.

4.2.3 Factors other than temperature affecting egg development

Very few studies have so far described the effect of factors other than temperature on egg development, although it is accepted that such factors exist and also affect the probability of survival. Stratoudakis *et al.* (1998) and Steer *et al.* (2002) have demonstrated the impact of factors other than temperature (vertical position within multi-layer mats, salinity) to the development and mortality rates in the demersal eggs of herring (*Clupea harengus*) and southern calamary (*Sepioteuthis australis*) respectively. In the case of pelagic fish eggs, egg size is known to affect mortality rates (Pépin, 1991), while also acting as an index of egg condition resulting from parental investment. More recently, egg size in combination with salinity and oxygen concentration have been reported as crucial to the survival and development of cod eggs (Vallin and Nissling, 2000). Finally, several studies

on the biochemical composition of pelagic fish eggs and larvae in relation to environmental and parental effects have been recently carried out around the Iberian peninsula (Riveiro *et al.*, 2000, Guisande *et al.*, 1998, Verea *et al.*, 1997). Parental condition and age seem to affect egg size and biochemical composition in pelagic fish, although results need to become more conclusive.

To the best knowledge of this Study Group, no incubation experiments so far, have taken into account factors other than temperature in the estimation of development rates. There is a potential scientific field for studying the relationship between egg development (and mortality) and environmental variables other than temperature, as well as taking into account biotic variables like parental condition. Such studies may help to understand environmentally-driven recruitment variability, which is largely dependent on variability in egg, larval and post-larval survival. Further, accounting for variability in egg development due to factors other than temperature may also help to improve egg ageing for DEPM purposes. Due to the Bayesian nature of the ageing method presented in Section 3.2.1, any new information on incubation experiments that include other environmental and/or biological variables can be incorporated in the ageing procedure, with a potential improvement in ageing precision.

4.2.4 Assumptions on daily spawning

For the application of the DEPM to small pelagic fish species with daily spawning synchronicity, time of peak spawning must be known to age eggs according to their observed stages and sampling time (e.g., Lo, 1985; Bernal *et al.*, 2001). This procedure is necessary to obtain egg densities and mean ages for each daily cohort in the sample and thus estimate daily egg production and mortality. In addition, the new Bayesian approach for assigning ages to egg stages (see Section 3.2.1) requires a probability function of spawning time. Assuming a normal distribution, this implies, in addition to the time of peak spawning, that the standard deviation of daily spawning activity from the peak is also known to identify uniquely the daily spawning probability density function (SPDF). In the remaining of this section, the information available for estimating these two parameters for anchovy and sardine is revised and gaps in current knowledge are highlighted.

In the case of anchovy, Motos (1994) used observations on the reproductive state of spawning females and on the prevalence of stage I eggs in the plankton (data from 1989 to 1992) to suggest that peak spawning occurs around midnight. This estimate of peak spawning time has been used in the traditional application of the DEPM to anchovy until now. However, data from another decade are now available, allowing a revision of this information based on a considerably wider set of data than those originally available to Motos (1994). The reproductive state of adult females is reviewed using histological data from the surveys of 1990–1992, 1994, 1995, 1997, 1998, 2001, and 2002. The incidence around midnight of females that are to ready spawn or that have already spawned in the night was analysed as in Motos (1994), in order to identify the exact time that those already spawned are more frequent in the sample those that are still to spawn in the same night. In addition, the incidence of stage I in the plankton around midnight was also analysed as indicative of the effective realisation of the spawning act by adults.

Figure 4.2.4.1 shows the evolution of the gonad state of spawning females in samples collected from 18:00 to 6:00 (data pooled across years). The transition of the predominance of pre-spawning (hydrated) females to post-spawning females (gonads with POFs) occurs between 23 and 24 hours, while between 23.5 and 24 hours post-spawning females attain about half of the spawning females. Overall, the presence of spawning females (hydrated and POFs) reach its maximum between 23 and 24 hours and particularly between 23.5 and 24 hours. The range of spawning times as revealed by spawning females mainly extends from 21 to 1.5 hours (i.e., a spawning range of 4.5 hours), being negligible the spawning occurring before or after. However, a minor, but persistent across years, secondary peak of spawning seems to occur between 4 – 5 in the morning.

Figure 4.2.4.2 shows the incidence of stage I eggs per surface unit during the 1994–2002 surveys, overlaid to the expected stage I distribution arising from the fitting of a normal distribution to the spawning time. This direct fitting suggests that a peak spawning time at 23.25 hours (23 hours and 15 minutes) with a 2 hours standard deviation would best explain the observed stage I egg distribution if duration of that stage is 1.33 hours. However, considering only the period 1996–2002, the optimum peak spawning time would be 23:00 hours with a SD of 1.7. It should be noted that both estimates are based on the restrictive assumption that stage I eggs last 1.33 hours, but this is a minimal duration of that stage and therefore the indication from those eggs is that peak spawning is to be considered to take place at the latest at 23:15. Overall, revised adult and egg data seem to confirm that peak spawning for anchovy in the Bay of Biscay must be between 23 and 24 GMT hours, with the best estimate of SPDF from these data being ~N(23, 1.25).

In the case of sardine, existing DEPM applications have assumed a 19:00 GMT peak in spawning activity (Cunha *et al.*, 1992; García *et al.*, 1992). However, the assumption was based on less exact data than in the case of anchovy, although there is little doubt that sardine also shows considerable daily synchronicity in spawning, most likely towards dusk (Ré *et al.*, 1988; Bernal *et al.*, 2001). Zwolinski *et al.* (2001) showed that the gonadosomatic index and the density and diameter of hydrated oocytes within the gonad follow a consistent daily pattern in female sardine that are expected to spawn on the same day (Figure 4.2.4.3). The same Figure also shows that until 18:00 hydrated females did not show any evidence that spawning had already started (i.e., no POFs were found during the histological inspection of the gonads). On the other hand, plotting the hourly distribution of stage I eggs from all Iberian DEPM surveys (Figure 4.2.4.4) demonstrates a peak in egg density around 22:00 GMT. This matches well the observed daily distribution of sardine spawning in a fixed location repeatedly sampled within a day (Ré *et al.*, 1988) and with more recent evidence from intensive adult sampling during the end of the day (Ganias *et al.*, 2003b). Figure

4.2.4.2 also shows that a SPDF with 0.5h standard deviation seems to fit more adequately the data from a single location (Ré et al., 1988), while one with 1h sd seems more appropriate for data collected across DEPM surveys. Overall, this evidence seems to contradict the currently assumed location of the SPDF for sardine, but more detailed information is needed for the group to recommend an alteration to the assumed SPDF (which is currently set as N~(19,1)). This is because changes in the duration of daylight along the spawning season (the data of Ré et al. (1988) were collected in May) and geographic differences (the data of Ganias et al. (2003b) are from the Aegean Sea) may partly contribute to the observed discrepancies.

4.3   Sardine reproduction off Iberia

This section reviews information on the reproductive activity of sardine off Iberia. Section 4.3.1 describes sardine maturation, its classification and appropriate use for spawning biomass estimation, while Section 4.3.2 identifies metrics of spawning activity and reports recent data on the seasonal dynamics of sardine spawning off Portugal.

4.3.1   Maturity

In recent years, the macroscopic classification of sardine maturity in the Atlanto-Iberian stock has been based on maturity scales adapted from Pinto and Andreu (1957 – described in Table 4.3.1.1 and Figure 4.3.1.1). The original scale was developed from extensive macroscopic and microscopic analyses of female gonads, however the consistency in use among applications (e.g., DEPM sampling, acoustic estimation and stock assessment) and institutes, as well as the overall adequacy of the scale to describe the maturation process of sardine have not been reviewed for a long time. Within DEPM, the macroscopic classification of the ovaries is not critical, in the sense that most sampled ovaries are analysed microscopically and any misclassification can be eventually corrected. However, only macroscopically identified mature females are analysed histologically and a poor definition or large misclassification rate of mature females may introduce bias in the estimation of adult parameters. More importantly, the boundary between macroscopically immature and mature individuals is defined in a slightly different way for stock assessment (stage III and above – ICES, 1982) and DEPM purposes (stage II and above). Since spawning-stock biomass (SSB) estimates from DEPM are used to tune the assessment model, the criteria used to define maturity in DEPM and the remaining data entering the assessment model have to be consistent.

   In order to clarify the above issues, various terms that are often used to describe the reproductive state of small pelagic fish are defined (Section 4.3.1.1) and macroscopic maturity stages are compared to the microscopic aspect of sardine gonads (Section 4.3.1.2). Finally, Section 4.3.1.3 synthesizes the findings from these comparisons to identify the best methodology currently available for the coherent estimation of SSB in surveys and stock assessment.

4.3.1.1   Clarification of definitions

A series of terms related to reproductive state are often used interchangeably without particular attention to their meaning. This can lead to confusions, particularly when definitions are lacking or are not sufficiently explicit. For that, the group decided to adopt the following definitions, based on explicit criteria. It should be noted that these definitions are used for sardine and anchovy, species that are known to be indeterminate, serial (batch) spawners with protracted spawning seasons.

   The first set of definitions refers to the spawning history of the individual, separating fish that have at least once contributed to the spawning population from others.

   Criterion: spawning history, refers to life cycle;

Virgin: an individual that has never spawned in its life;
Adult: an individual that has spawned at least once in its life.

The second set of definitions refers to the actual state of the fish gonad, separating fish that are clearly outside their spawning season from those that have recently, are actually or will soon be spawning.

   Criterion: gonadal development refers to annual reproductive cycle;

Immature: virgin and adult individuals with no signs of gonad development or reproductive activity (i.e., individuals outside their spawning season);

Mature: adult (but in some cases also virgin) individuals that have clear signs of gonad development or reproductive activity (i.e., individuals within their spawning season).

The third set of definitions refers mainly to mature individuals within the reproductive season, separating those that have ceased their activity for the current season from others.

   Criterion: spawning activity, refers to reproductive season (see also Section 4.3.2.1);

Inactive: individuals that do not show signs of recent or eminent spawning activity (e.g., a female gonad containing only unyolked and atretic oocytes);

Active: individuals that show signs of recent or eminent spawning activity.

According to the above definitions, it is plausible that a virgin fish is mature (during its first maturation) and an adult fish is immature (a spawner well outside its reproductive season). Moreover, it is also possible that a virgin and mature fish is inactive (a first time spawner that has not managed to mobilize sufficient energy resources to complete gonad development and release even the first batch of oocytes).

4.3.1.2 Comparison of macroscopic and microscopic maturity stages

In order to evaluate the criteria of macroscopic identification (Table 4.3.1.1), the macroscopic and microscopic stages of 177 sardine ovaries collected during an acoustic survey (Portuguese November 2000 survey) were compared. A similar analysis was performed with data from the Portuguese DEPM surveys of 1997, 1999 and 2002 (the number of gonads analysed was 460, 636 and 574, respectively). It should be noted that histological examination in the two sets of samples was performed with a different objective, so different microscopic criteria were used in the two cases. In the sample from the acoustic survey (specifically collected for the needs of this comparison), the six microscopic stages were based on the characteristics of the most advanced oocyte stage (West, 1990) and other morphological aspects of the ovary (e.g., organization, presence of POFs) to match exactly the 6 macroscopic stages. In the samples from the DEPM surveys (where microscopic staging formed part of the routine analysis for POF detection), gonads were classified according to the four stages of Hunter and Macewicz (1985).

The correspondence between macroscopic and microscopic gonad staging from the November 2000 acoustic survey and the DEPM surveys are presented in Tables 4.3.1.2.1 and 4.3.1.2.2 respectively. Overall agreement between macroscopic and microscopic classification of sardine maturity was low (56% in data from the November 2000 survey), mainly due to poor consistency within initial development (stages I and II) and post-spawning (stages V and V–III). Other studies (Grilo, 2002) confirm the poor precision of stage II (71% misclassification) and III (67% misclassification), these misclassification rates increasing considerably in fixed gonads. The main problem for macroscopic stage I is confusion with stage II (25% of stage I ovaries were classified microscopically as stage II). The inverse can also occur, with a considerable fraction of macroscopic stage II gonads being microscopically classified as stage I (Table 4.3.1.2.2). This is not unexpected, taking into account that only small changes in volume or external aspect of the ovary take place during the appearance of pre-vitellogenic vesicles. Misclassification rates were also large for stages III and V–III, mainly due to the confusion between these two stages. Microscopically, these stages are different only during a short period after spawning (generally up to three days) when POFs are visible. Macroscopically these two stages are very similar and several external factors can turn a stage III to appear as a stage V–III gonad (e.g., long time from capture to observation, freezing and defrosting prior to observation, etc.). When the two stages are merged, the percentage of agreement between macroscopic and microscopic classifications increases to values above 90% (as shown by the DEPM data in Table 4.3.1.2.2, where the two stages are not separated in the microscopic scale). Finally, the large misclassification in stage V highlights the need to revise both its macroscopic and microscopic characteristics.

The above results indicate several inadequacies in the application of the existing macroscopic maturity scale for sardine. Some of these are relatively easy to amend, as

for example the simplification of the scale by the fusion of the III and V–III stages. However, the group decided to continue with the macroscopic scale of 6 stages and adopt a similar microscopic maturity scale (Table 4.3.1.1), in order to perform additional comparisons using also data collected in other periods of the annual reproductive cycle. This scale will be improved as more detailed microscopic information is gathered from analysis of maturity in different parts of the year (gonads between April and September 2002 are already available at IPIMAR), while a similar analysis with the DEPM histological data of IEO is also anticipated.

4.3.1.3 Maturity stages to be included in the estimation of spawning biomass

According to the definitions in Section 4.3.3.1 and the microscopic characteristics of maturation stage II (Table 4.3.1.1), correctly classified (macroscopically) stage II individuals are mature and will very probably spawn in the near future. Hence, such individuals should form part of the potential SSB that is estimated during analytical assessment (currently these individuals are considered immature for assessment purposes). On the other hand, the DEPM aims to estimate SSB at the time of the survey, by dividing the observed total daily egg production over the fraction of the population biomass that has given rise to these eggs. Clearly, a stage II individual has not yet contributed to the observed egg production in that spawning season, so in the case of DEPM stage II individuals should be excluded from the estimation of adult parameters and SSB. These conclusions suggest that the existing practice for sardine (i.e., inclusion of stage II in DEPM but not in assessment), should most likely be inverted (i.e., inclusion of stage II in assessment but no in DEPM). Nevertheless, the Group recommends that the issue is further discussed in the light of additional biological information on sardine reproduction and a final decision is only taken when a satisfactory maturity scale is introduced. Until then, and to avoid repeated and relatively minor modifications in the input data to assessment, the Group recommends that the existing practice for SSB estimation is maintained both for DEPM and for assessment purposes. However, the potential for macroscopic misclassification demonstrated in Section 4.3.1.2 should be taken into account in future DEPM sampling.

4.3.2 Spawning season and dynamics

4.3.2.1 Spawning activity

Although the general characteristics of the spawning season (duration and peak) are generally invariant for pelagic fish populations in a given geographical region, spawning activity within a population is not necessarily synchronous and may depend on biological properties like age and energy reserves (Ganias, 2003). In the case of small pelagics, female spawning activity is usually defined by the presence of yolked oocytes and the intensity of artesia (Pérez and Figueiredo, 1992). For example, Hunter and Macewicz (1985) define a female anchovy (*Enrgaulis mordax*) as active when at least 50% of the yolked oocytes in the gonad are not atretic. On the other

hand, Ganias (2003) and Ganias *et al.* (2003a) showed that in the case of the Mediterranean sardine only females with 100% of atretic yolked oocytes can be safely considered inactive. Describing adequately spawning activity and identifying the biological factors that can influence the spawning dynamics of a population is an area of research that deserves attention, as they can provide useful insights to population dynamics and help in the planning and interpretation of DEPM applications.

Spawning activity is best described by histological examination of the gonads. However, histology is a costly operation and can become prohibitively expensive in pelagic fish with a protracted spawning season (in the case of sardine this can be 5–6 months). A cheaper alternative is to describe spawning activity as a function of the gonadosomatic index (GSI), which is based on biological information that is routinely collected (gonad and gonad-free weight). Figure 4.3.2.1.1 shows an example of an activity ogive developed for the Mediterranean sardine (Ganias, 2003). A histological sample was used to fit a logistic curve and identify GSI_{50} (i.e., the value of GSI where 50% of the females are active) and this value was then applied to GSI data collected along the spawning season to describe the dynamics of sardine spawning activity as a function of time (Figure 4.3.2.1.2) and biological properties (size and hepatosomatic index). Clearly, this application shows promising results and the method could also be used to understand better the spawning dynamics of sardine in the Iberian Peninsula.

4.3.2.2 Spawning dynamics off Portugal

In 1988 and 1997 the Iberian DEPM surveys for sardine were performed during March/April both in Portugal and in Spain. In 1999 the Portuguese survey was performed in January, introducing logistic problems in sampling (adult samples could no longer be collected in the acoustic survey) and a 2 months gap between the survey in northern Spain and the remaining stock area. The change of timing in the Portuguese survey was based on revision of a decade of biological data that appointed to a winter (December/January) peak in spawning activity off Portugal. In the following two years, the same seasonal pattern was observed (Zwolinski *et al.*, 2001), so the timing for the 2002 Portuguese survey was maintained (ICES, 2002). Nevertheless, during January 2002 several indicators suggested an irregular regression of sardine spawning activity, coinciding with a particularly low estimate of spawning fraction (see Section 2.4.3). As a result, the anticipated improvements in the precision of spawning fraction and stock biomass estimation in 2002 were not observed, despite the considerable increase of sampling effort (from 36 in 1999 to 74 independent fish samples in 2002). Apart from the obvious implications to DEPM survey design and estimation, these observations also highlight the need for a better understanding of the biological mechanisms that control sardine spawning dynamics.

In addition to the particularly low estimate of spawning fraction, estimation of batch fecundity for the 2002 Portuguese DEPM survey also revealed the lowest levels of relative fecundity observed so far for sardine. Further, unlike previous surveys, more than 10% of the macro-

scopically classified female gonads were at stage V (post-spawning), and this inactivity was confirmed by microscopic examination (see Table 4.3.1.2.2). Inactive females were observed across the length range of 12–25 cm, with the proportion inactive raising to very high levels for fish larger than 22 cm (although these were caught in a few hauls). The prevalence of atresia was also very high and, although prevalence is not a sufficient indicator of inactivity (see Section 4.3.2.1), it is interesting that more than 90% of the gonads microscopically classified as stage I and II showed evidence of atresia (i.e., inactive fish according to the definitions in Section 4.3.1.1). Finally, the mean female gonadosomatic index (GSI) was uncharacteristically low in January 2002 and its evolution along the 2001/2002 spawning season (Figure 4.3.2.2.1) confirms a departure from the typical seasonal pattern (e.g., Zwolinski *et al.*, 2001).

Cunha *et al.* (2003) describes the seasonal evolution of several sardine biological properties during the spawning season of 2001/2002, based on samples collected from September 2001 to June 2002 at the main spawning grounds off northern (Figueira da Foz) and southern (Olhão/Portimão) Portugal. Despite the limited intensity of sampling (data from 5 hauls were collected every two months from each area) this study provides useful insights to the sardine spawning dynamics along the 2001/2002 spawning season. Figure 4.3.2.2.2 confirms previous information (Zwolinski *et al.*, 2001) that spawning of sardine is more protracted in southern Portugal and that from late autumn onwards most female fish are sexually mature. Estimates of spawning fraction also suggest an earlier and longer spawning season off the southern coast when compared to the north (Figure 4.3.2.2.3). However, with the exception of November in the south and January in the north, all estimates of spawning fraction are below 10%, i.e., lower than all previous spawning fraction estimates off Portugal. It should also be noted that the estimate of spawning fraction in the north during January is probably an over-estimate, since the samples from this study were obtained from a region that had uncharacteristically high levels of spawning activity during the DEPM survey. Finally, contrary to what was expected, prevalence of atresia was highest during the peak spawning season. The massive presence of atresia started earlier in the southern coast (November) than in the north (January). These were also the respective months with the smallest mean length in the two areas (ca 16.5 cm for November in the south and ca 15 cm during January in the north).

Additional research in the seasonality and dynamics of sardine spawning in the north-eastern Atlantic is currently underway, as part of the EU project SARDYN (SARdine DYNamics and stock structure in the north-eastern Atlantic, Q5RS–2002–000818). Within this project, literature information and recent data on sardine spawning areas and seasons will be compiled from the north-eastern Atlantic to help in the clarification of stock structure and the delimitation of stock areas. Further, life history properties (including reproductive parameters) will be studied at fine spatio-temporal scales to describe the species dynamics and its potential links to environmental conditions.

4.4 POF dating and spawning fraction estimation

The spawning fraction (S – the proportion of the female population spawning per day) is one of the four parameters required for the DEPM estimation of spawning-stock biomass. Estimation of S is based on the post-ovulatory follicle (POF) method (Hunter and Macewicz, 1985), resulting from the histological examination of ovaries. POFs correspond to the epithelial/connective layer that surrounds a growing oocyte, which remains in the ovary after the ovulation (and consequent spawning) of the hydrated egg. These structures have been poorly studied in teleosts, but there is evidence that they are rapidly reabsorbed as the follicular cells develop phagocytic activity. A recent laboratory study on *Astyanax bimaculatus lacustris* (a freshwater characid with serial spawning along most of the year) describes the ultrastructure of POFs in the four days following induced spawning, in an attempt to elucidate the mechanisms responsible for their elimination during ovarian recovery (Drummond *et al.*, 2000). Immediately after spawning, POFs consisted of a convoluted follicular wall (a single layer of cells enveloped by a thick basement membrane and a thin theca of connective tissue) surrounding an irregular cavity (lumen). At initial phases of involution, some follicular cells were dissociated from their neighbours and detached from the basement membrane, showing typical apoptotyc figures. Four days after spawning the bulk of the POFs were reduced but not completely reabsorbed.

For DEPM applications, it is important to know the typical state of follicular structures during the first few days from spawning, in order to group POFs into daily cohorts. The most important morphological changes with time in POFs have been described for northern anchovy (Hunter and Macewicz, 1985) and have been used as a template for most DEPM applications on small pelagics worldwide (see Annexes 3 and 4). A similar study was performed for sardine (Pérez *et al.*, 1992a), but the results were not conclusive due to the small number of fish sampled. Information from previous applications of the POF method on several *Sardina* (Cunha *et al.*, 1992; García *et al.*, 1992, Pérez *et al.*, 1992a; ICES, 2000; Quintanilla and Pérez, 2000a) and *Sardinops* (Macewicz *et al.*, 1996; Ward *et al.*, 2001) populations, reveal various aspects of sampling and laboratory analysis that can affect accuracy and precision of the respective spawning fraction estimates:

a) sampling bias due to horizontal or vertical temporary segregation of spawners from the remaining population;

b) subjectivity in the identification of POFs and their assignment to histological classes;

c) subjectivity in the attribution of POF classes to daily spawning cohorts; and

d) uncertainty on the impact of temperature in the degeneration of POFs.

After the recent advances in egg production estimation, POF staging and aging and estimation of spawning frac-

tion probably consist the only aspects of DEPM estimation where subjective decisions and bias due to sampling and laboratory analysis can still occur. In the remaining part of this section the above problems are discussed and potential solutions are suggested. Section 4.4.1 considers the issue of sampling bias due to differences in the catchability of the spawning component of the population, while Section 4.4.2 reviews the issue of POF staging and aging in the light of the methodology currently adopted for staging and aging of pelagic fish eggs.

4.4.1 Sampling bias

A common feature in the spawning behaviour of most pelagic schooling fish like anchovies and sardines is the formation of spawning schools where individuals (females and males) synchronize spawning (Alheit, 1993). Spawning schools consist of a limited in space and/or ephemeral in time (Ganias *et al.*, 2003b) segregation of females with imminent and/or recent spawning activity (so called Day-0 females) surrounded by large numbers of males (Alheit, 1985; Picquelle and Stauffer, 1985; Axelsen *et al.*, 2000; Ganias *et al.*, 2003b). Sampling gears (trawls and purse seines) are supposed to sample randomly from spawning and non-spawning schools to provide estimates of spawning fraction that are representative for the population (Smith and Hewitt, 1985). However, in many surveys fish samples exhibit spatial and temporal heterogeneity with respect to the fraction of Day-0 females and sex ratio and this seems to be controlled by the spawning act (Smith and Hewitt, 1985). Due to this bias, Day-0 females are usually excluded from the composite estimates of S. However, this exclusion has a great economic impact on the adult survey because it discards sampling effort and laboratory analysis of samples corresponding to spawning schools. This problem might thus be avoided, simply, by sampling before or after the hours of bias, i.e., on either side of the spawning act.

An important aspect of the spawning fraction parameter is that it constitutes a population rather than an individual estimate. Parrish *et al.* (1986) and Ganias *et al.* (2003b) found that the spawning incidence was heavily age dependent in northern anchovy and the Mediterranean sardine respectively. This dependency of spawning fraction to fish age/size has rarely been studied for pelagic schooling fishes. In comparing all available spawning fraction estimates for several sardine and anchovy stocks around the world, Alheit (1993) noticed higher values for stocks sampled from the professional fishery and suggested that the commercial catches might be biased if fish form spawning aggregations are more vulnerable to the commercial fishery. Alternatively, when size has a significant effect on spawning activity, higher values of population spawning fraction might be due to commercial fishery selecting larger fish (Ganias *et al.*, 2003b). Thus, when age/size effects might be present, additional attention should be paid so that the samples used for the estimation of spawning fraction represent adequately the age/size structure of the sampled population.

4.4.2 POF staging and ageing

The process of POF staging, aging and attribution of spawners to daily cohorts has many similarities with egg staging, aging and attribution to daily cohorts of eggs. Both processes require information on the daily probability density function of spawning activity and the impact of temperature on physiological phenomena (egg development and POF degradation respectively), in order to attribute an age to a staged egg or a classified POF according to the time of sampling. In principle, the methodology developed for egg staging and ageing (see Section 3.2.1) could be also applied for the identification of POF cohorts for spawning fraction estimation. This would require:

- Uniform description of POF morphological classes/stages: Similar to what is done with egg staging, the main morphological characteristics of POFs along the first few days of involution could be identified to delimit discrete histological classes. Ganias *et al.* (2003b) showed that in species with daily spawning synchronicity this can be done even when the exact age of POFs is not known. Compiling histological samples of POFs from several surveys and from discrete daily periods can provide sufficient data to describe the main morphological characteristics of POF stages that are ordered from youngest to oldest. For example, Ganias *et al.* (2003b) using only samples from night-time (18:00

– 5:00) produced ordered POF stages with large morphological differences for sardine in the eastern Mediterranean.

- A model of POF stages as a function of age and temperature: Extending the work of Hunter and Macewicz (1985) and Fitzhugh and Hettler (1995), induced spawning of pelagic fish in captivity could be performed along a temperature gradient following the same principles used in egg incubation experiments (see Section 4.2). Although this is probably more demanding than an egg incubation experiment, maintaining pelagic fish in captivity and inducing spawning has already been successful for several sardine and anchovy species. The data from such an experiment could then be analysed with multinomial models to obtain the probability of being at a POF stage given observation time (age) and temperature in exactly the same way as described in Section 4.2.2.

The above information could then be used with the spawning probability density function (Section 4.2.4) and the staged POFs from a survey to obtain an "age" for each spawner (in this case age refers to time from spawning) and to group spawners in daily cohorts according to the Bayesian method and software described in Sections 3.2.1 and 4.2.2.

Table 4.1.2.1.1. Summary of the year effects by vessels (as reflected by the differences in the intercepts of least squares fitted lines by years forcing common slopes and from the GLM fitted models by vessels). (from Uriarte *et al.*, 2003).

Sardine Year Effect	Slopes		Intercepts			
	Common Value	P(b00=b01)	Difference	P(Dif=0)	CD (R2 adjusted)	Stand.Error of Reg.
Investigador	0.9619	0.0029	**0.3669**	0.0000	65.8	0.7548
Thalassa	0.8578	0.7922	**-0.5000**	0.0000	64.3	0.8754

Table 4.1.3.1. Comparison between presence and absence of sardine eggs in the samples obtained with CalVET and CUFES in the Portuguese 2002 DEPM sardine survey.

	CalVET (+)	%	CalVET (-)	%		%
CUFES (+)	154	32	84	18	Concordance	75
CUFES (-)	31	6	201	43	Discordance	25

Table 4.1.3.2. Comparison of number of stations with sardine eggs obtained with CUFES at, before, and after CALVET in the Spanish 2002 DEPM sardine survey (P – presence; A – absence).

Comparison	P-P	P-A	A-P	A-A	Agreement (%)
CUFES (At)/CUFES (Before)	33	1	2	19	96
CUFES (At)/CUFES (After)	32	2	7	14	85
CUFES(Before)/CUFES (After)	34	1	5	15	91

Table 4.1.3.3. Sardine eggs presence/absence from CalVET and CUFES (at, before, and after CALVET stations) during the Spanish 2002 DEPM sardine survey (P – presence; A – absence).

Comparison	P-P	P-A	A-P	A-A	Agreement (%)
CALVET/CUFES (At)	33	5	1	16	91
CALVET/CUFES (Before)	34	4	1	16	93
CALVET/CUFES (After)	35	3	4	13	89

Table 4.2.1.1. Percent of agreement during the first and second staging of anchovy (Ee) and sardine (Sp) eggs from CUFES and PAIROVET (PV) in the San Sebastian staging workshop.

Agreement (%)	Ee CUFES	Sp CUFES	Ee PV	Sp PV
1st reading	82.1	89.1	71.5	77.5
2nd reading	92.5	96.8	86.4	85.7

Table 4.2.1.2. Percent of agreement by development stage during the first and second staging of anchovy and sardine eggs from PAIROVET in the San Sebastian staging workshop.

Agreement (%)	Anchovy		Sardine	
Stages	1st staging	2nd staging	1st staging	2nd staging
1	75	88	-	-
2	83	89	87	84
3	61	81	65	81
4	76	96	60	57
5	61	78	74	89
6	61	100	87	95
7	68	92	85	85
8	74	75	78	83
9	70	83	72	84
10	73	92	74	76
11	64	81	76	87

Table 4.2.1.3. Percent of agreement by development stage during the first and second staging of anchovy and sardine eggs from CUFES in the San Sebastian staging workshop.

Agreement (%)	Anchovy		Sardine	
Stages	1st staging	2nd staging	1st staging	2nd staging
1	89	97	60	84
2	63	81	78	-
3	90	91	99	100

Table 4.2.1.4. Summary (number of stations and eggs) of total, disintegrated and only disintegrated sardine eggs (i.e., stations where only disintegrated eggs were found) for each year and national survey.

Year	Country	Stations			Eggs		
		Total	Dis	Only Dis	Total	Dis	Only Dis
1988	P	309	9	9	1 307	19	19
	S	516	0	0	3 922	0	0
1990	S	475	0	0	1 494	0	0
1997	P	373	50	2	1 454	179	4
	S	515	0	0	1 465	0	0
1999	P	413	57	2	5 110	1 191	7
	S	402	16	0	2 340	28	0
2002	P	484	19	5	2 585	72	6
	S	313	21	4	1 939	33	4

Table 4.2.2.1.1. Summary of anchovy incubation experiment (Motos, 1994): mean temperature (°C, corresponding coefficient of variation in brackets) for the 8 tubes used in each of the incubations and for both incubations together.

Incubation	A	C	E	G	I	K	M
	8.52	10.88	13.12	15.53	17.81	19.96	21.68
Incubation 2	(0.04)	(0.05)	(0.03)	(0.02)	(0.02)	(0.02)	(0.02)
	8.46	10.72	13.15	15.47	17.81	20.04	21.93
Incubation 3	(0.05)	(0.04)	(0.04)	(0.04)	(0.03)	(0.03)	(0.03)
Incubations 2 and 3	8.49	10.79	13.13	15.5	17.81	20.0	21.81
	(0.05)	(0.05)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)

Table 4.2.2.2.1. Summary of sardine incubation experiment in the Gulf of Cadiz during 2002: mean temperature (coefficient of variation in brackets), mean number of eggs sampled in each event and time to hatch (i.e., time at which all alive eggs have passed to larval stage) for all incubators.

Incubator	Mean T °C (cv)	Mean Sampled eggs	Time to hatch (h)
D	10.75 (0.10)	27	129.17
G	13.00 (0.05)	28	96.08
J	15.46 (0.05)	33	84.03
M	16.98 (0.05)	33	63.00

Table 4.3.1.1. Macroscopic and microscopic maturity scale for female sardine.

MATURITY STAGE		MACROSCOPIC DESCRIPTION	MICROSCOPIC DESCRIPTION
I	**VIRGIN AND RESTING**	Invisible or very small cord-shaped ovaries; translucent with almost no colour.	Unyolked oocytes as the unique type of oocytes present in the gonad.
II	*DEVELOPING*	Wider ovaries occupying 1/4 to 3/4 of body cavity; opaque with pinkish or yellow colour . Visible oocytes are not present.	The most advanced batch of oocytes are partially-yolked ones.
III	*PRE-SPAWNING*	Bigger ovaries occupying 3/4 to almost fitting body cavity; opaque with yellow or orange colour . Small opaque oocytes are visible.	The most advanced batch of oocytes are yolked ones.
IV	*SPAWNING*	Large ovaries occupying the full body cavity; fully or partially translucent with gelatinous aspect. Hyaline oocytes are visible (some small opaque oocytes can be visible).	The most advanced batch of oocytes are hydrated ones.
V	*PARTIAL POST-SPAWNING (recovering to Stage III)*	Deflated and flaccid ovaries occupying about 3/4 of body cavity; with some ruptured blood vessels that gives them a bloodshot aspect. Some small opaque oocytes are visible (some hyaline oocytes can be present).	Post-ovulatory Follicles may be present. Some ruptured blood vessels and free haemocytes present spread all over the ovary tissue (light haemocyte infiltration). Presence of all oocyte stages is possible.
VI	*ULTIMATE POST-SPAWNING*	Very deflated and flaccid ovaries occupying from about 3/4 to 1/4 of body cavity; with many ruptured blood vessels that gives them a reddish colour. Some small opaque oocytes can be visible (no hyaline oocytes are present)	Post-ovulatory Follicles may be present. Many ruptured blood vessels and free haemocytes invading the ovary tissue (generalised haemocyte infiltration). No yolked oocytes, or most of them in an atretic state.

Table 4.3.1.2.1. Correspondence (%) between macroscopic and microscopic classifications of female sardine maturity (data collected during the November 2000 Portuguese acoustic survey).

Macroscopic maturity stage	Microscopic maturity stage						N
	I	II	III	IV	V	V-III	
Stage I	**75.5**	24.5					49
Stage II	6.7	**60.0**	20.0		6.7	6.7	15
Stage III			**50.0**			50.0	6
Stage IV			4.2	**95.8**			24
Stage V		36.4	27.3		**0.0**	36.4	11
Stage V-III			56.9	5.6		**37.5**	72

Table 4.3.1.2.2. Correspondence (%) between macroscopic and microscopic classifications of female sardine maturity in DEPM surveys (in 1999, the macroscopic stages were attributed to formol-fixed, instead of fresh, gonads).

Survey	Macroscopic maturity stage	Microscopic maturity stage				N
		I	II	III	IV	
March 1997	I					0
	II	66	28	7		61
	III	3	4	92	2	253
	IV	3	0	3	93	30
	V					0
	V-III		1	99		116
January 1999	I	38	13	50		8
	II	26	9	65		23
	III	1	1	93	6	563
	IV			24	76	42
	V					0
	V-III					0
January 2002	I	100				1
	II	50	33	17		6
	III			100		12
	IV			2	98	129
	V	22	51	28		65
	V-III			5	95	361
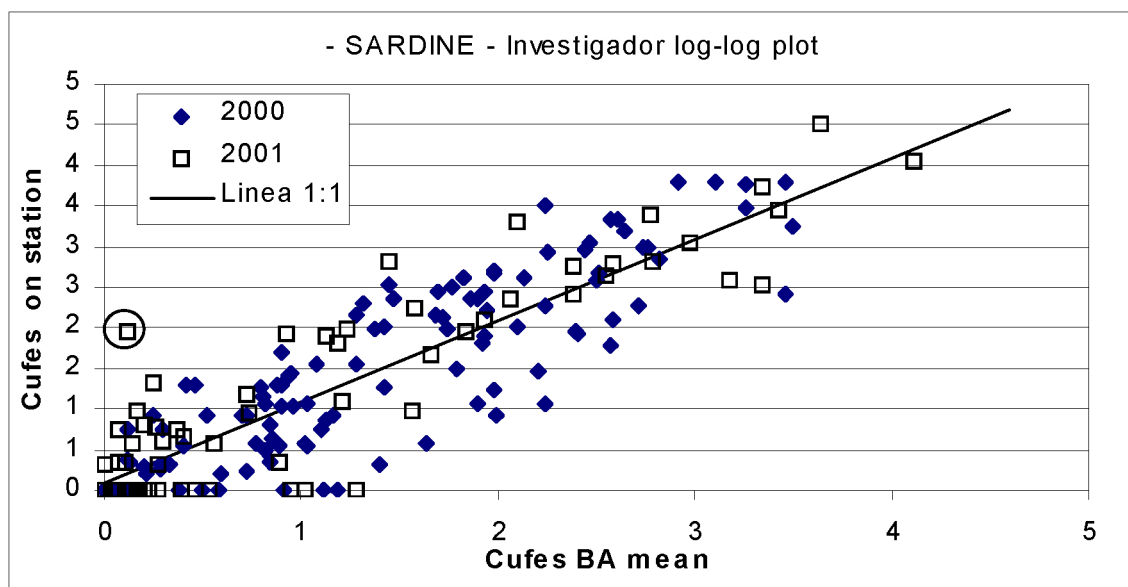
Figure 4.1.2.1.1. Comparison of CUFES sardine egg densities (eggs/m³, log-scale) in underway (before and after the PAIROVET haul - CUFES BA mean) and stationary (4 min during the PAIROVET haul (CUFES on station) samples from two years (2000 and 2001). Line is 1:1 relationship and encircled points are removed outliers (from Uriarte *et al.*, 2003).
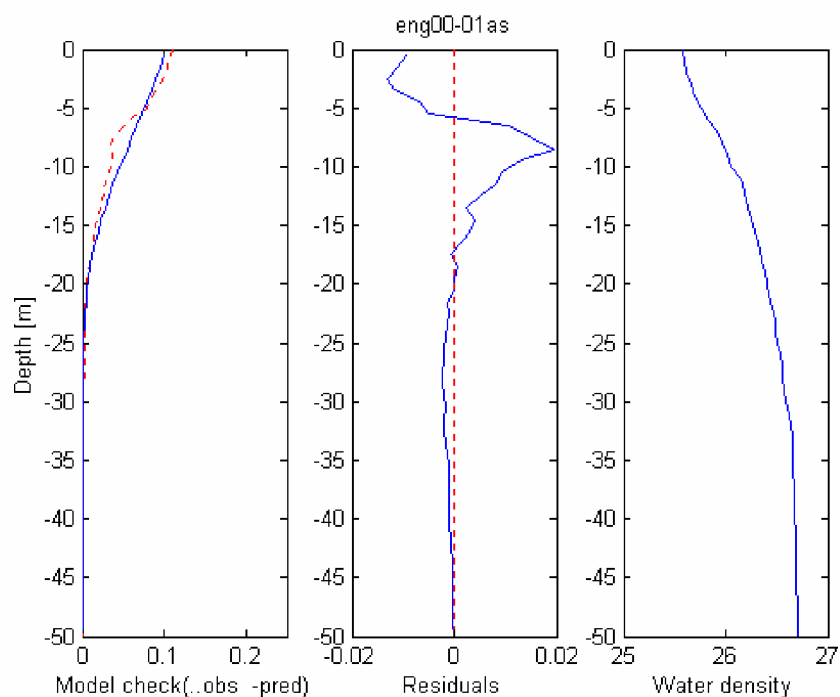
Figure 4.1.2.2.1. Example of vertical egg distribution fitting to the average of LHPR hauls from the sampling in 2000 and 2001 for anchovy in waters of high salinity at surface (Boyra *et al.*, in press).
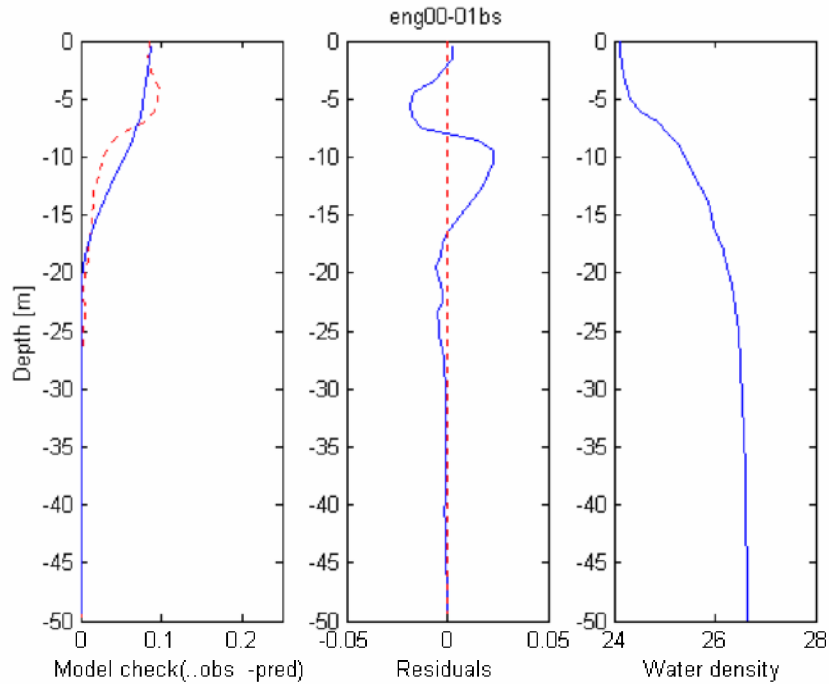
Figure 4.1.2.2.2. Example of vertical egg distribution fitting to the average of LHPR hauls from the sampling in 2000 and 2001 for anchovy in waters of low salinity at surface (Boyra *et al.*, in press).
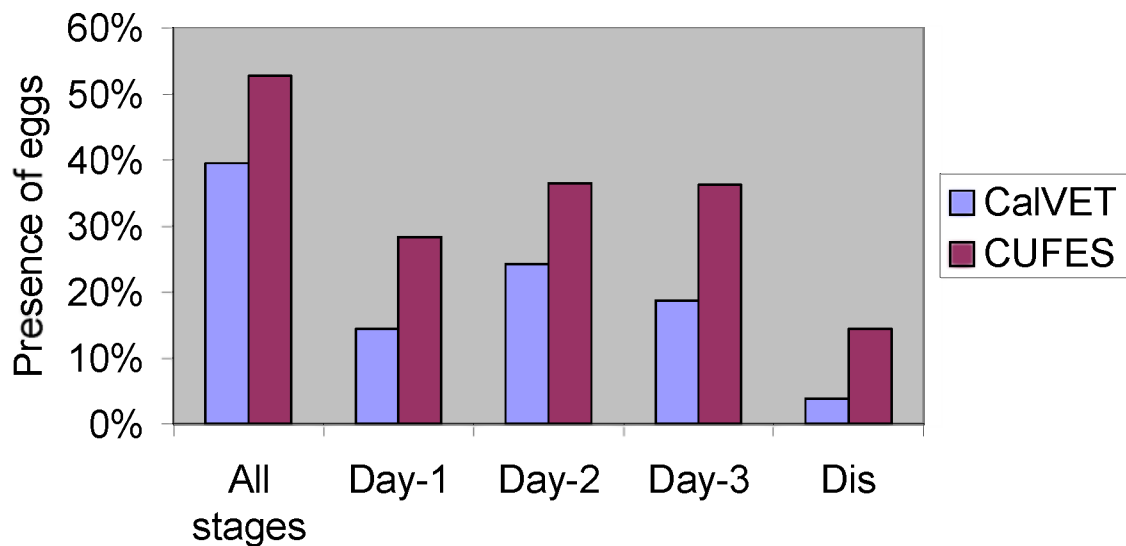
Figure 4.1.3.1. Comparison of the proportion of stations where sardine eggs (all stages and separately by broad categories of development) were detected by CalVET (blue columns) and CUFES (red columns) during the 2002 Portuguese DEPM survey.
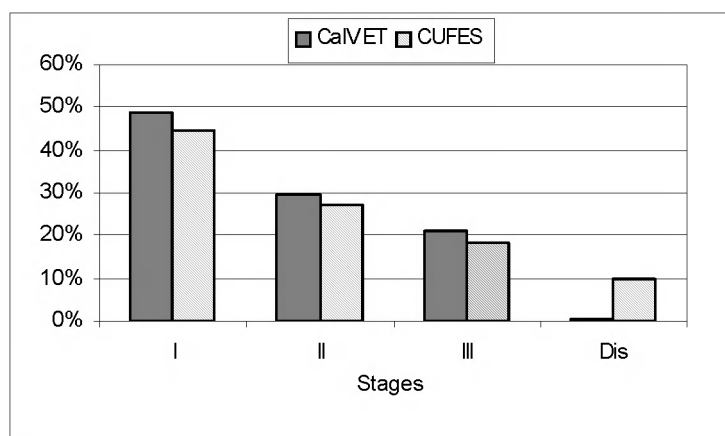
Figure 4.1.3.2. Percentage of sardine eggs at stage (broad stages for CUFES applications) in CalVET and CUFES in the calibration exercise performed during the Spanish 2002 DEPM survey.
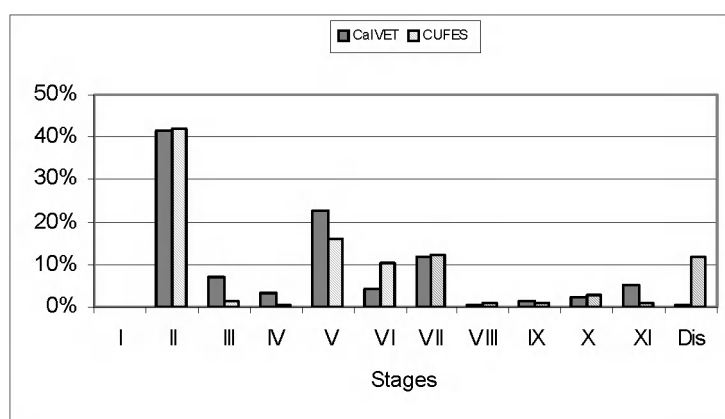
Figure 4.1.3.3. Percentage of sardine eggs at stage (standard stages for DEPM applications) in CalVET and CUFES in the calibration exercise performed during the Spanish 2002 DEPM survey.
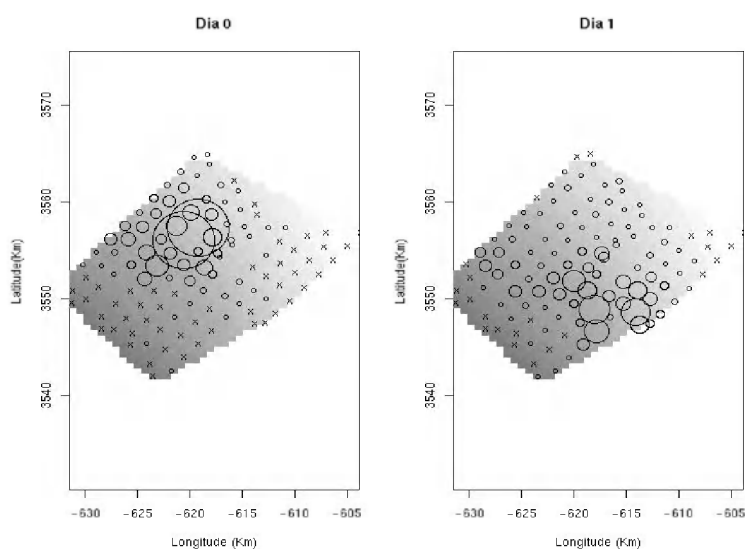
Figure 4.1.4.1. Distribution of two daily cohorts of sardine eggs (hatched during the night of sampling in the left and 1 night before in the right) within a restricted area (approximately 120 nm^2) in the Gulf of Cadiz. Circles indicate egg presence (with radius proportional to egg density) and crosses egg absence.
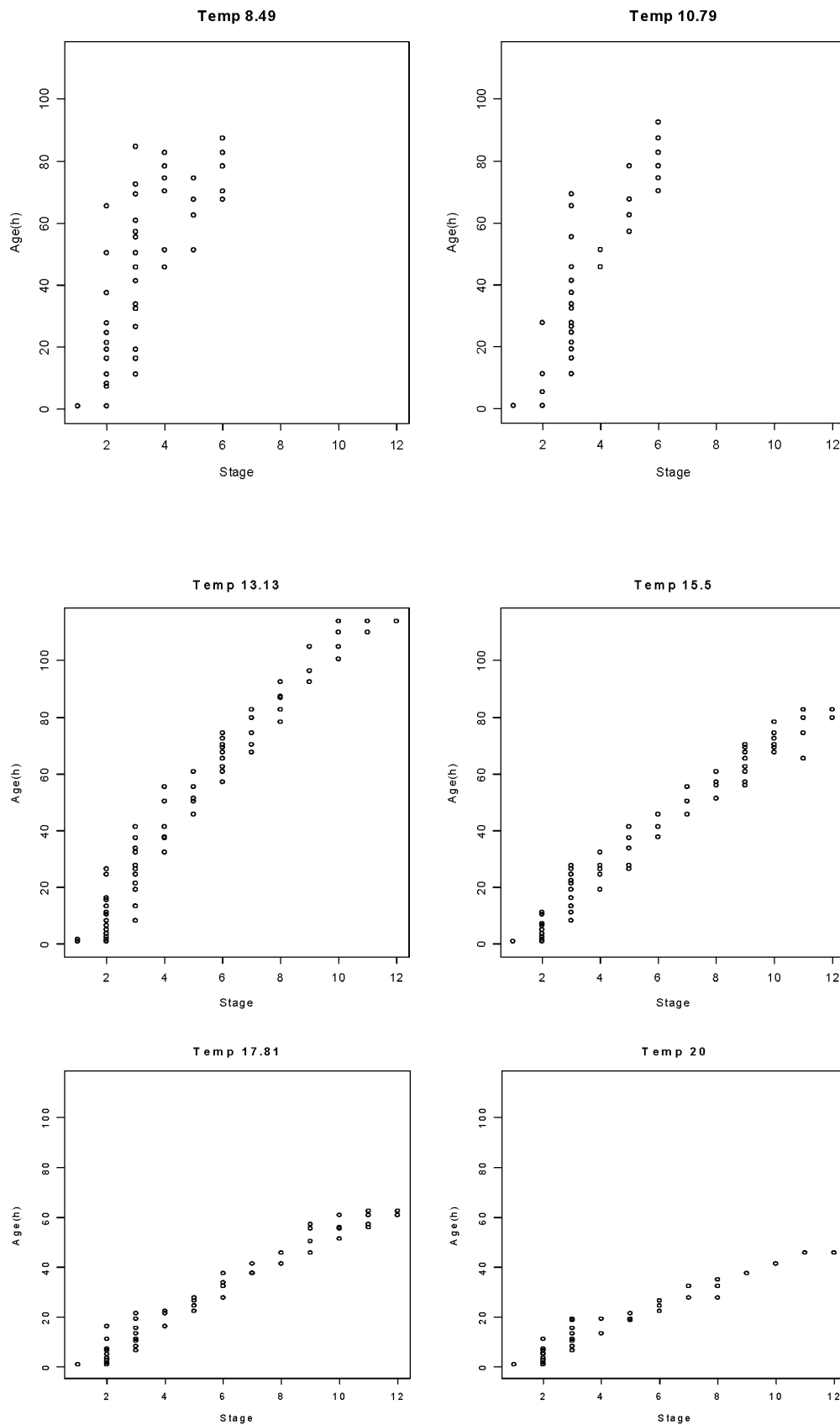
Figure 4.2.2.1.1. Observed age (in hours) for each stage and temperature (data from anchovy incubation experiment).
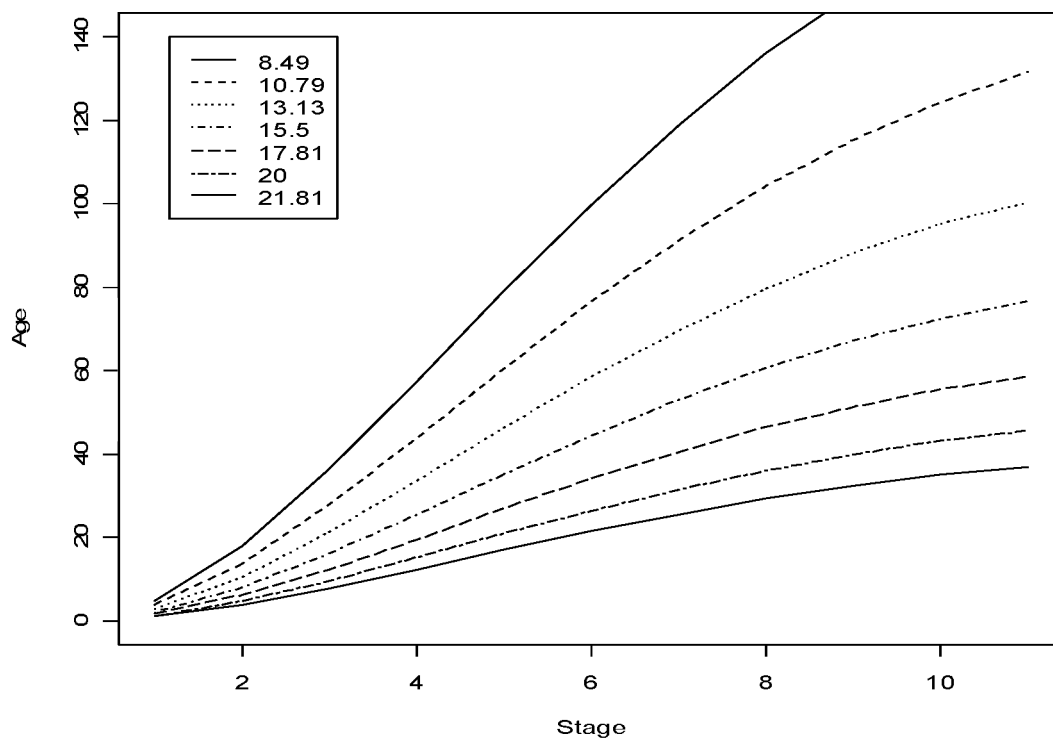
Figure 4.2.2.1.2. Mean age *vs.* stage from Lo's model fitted to anchovy incubation experiment data. Each line represents a different incubation temperature.
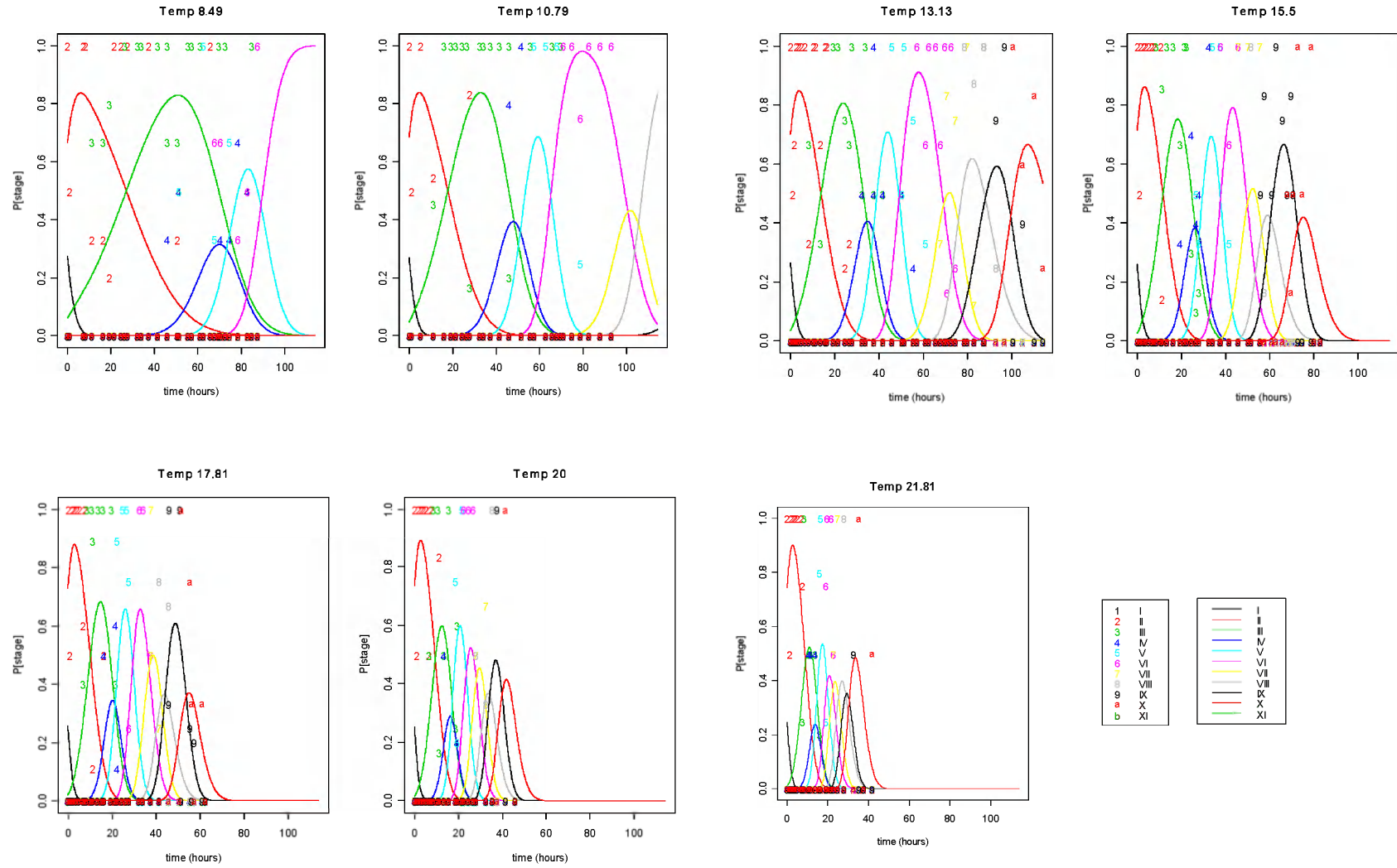
Figure 4.2.2.1.3. Probability of being at stage along observation time (age) from models fitted to anchovy incubation data (one incubation temperature in each panel).

Temp 10.75556

Temp 13.00278

Temp 15.45937

Temp 16.97600
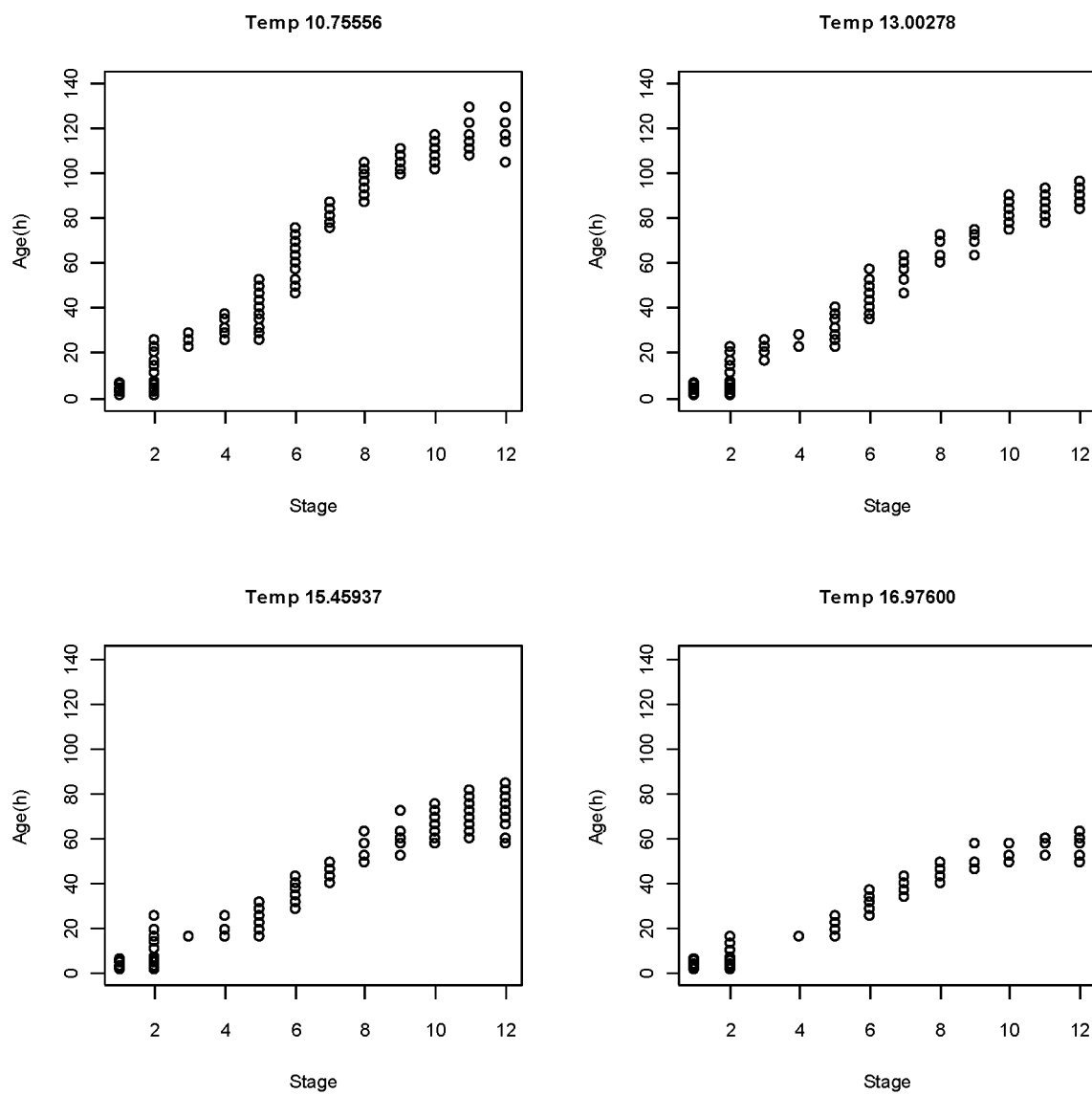
Figure 4.2.2.2.1. Observed age (in hours) for each stage and temperature (data from sardine new incubation experiment).
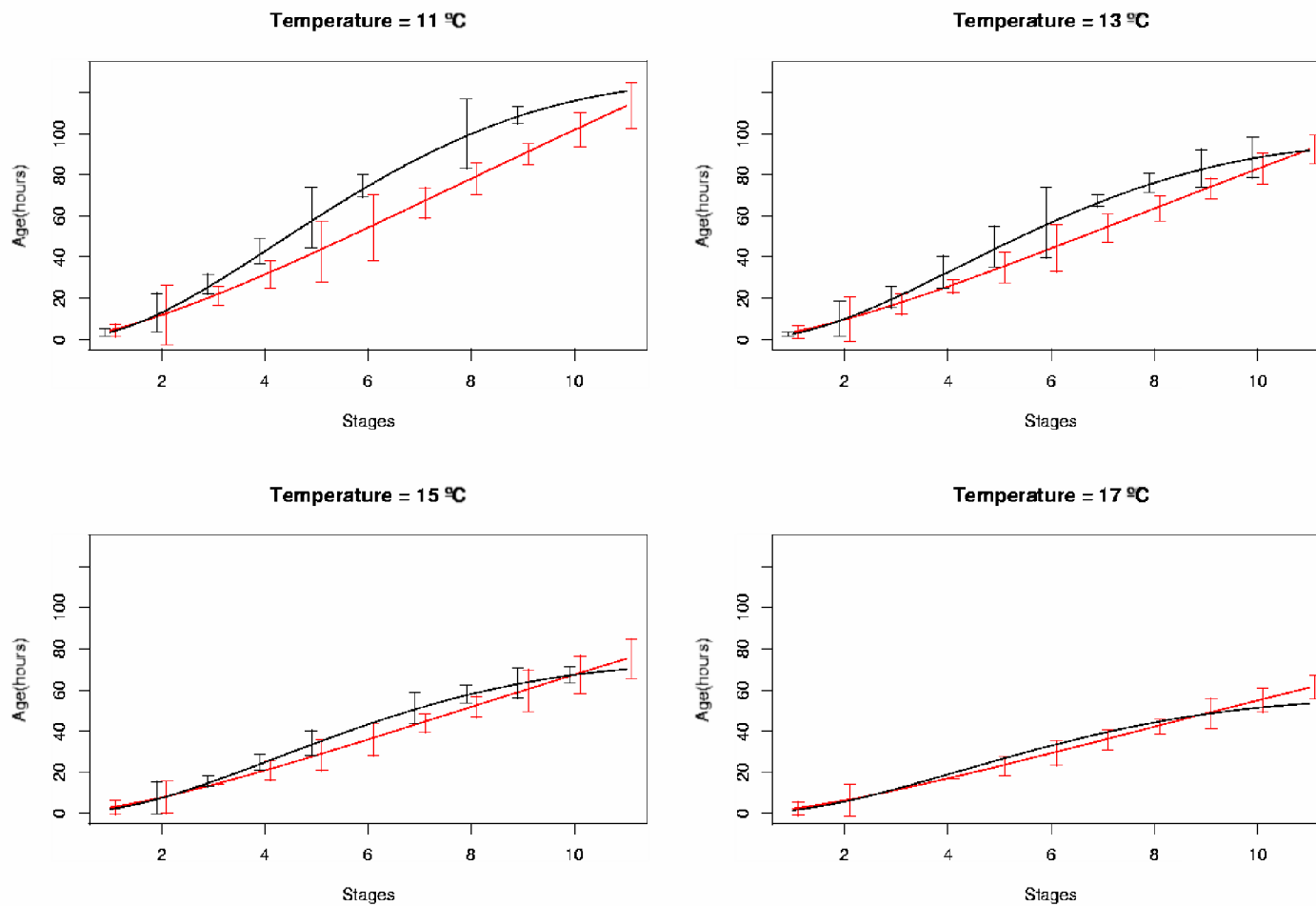
Figure 4.2.2.2.2. Lo's model applied to Cadiz (red line) and Cantabric area (black line) incubation data. Vertical lines indicate estimates of stage duration obtained directly from the data following Lo (1985). (No observations for temperature=17ºC available n the Cantabric area data set).

Temp 10.75556

Temp 13.00278

Temp 15.45937

Temp 16.976
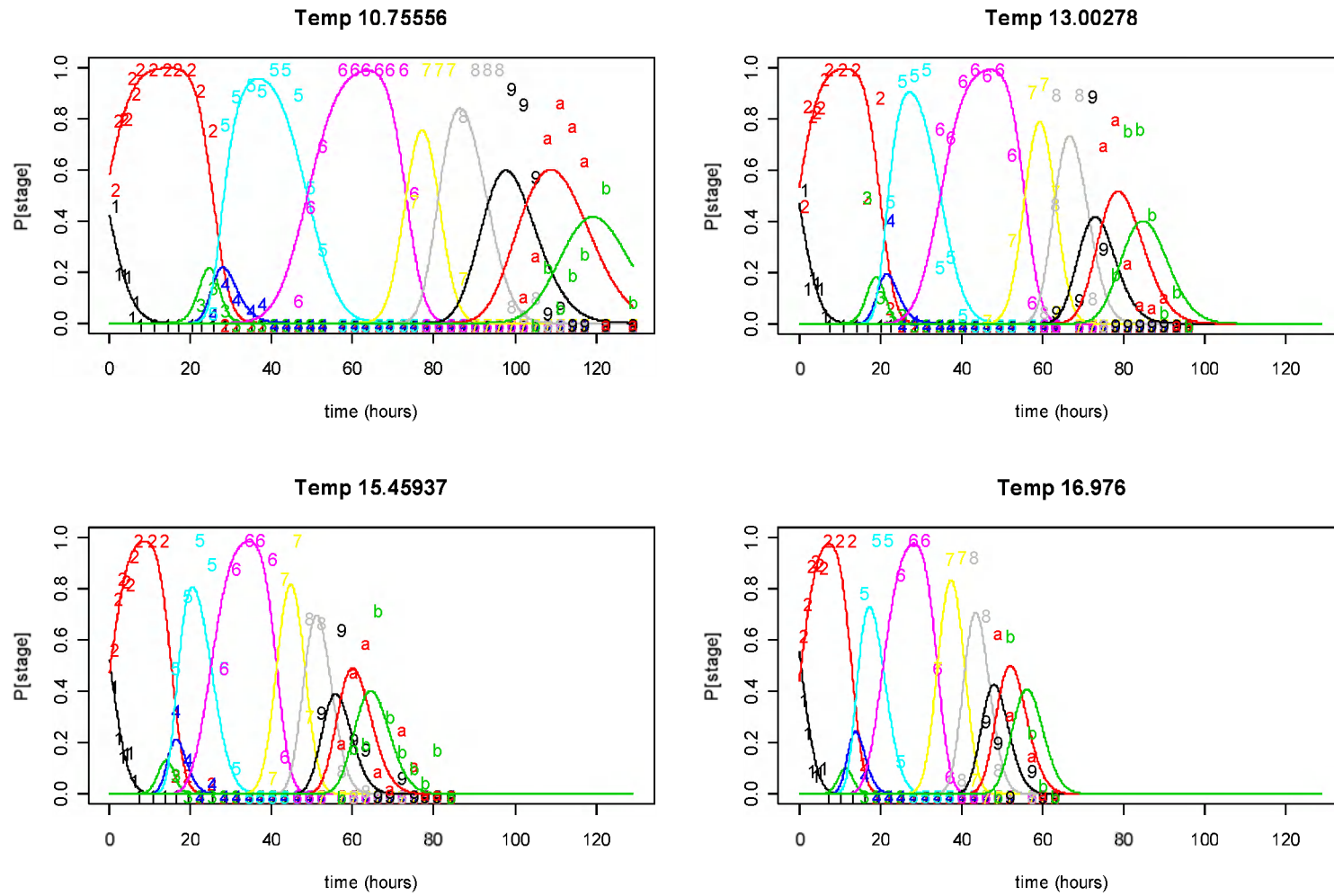
Figure 4.2.2.2.3. Multinomial model of probability of being in a given stage given age and temperature fitted to the sardine egg incubation data Cádiz data using the *depmodel* package.
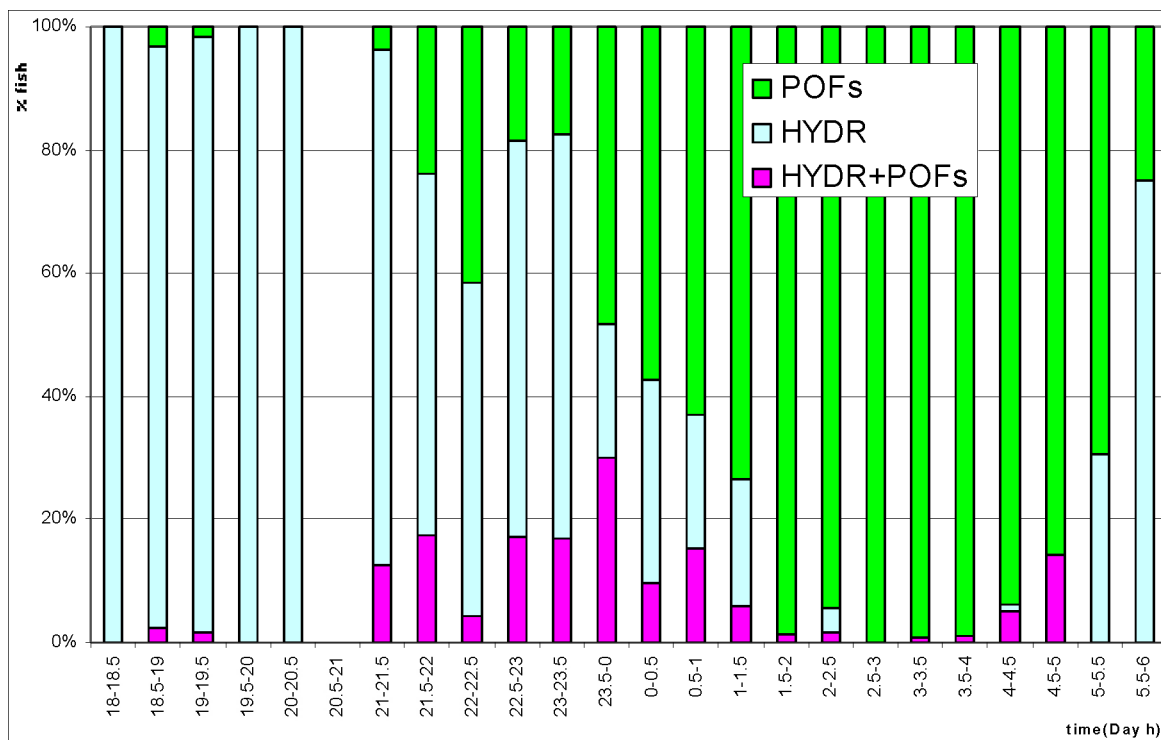
Figure 4.2.4.1. Half-hourly distribution of anchovy gonad states among the spawning females during the period 18:00 – 05:00 GMT (data from several DEPM surveys).
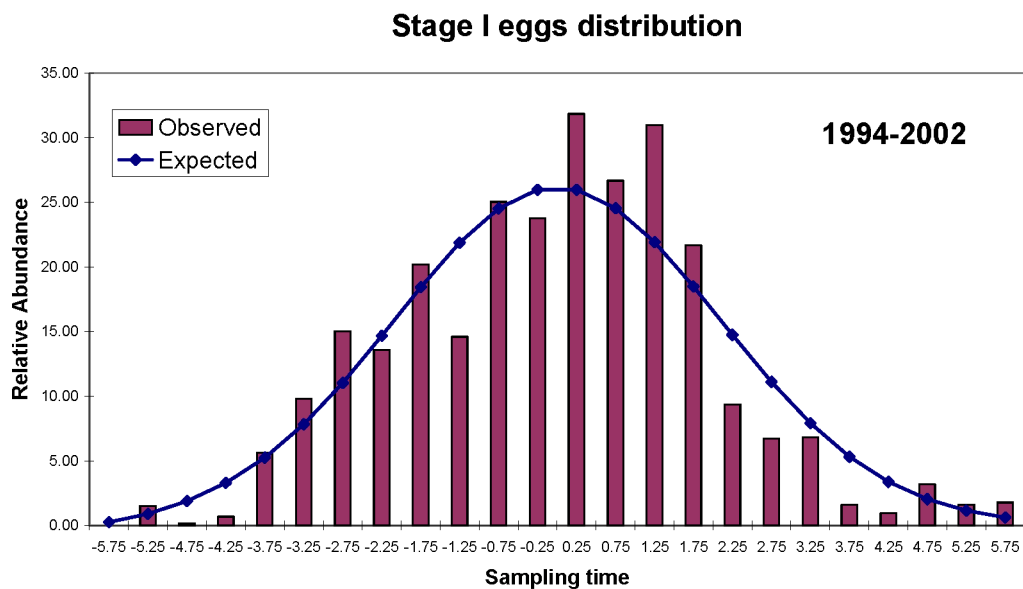
Figure 4.2.4.2. Distribution of Stage I anchovy eggs per surface unit versus sampling time in hours (in distance to midnight) and expected distribution according to the assumption of a normal curve to the distribution of spawning time (fitted parameters N (-0.76, 2.04)). Data from DEPM surveys 1994–2002.
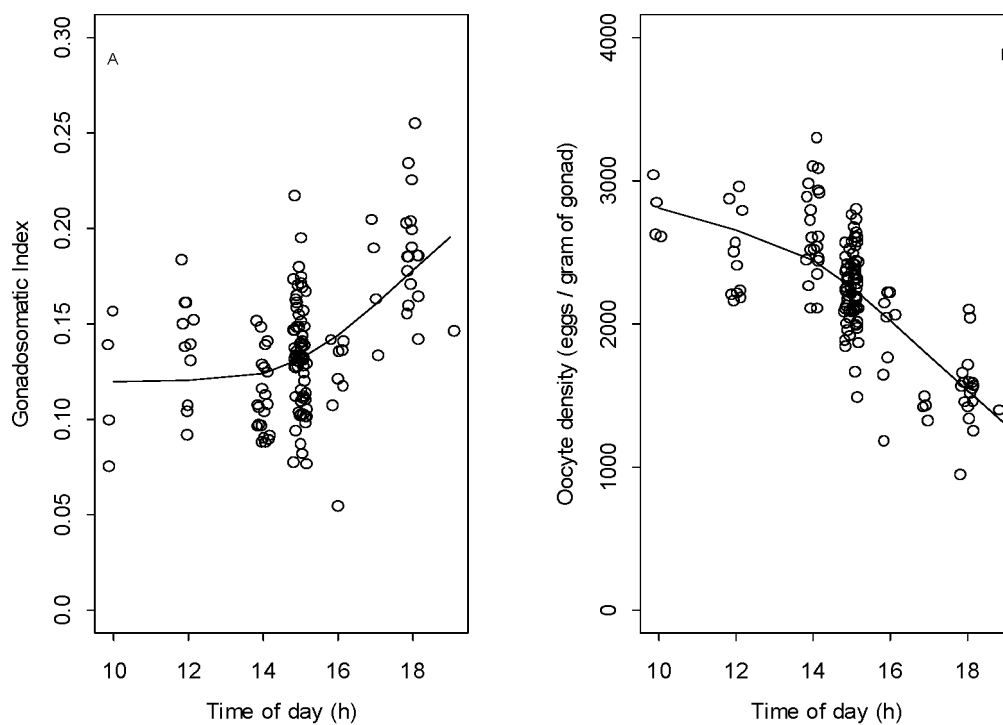
Figure 4.2.4.3. Diurnal development of gonadosomatic index (a) and hydrated oocyte density (b) for female sardines sampled during the day of anticipated spawning (from Zwolinski *et al.*, 2001).
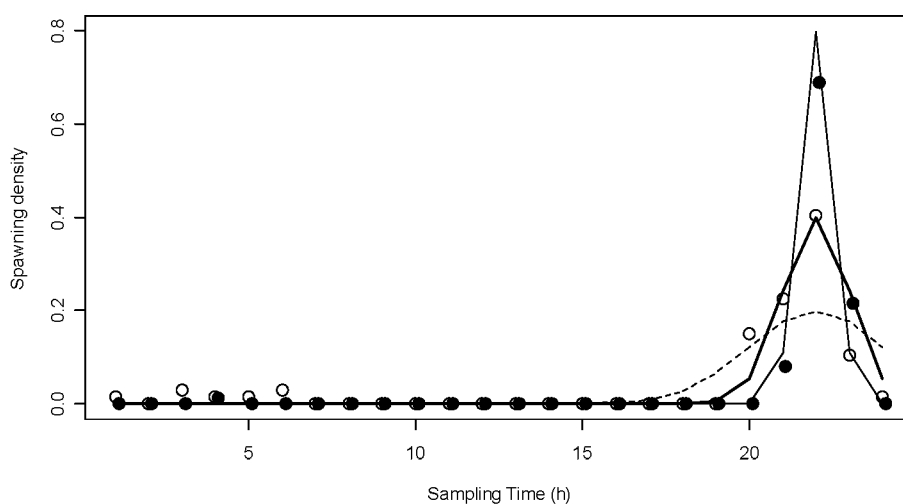
Figure 4.2.4.4. Proportion of stage I sardine eggs caught hourly during DEPM surveys (open circles) and during sampling in a fixed location (closed circles, data from Ré *et al.*, 1988). Lines correspond to a normal density functions with a 22 hr. mean and standard deviation of 0.5 (thin continuous), 1 hr. (thick continuous) and 2 hr. (broken) respectively.
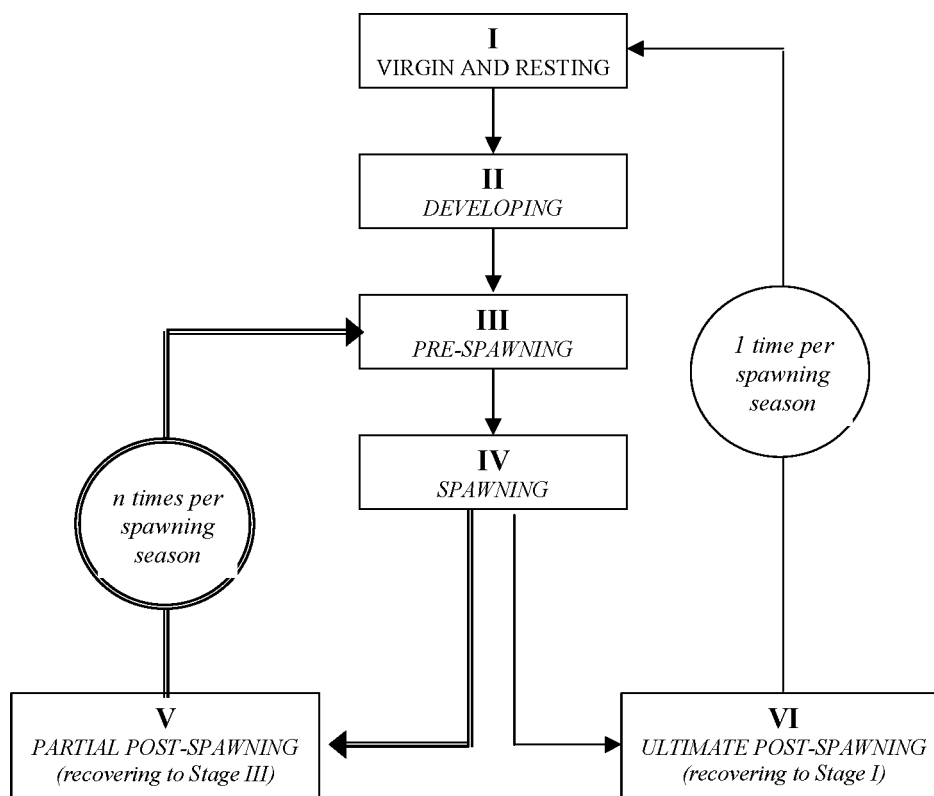
Figure 4.3.1.1. Female Sardine Maturity Cycle (based on the scale of Table 3.3.1.1).
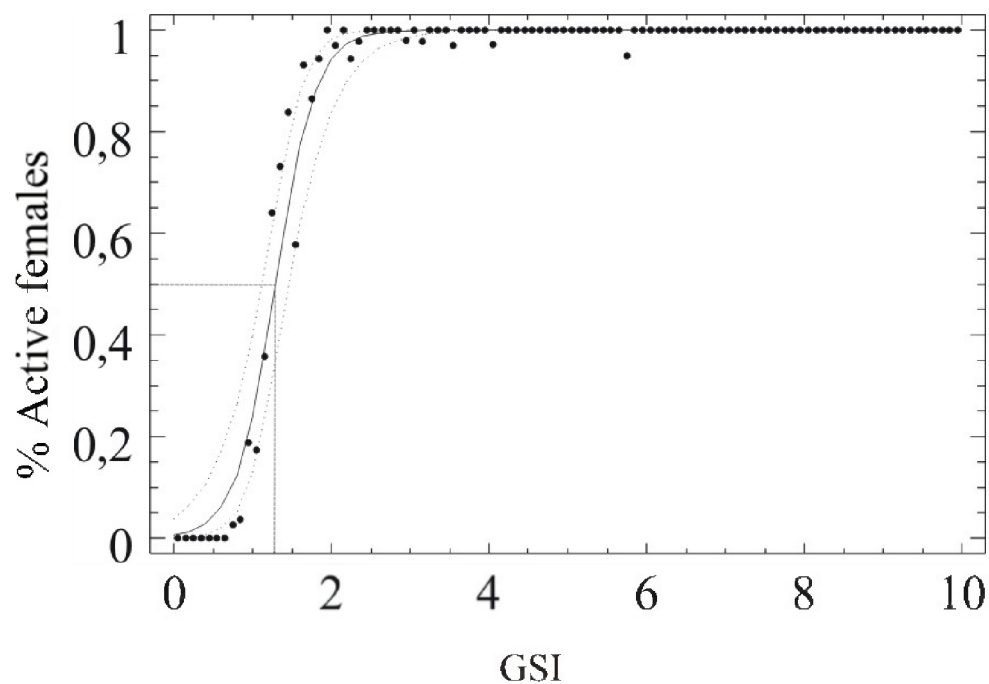
Figure 4.3.2.1.1. Proportion of active female sardines as a function of their gonadosomatic index (GSI) and respective ogive to identify the value of GSI at which half of the sardine females are active (from Ganias, 2003).
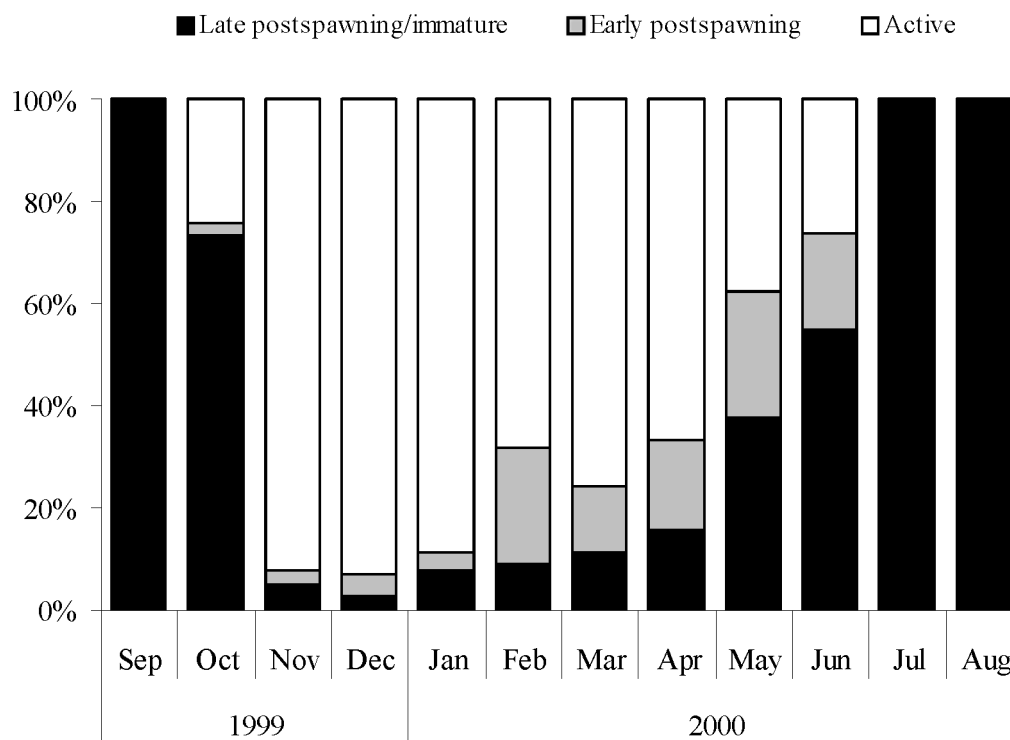
Figure 4.3.2.1.2. Evolution of the monthly fractions of active, early postspawning and late postspawning/immature female sardines in the Mediterranean (from Ganias *et al.*, submitted).
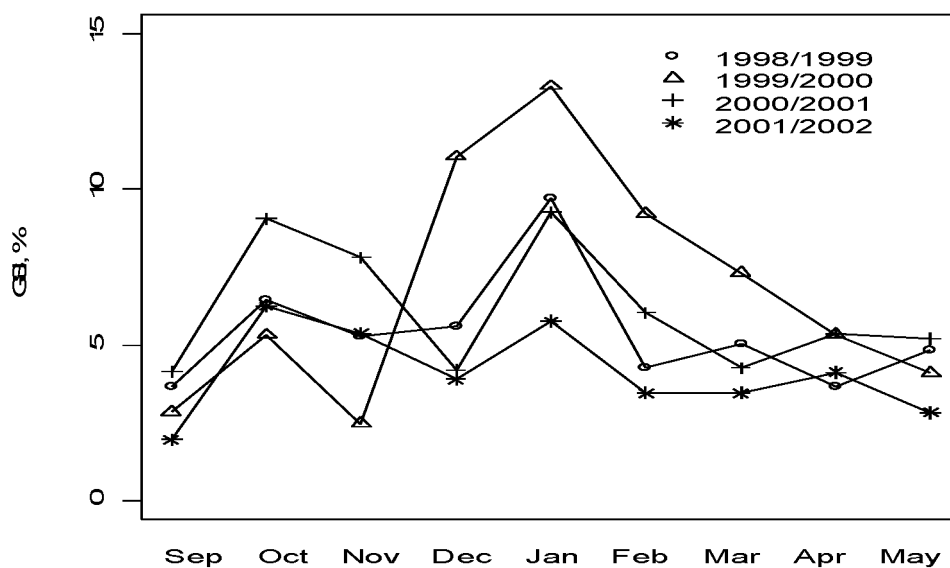
Figure 4.3.2.2.1. Average monthly female gonadosomatic index (GSI) along the sardine spawning season off Portugal in recent years (data from Portuguese market samples).
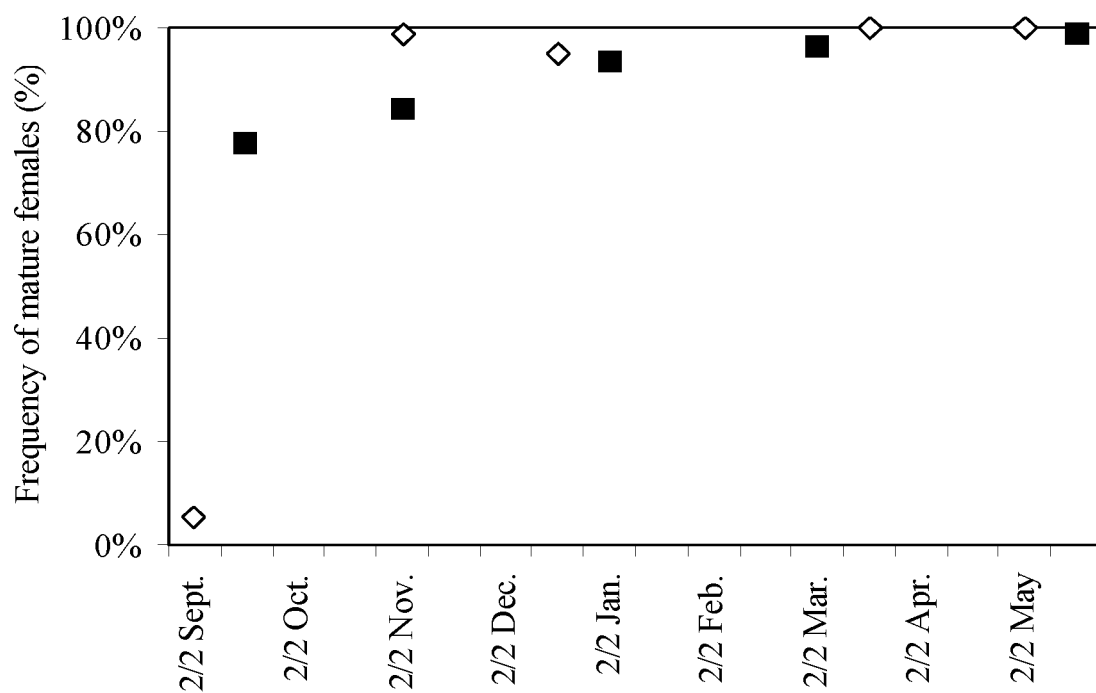
Figure 4.3.2.2.2. Evolution of female sexual maturation at the northern (diamonds) and southern (black squares) coasts during 2001/2002 spawning season.
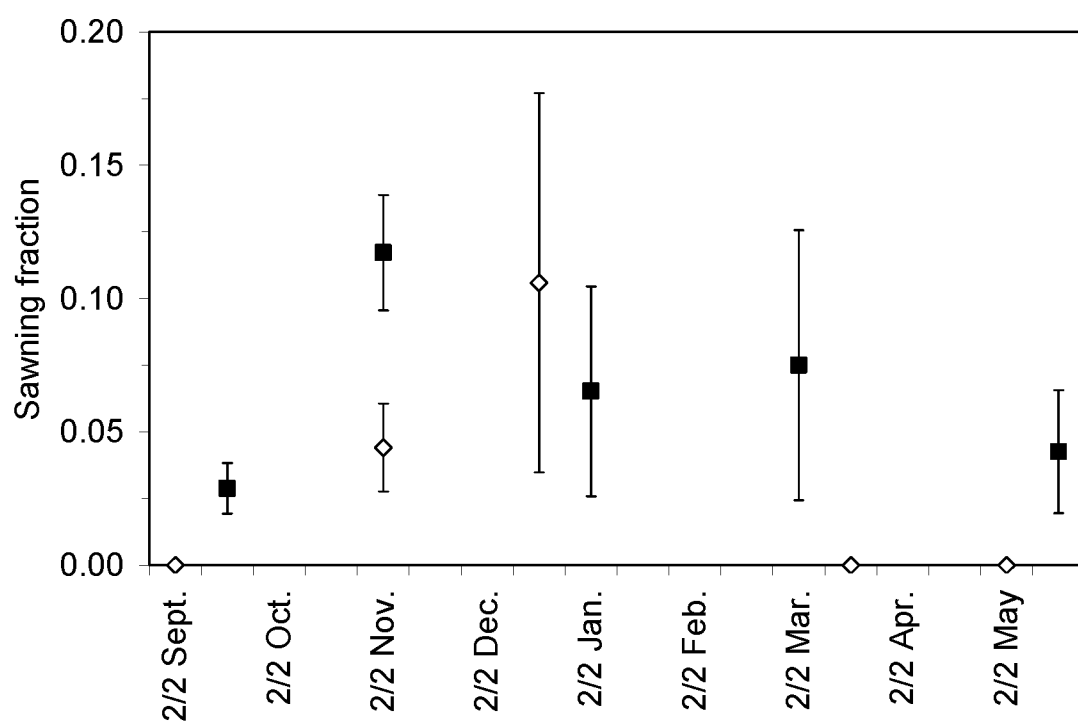
Figure 4.3.2.2.3. Temporal progression of spawning fraction along the northern (diamonds) and southern (squares) coasts during 2001/2002 spawning season.

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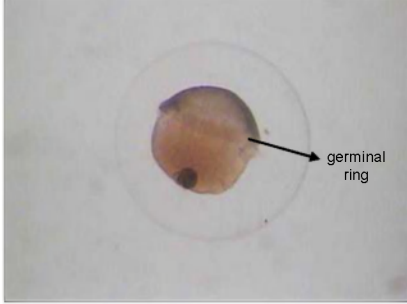Gu, C., and Wahba, G. 1991. Minimizing GCV/GML scores with multiple smoothing parameters via the Newton
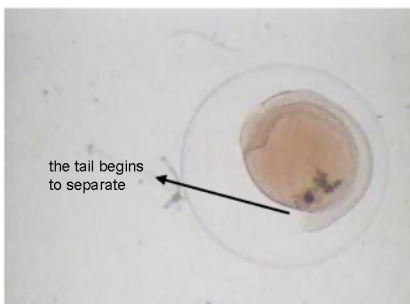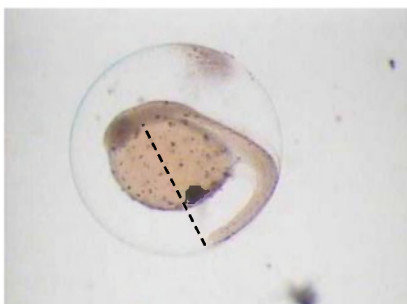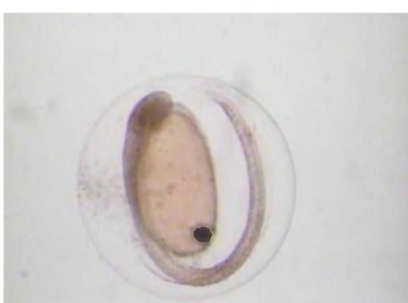
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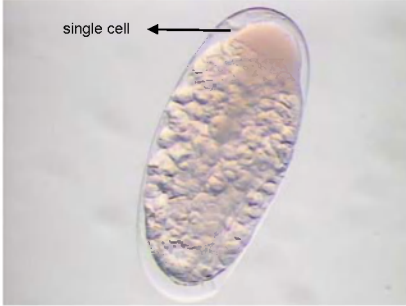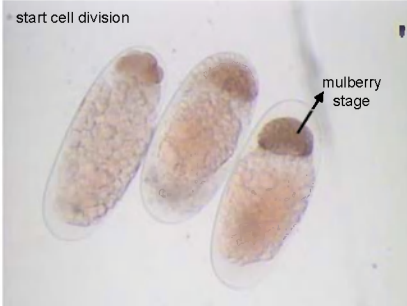
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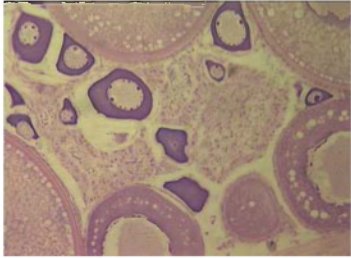
6   Annexes
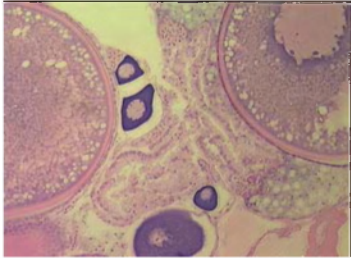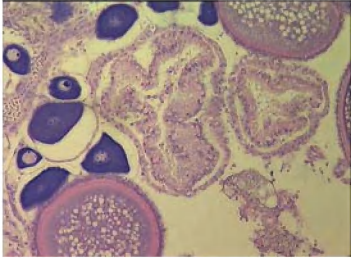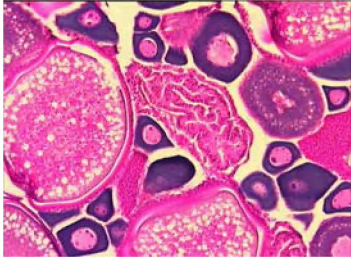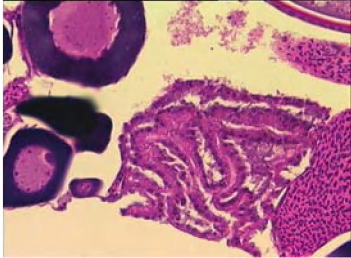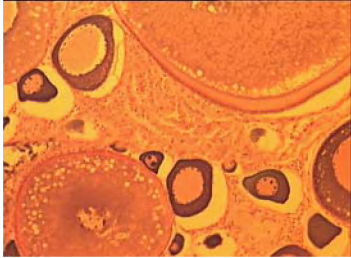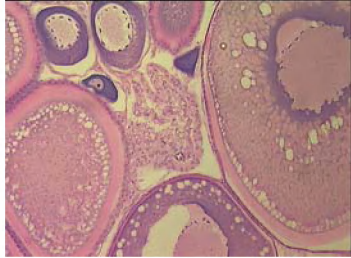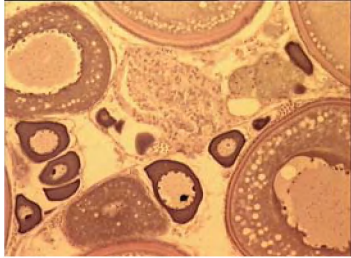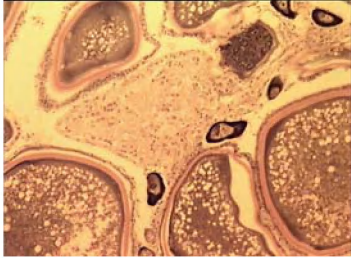
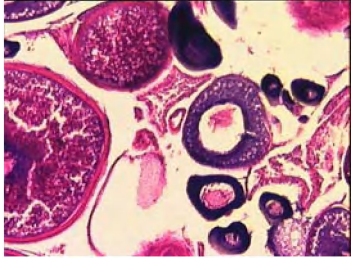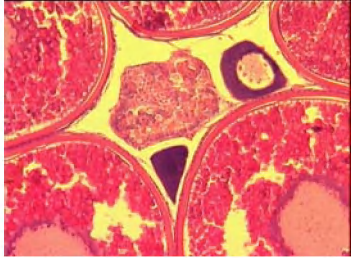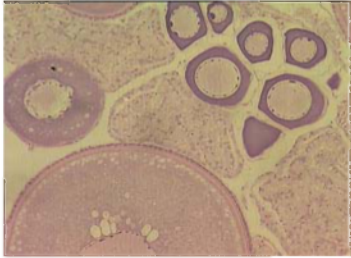**Annex I: Reference collection of sardine (*Sardina pilchardus*) egg stages. Staging based on Ahlstrom (1943).**

Stage I	Stage II	Stage III
	blastodermal cap	germinal ring

Stage IV	Stage V	Stage VI
embryo	not optic vesicles	optic vesicls

Stage VII	Stage VIII	Stage IX
the tail begins to separate	the tail is free for a length greater than the length of the head (about 1/3 of the body)	

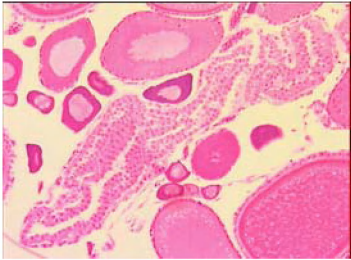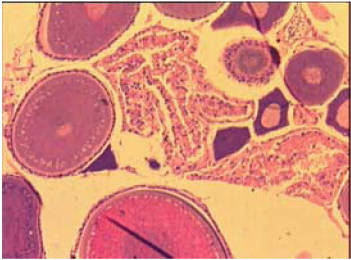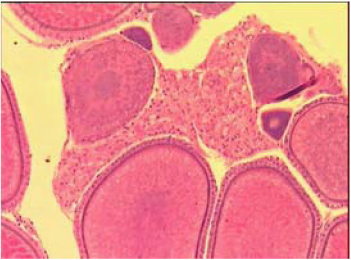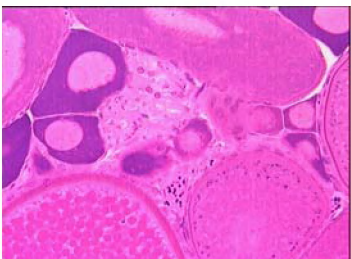| Stage X	Stage XI	
the plane of orientation has been rotated a complete 90°		

Annex II: Reference collection of anchovy (*Engraulis encrasicolus*) egg stages. Staging based on Moser, and Ahlstrom (1985).

Stage I	Stage II	Stage III
single cell ←	start cell division / mulberry stage	blastodermal cap
Stage IV	**Stage V**	**Stage VI**
1/3 / 2/3	> 2/3	clausure of the blastopore
Stage VII	**Stage VIII**	**Stage IX**
the tails begind to separate	greater than 1/2 head length	end of the tail / 1/4
Stage X	**Stage XI**	
1/2	3/4	

Annex III: Reference collection of sardine (*Sardina pilchardus*) POF daily cohorts.

Sardine Day-0	Sardine Day-0	Sardine Day-0
Sardine Day-1	Sardine Day-1	Sardine Day-1
Sardine Day-2	Sardine Day-2	Sardine Day-2
Sardine Day-3	Sardine Day-3	Sardine Day-3

Annex IV: Reference collection of anchovy (*Engraulis encrasicolus*) POF daily cohorts.

Anchovy Day-0	Anchovy Day-1	Anchovy Day-2
	Anchovy Day-3	

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