

REPORT OF THE
Study Group on the estimation of Spawning Stock Biomass
of Sardine and Anchovy

Malaga, Spain
23–27 June 2003

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1 INTRODUCTION

1.1 Terms of Reference

During the ICES Annual Conference in Copenhagen, Denmark (89th Statutory meeting in October 2002) was decided that the second meeting of the Study Group on the estimation of the spawning biomass of sardine and anchovy (SGSBSA) would take place in Malaga (Spain) between 23-27/6/2003 (dates revised with the agreement of ICES) with the objective to:

- i) provide final 2002 DEPM estimates for sardine and anchovy in Atlantic European waters;
- ii) complete the review of previous sardine DEPM estimates and provide a clear and synthetic description of the uncertainties associated with each estimate;
- iii) decide the most appropriate timing of future DEPM surveys for the Atlanto-Iberian stock of sardine;
- iv) consult recent developments in the use of CUFES and GAMs to consider their most appropriate application in DEPM surveys and estimation;
- v) update work on egg stage-age models, POF dating and spawning fraction estimation;
- vi) revise the maturity ogive of sardine based on past and 2002 DEPM histological information, in order to clarify its appropriate use in analytical assessment;
- vii) develop protocols and criteria to ensure standardization of all sampling tools and survey gears.

1.2 Participants

The study group met in Malaga (Spain) from 23-27 June 2003 with the following participation:

Bernal, Miguel	Spain
Cunha, Emilia	Portugal
Ibaibarriaga, Leire	Spain
Franco, Concha	Spain
Ganias, Konstantinos	Greece (observer, part time)
Garcia, Alberto	Spain (part time)
Jimenes, Paz	Spain
Lago de Lanzós, Ana	Spain
Nunes, Cristina	Portugal
Pérez, José Ramón	Spain
Santos, Maria	Spain
Quintanilla, Luis	Spain (part time)
Silva, Alexandra	Portugal
Stratoudakis, Yorgos (Chair)	Portugal
Uriarte, Andres	Spain
Vila, Yolanda	Spain

1.3 Progress with respect to recommendations of previous meeting

Considerable progress has been made in relation to most of the recommendations of the previous Study Group meeting (Lisbon, 2001), mainly as a result of contributions from national and international research projects. The 2002 surveys for anchovy and sardine took place according to the plan set in the Lisbon meeting and with the financial support of the EU Program of National Sampling (priority 1) in Spain and Portugal respectively. All egg and adult data have been prepared and collated in time for the final analysis during the Malaga meeting. Statistical methodology for the most appropriate use of egg incubation data in stage/age models, the ageing of staged eggs and the modeling of daily egg production and mortality through GAMs have been developed as part of the EU project on GAMs (Study 99/080, <http://www.ruwpa.st-and.ac.uk/depm/index.html>) that was concluded in May 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). A new sardine incubation experiment was performed by IEO as part of national research activities, while the study of the seasonality in sardine spawning off Portugal and the comparison of macroscopic with microscopic stages of sardine maturity were performed as part of the research project PELAGICOS (<http://ipimar-iniap.ipimar.pt/pelagicos>) at IPIMAR. Finally, novel work on the staging and dating of sardine post-ovulatory follicles and the estimation of spawning fraction was performed as part of a PhD thesis in Greece (Kostas Ganias, University of Thessaly) that was presented at the meeting. Within the report we present these advances, although it should be taken into account that much of this information forms part of manuscripts that are in the process of being submitted for publication.

1.4 Report structure

Chapter 2 is dedicated to the application of GAMs in DEPM estimation, summarizing and extending the findings of the recent EU project on GAMs (Study 99/080) that most SG members participated. Chapter 3 considers advances in other methodological aspects of DEPM estimation. The first part of this chapter 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. Chapter 4 provides a brief description of the 2002 DEPM surveys for sardine (Iberia) and anchovy (Bay of Biscay) and estimates egg production and stock biomass using the traditional methodology. Chapter 5 reviews survey details, data and estimation from all sardine DEPM surveys in Iberia to identify the best course of action for the use of these estimates in stock assessment. Chapter 6 briefly describes the ichthyoplankton sampling methodologies in DEPM and identifies minor issues where additional effort in the standardization of gear performance is required. Chapter 7 summarizes the main findings and conclusions with respect to the terms of reference for this meeting, while chapter 8 lists the recommendations resulting from it. At the end of the report (after the list of references) the abstracts of all working documents presented to the meeting are included.

2 GAMS IN DEPM ESTIMATION

This chapter is dedicated to the use of generalized additive models (GAMs) in DEPM estimation. Section 2.1 summarizes aspects of modeling that have received particular attention during the recent EU project on GAMs and section 2.2 briefly describes the most important methodological innovations resulting from this project. Section 2.3 demonstrates the use of GAMs in the estimation of egg production and compares GAM-based with traditional estimates for sardine and anchovy. Section 2.4 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 2.5 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 near future.

2.1 Introduction

GAMs have been proven to be a promising tool for modelling egg distribution and estimating egg production (e.g. Borchers et al. 1997; Bernal 1999; Stratoudakis et al. 2003), but several methodological problems remained to be solved prior to the use of the method in DEPM estimation (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 has been done on a relatively *ad hoc* basis with elements of subjectivity;
- ✓ modelling in a co-ordinate independent way in a GAM (fish don't know that we're choosing to use a latitude and longitude as co-ordinates – so 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 overdispersion 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 this SG 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 SG. 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 recently performed both during the above-mentioned EU project and afterwards by the SG members.

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

$$E [N_i] = g^{-1} (\text{offset} + s (\mathbf{x}_1 , \text{by} = P_0) + s (\mathbf{x}_2 , \text{by} = \text{age})) ,$$

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 ;

$s (\mathbf{x}_1 , \text{by} = P_0)$ = the smooth function that describes the relationship between egg production (on a log scale) and the set of covariates \mathbf{x}_1 (for example $s (\text{Lat}, \text{Long}, \text{by} = P_0)$);

$s (\mathbf{x}_2 , \text{by} = \text{age})$ = the smooth function that describes the relationship between egg mortality and the set of covariates \mathbf{x}_2 (for example $s (\text{Depth}, \text{by} = \text{age})$).

Estimates of *age* and N_i can be obtained directly from the samples, using an ageing procedure (section 2.2.1). 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 “wiggleness” of the smooth functions used is automatically chosen by the new GAM software using General Cross-validation (section 2.2.3). 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, $s (\text{Lat}, \text{by} = P_0) + s (\text{Long}, \text{by} = P_0)$) or higher dimension smoothers (for example, $s (\text{Lat} , \text{Long}, \text{by} = P_0)$) has to be chosen by the scientist. A brief description of the methods used to estimate egg production using GAMs is detailed in sections 2.2.1 to 2.2.5 below.

2.2 Methodology

2.2.1 Egg ageing

Estimating egg production in fish species that show fast egg development rates, such as sardines and anchovies, require 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 abundance of daily cohorts given their mean age (e.g. Piquelle and Stauffer 1985). As only egg stages, and not ages, are observed, 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 Pepin 1990), and to a lower degree on other parameters (see section 3.2.5). 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 known 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 3.2.4), 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 day, 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; $f_i(a | \tau)$. 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):

$$\hat{p}(a | s, \tau, T) \propto f_i(a | \tau) p(s | a, T)$$

and, thus, assign an age to every staged egg by sampling from the posterior distribution $\hat{p}(a | s, \tau, T)$ 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. 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 a 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 in 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 3.2.2 and 3.2.5 respectively.

2.2.2 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 4.1.1 and 4.2.1 for decisions in recent sardine and anchovy surveys respectively). Design-based analysis of these surveys allocates non-overlapping areas to each sample point (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 SG members and within the EU GAMs project. Assumptions and protocols to define survey area were unified and a software to semi-automatically define survey areas was produced within the project. The main characteristics of this software are described in section 2.2.5, 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 inlets like estuaries or Rias (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 2.2.5.

2.2.3 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 “wiggleness” 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 efficiency 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 wiggleness 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.

2.2.4 Model prediction and variance estimation

Predictions from a fitted GAM can be readily obtained using the software described in section 2.2.5 in either the sampled grid or in any regular grid (see section 2.2.2). 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 2.2.2).

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 SSB estimates 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 interval for SSB can in principle be obtained from a full GAM-based DEPM analysis (see section 2.4 and 2.5).

2.2.5 Software

Three packages that allow to fit 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 to fit 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 2.2.1);
- ✓ 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 2.2.1);
- ✓ 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 2.1.
- ✓ 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*, but are expected to be included in following versions.

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 2.2.2;
- ✓ 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.

2.3 GAM-based estimation of egg production

This section summarizes the methodology and main results from the fitting of GAMs to sardine (section 2.3.1) and anchovy (section 2.3.2) eggs from DEPM surveys. 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.

2.3.1 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 2.2.1 for age assignment that relies on data from an incubation experiment (see section 3.2.2.2) and an assumed daily probability density function of spawning ($\sim N(19,1)$, but also see section 3.2.4). 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 m²). This is 0.05 m² 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 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 subareas 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 stored in separate files for models fitted to resamples of both survey and incubation data or of only survey data (the difference between the two giving an indication of the variation due to ageing).

Table 2.3.1.1 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 noting that in the first 3 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 2 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 2.3.1.1 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 2.3.1.2). Table 3 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 2.3.1.1 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 2.3.1.3 shows the traditional and GAM-based estimates of egg production together with the estimated CVs. Figure 2.3.1.2 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 2.3.1.3 and Figure 2.3.1.2 show that in the three years that identical data sets were used for traditional and GAM-based estimation, 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 - Table 3) 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 low (but more realistic given the data) precision.

Figure 2.3.1.3 shows the fitted egg production in comparison to 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 et al. 2001; 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 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.

2.3.2 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 (*Efareg*): 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 is a correcting variable for standardization, which enters in 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 2.3.2.1 shows the number of observations (N), the number of knots (k) and the % 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 2.3.4.1 show 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 Gironde) 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:

- $s(\text{Long}, \text{Lat})$ for describing the spatial location. Two bivariate smooths, $s(\text{Long}, \text{Lat})$ and $s(\text{Alongdist}, \text{Perpdist})$, were available for representing the geographical location of the points. However, it was decided to take only one of them. Given the “rectangular form” of the Bay of Biscay the differences between both options were minor, but $s(\text{Long}, \text{Lat})$ was selected as the more natural one.
- $s(\text{Sst.fit}, \text{Sss.fit})$ for describing the environmental situation. Given that Sst and Sss were highly correlated, a bivariate smooth was considered.
- $s(\text{Log}(\text{Depth}))$. Depth was log transformed due to its skewed distribution.
- $s(\text{Log}(\text{Depth}), \text{Alongdist}/87)$ 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 depth gradient is very steep, being the 200 m depth contour line very close to the coast, whereas in the French coast Depth 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.

- $s(\text{Long}, \text{Lat})$ for describing daily mortality rate varying very smoothly in space

Models considered and correspondent names are shown in Table 2.3.2.2. All models were fitted using the package `depmodel` (section 2.2.5). 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 2.2.1, with the multinomial model for the distribution of stage given age and temperature as described in section 3.2.2.1 and spawning time distribution assumed to be normal with mean at 23 and standard deviation of 1.25 (section 3.2.4).

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 overdispersion. 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 2.3.2.3.

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 2.2.5).
- c) predicting on a regular grid (denser than the survey grid) using the sea area computed by geofun.

Table 2.3.2.4 shows the GAM-based total egg production estimates from model m4, together with the traditional DEPM estimates. 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 2.3.2.2 show 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 2.3.2.5 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. So, incubation experiment didn't add much variation to the final egg production estimate. Figure 2.3.2.3 shows the daily egg production estimates given by the traditional DEPM and by the selected 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 unbiased. The GAM method CVs are very plausible, between 13% and 21%, and are more reliable.

2.4 Adult DEPM parameters and SSB estimation through GAMs: 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.

2.4.1 Sardine

Responding to an SGSBSA recommendation (ICES 2002), 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 sections 4.1.1 and 4.1.3) 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 2.3.1 (apart from *Efarea*). *Alongdist* and *Perpdist* were estimated by geofun (section 2.2.5), 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 2.4.1.1 provides a summary of the model selected for each variable and Figure 2.4.1.1 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 2.4.1.2 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 2.4.1.2 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 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).

2.4.2 Anchovy

For anchovy, adult sampling in 2002 supplied a total of 35 samples (sections 4.2.1 and 4.2.3), 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. 1999). 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 automatically 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 2.4.2.1) 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 2.4.2.1 shows the standard checking for this later model whereas Figure 2.4.2.2 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. Figures 2.4.2.3 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 2.4.2.2). 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:

$$R' = \frac{w_f}{(w_f + w_m)}$$

Males have a slightly lower weight than females according to a fitted linear model (Figure 2.4.2.4).

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 (P_0): 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 GAM models for the estimation of P_0 are presented in Table 2.4.2.3.

Egg production surface (Figure 2.4.2.5) 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 2.4.2.4. 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 2.4.2.4 presents the final total egg production and SSB estimates for Bay of Biscay anchovy in 2002 and Figure 2.4.2.6 shows the spatial distribution of SSB.

2.5 Conclusions and recommendations for future applications of GAMs in DEPM estimation

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. As it has been shown during the SG meeting, GAMs can also be an effective means of estimating the distribution of adult parameters and SSB within in the spawning region, avoiding ad-hoc decisions on post-stratifications and providing the potential for additional improvements in the precision of the SSB 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 during the SG meeting 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 project and the application of the depmodel software during the SG meeting 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, this Group believes that GAMs are in a position to eventually substitute the traditional estimator as the recommended one 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 good understanding of the underlying fitting methodology, the software outputs and the dynamics of the species under study. For that, the Group recommends that a group of scientists meet before the next SG meeting to perform GAM-based estimation of egg production and SSB for all years for which data are available for anchovy and sardine. Final decisions on whether GAMs can safely substitute the traditional estimator in routine DEPM estimation will be taken during the next SG meeting in the light of the progress reported that far.

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 2.3.1.1 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	2298	9.892	9.585	47.6	0.0138 (0.0024)
1990	s(Lat, Long, 23) + s(log(Depth), Temp, 28) + age	1136	4.975	4.746	60.3	0.0191 (0.0042)
1997	s(Along, Perp, 18) + s(log(Depth), 4) + s(Temp, 8) + age	2049	11.580	11.404	49.9	0.0131 (0.0045)
1999	s(Along, Perp, 43) + s(log(Depth), Temp, 14) + age	2270	24.447	23.816	57.5	0.0057 (0.0035)
2002	s(Along, Perp, 54) + s(log(Depth), Temp, 18)	1994	11.778	11.347	43.2	0

Table 2.3.1.2 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 2.3.1.3 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 10¹² 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 2.3.2.1 Number of observations, number of knots and % deviance explained for the Sst and Sss models used in anchovy GAMs.

Year	Sea Surface Temperature			Sea Surface Salinity		
	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 2.3.2.2 Models considered and correspondent abbreviated names in GAMs of anchovy egg production.

Daily egg production	Mortality	
	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 2.3.2.3 Summary statistics of selected model (m4) for the estimation of anchovy egg production from 1996 to 1999.

Year	n	GCV	Scale	% Deviance	Z
1996	1118	9.562	8.923	8.923	0.014
1997	1736	8.569	8.173	8.173	0.010
1998	1899	14.563	14.001	14.001	0.007
1999	899	7.924	7.283	7.283	0.007

Table 2.3.2.4 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 2.3.2.5 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.

	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 2.4.1.1 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 2.4.2.1 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 2.4.2.2 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 2.4.2.3 Summary statistics of the fitted GAMs for anchovy daily egg production.

	Model	n	GCV	Scale	% Deviance	Z
model 0	s(Long,Lat)	1494	8.8712	8.7069	57.7	0.0077
model 1	s(Long,Lat) + s(Sst, Sss)	1494	8.2974	8.2974	64.6	0.0080

Table 2.4.2.4 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	29681153393
model 1	2.420E+12	2.418E+12	2.305E+12	29655692907

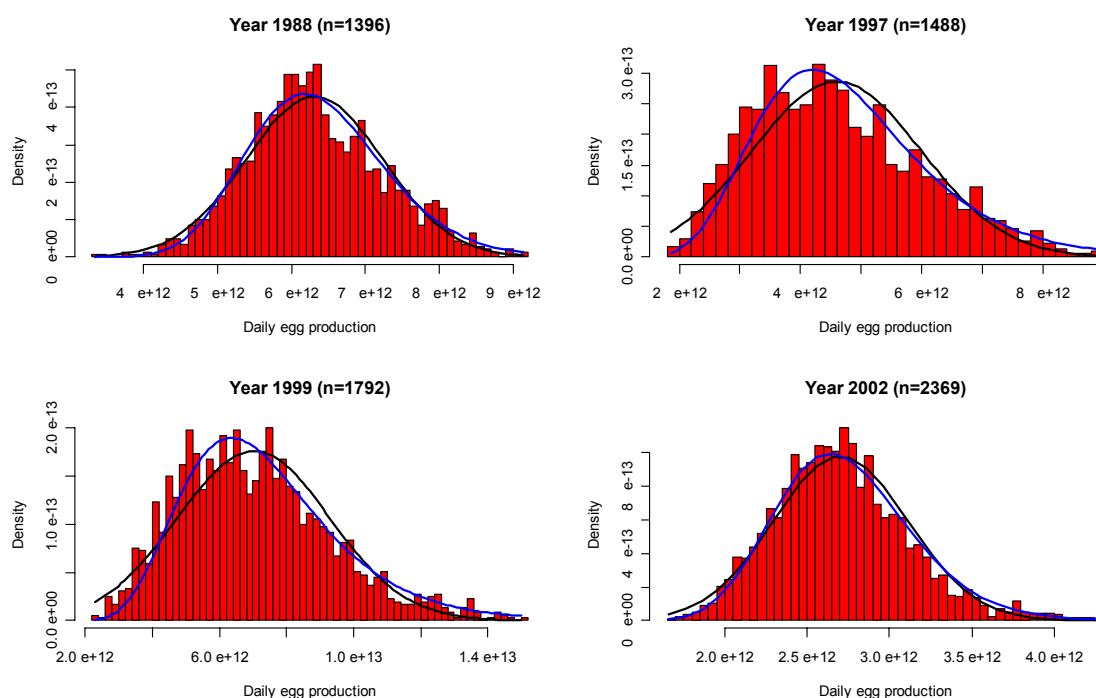


Figure 2.3.1.1 Distribution of bootstrap estimates of sardine daily egg production, after removing observations more than 3 standard deviations larger than the bootstrap mean. Continuous black/blue line corresponds to normal/log-normal density for a mean/log-mean and standard deviation equal to that of the bootstrap sample.

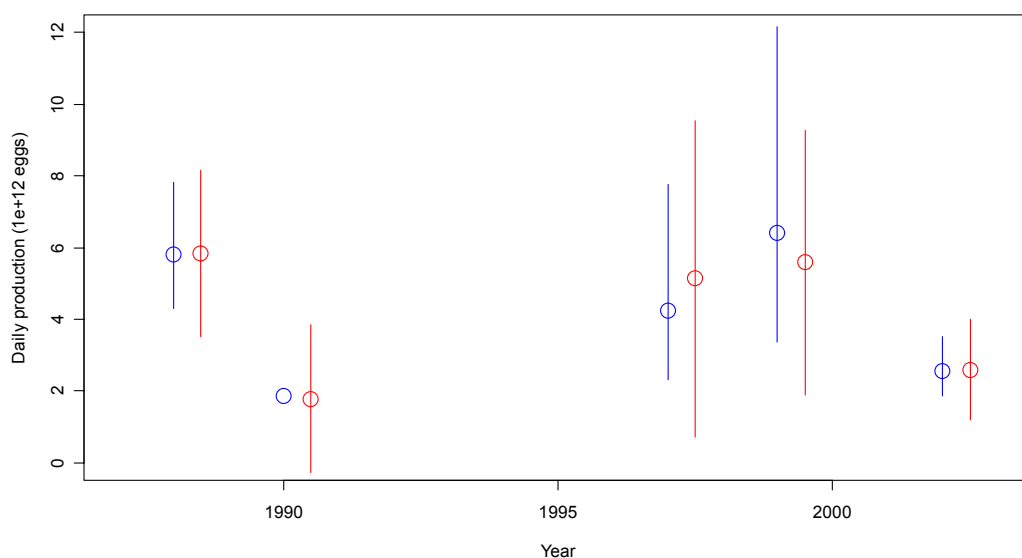


Figure 2.3.1.2 Time-series of estimates of sardine daily egg production based on the traditional method (red) and the new GAM method (blue). Lines are estimated 95% confidence intervals (CIs), which for the traditional method are based on an assumed normal distribution and in the GAM-method on a log-normal 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.

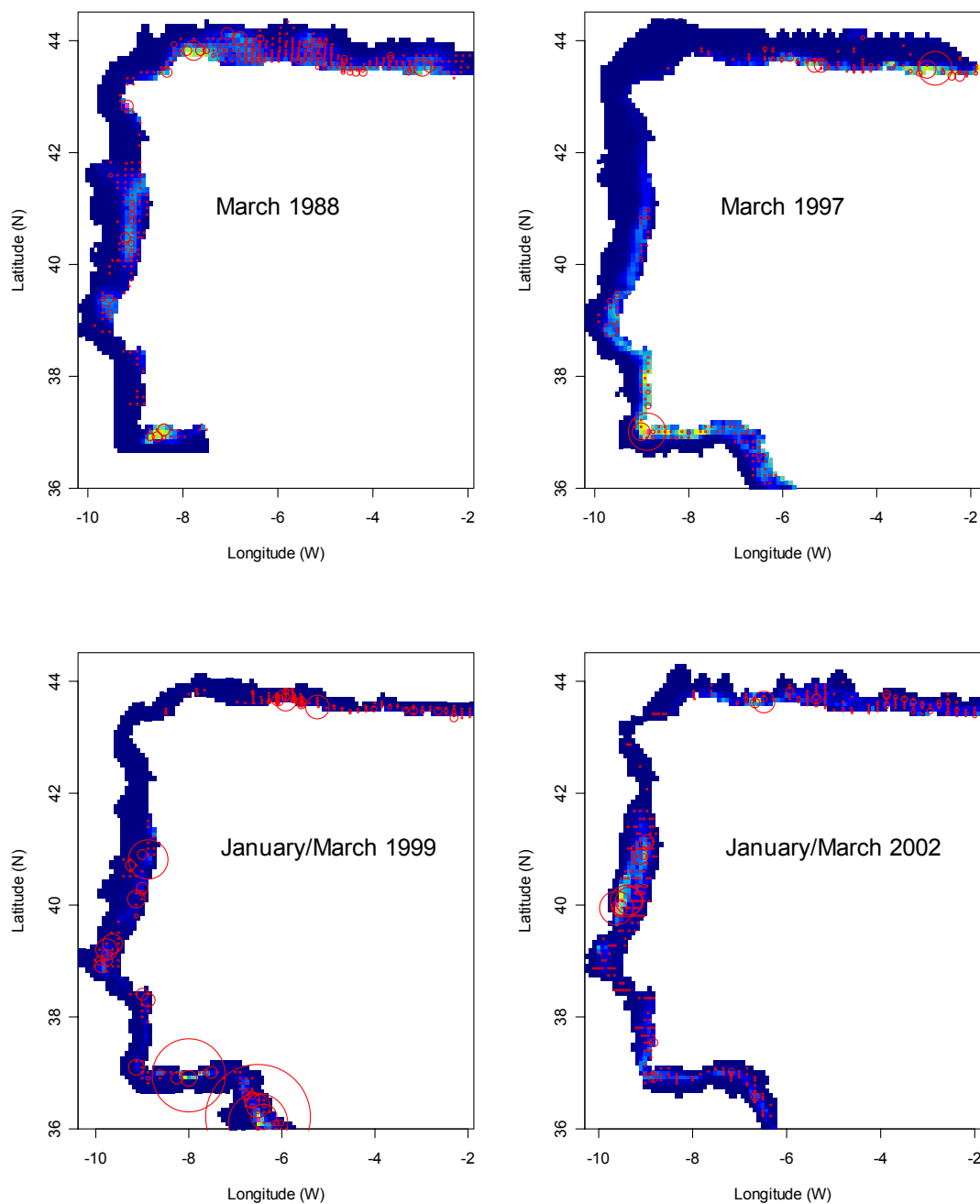


Figure 2.3.1.3 Modelled distribution of daily egg production (colour scale) and observed total number of sardine eggs (red circles) during the 1988 (upper left), 1997 (upper right), 1999 (bottom left) and 2002 (bottom right) Iberian DEPM surveys. Diameter of circles is proportional to the total number of eggs in a station.

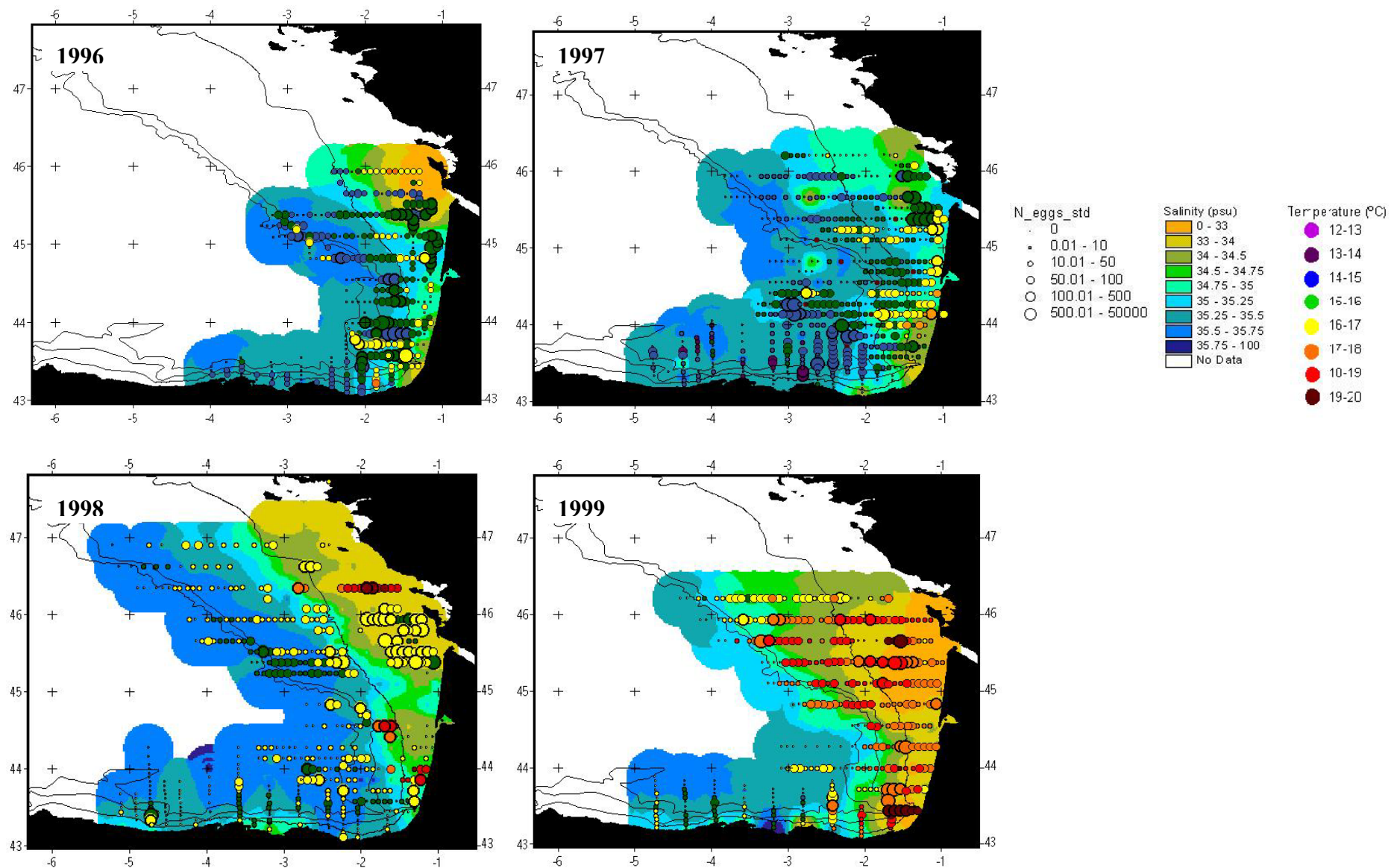


Figure 2.3.2.1: Environmental situation and egg abundance for 1996-1999 surveys. Background colour represents the sea surface salinity. Circles correspond to survey stations. Size of the circles is proportional to egg abundance and colour of the circles is given according to the sea surface temperature at each station.

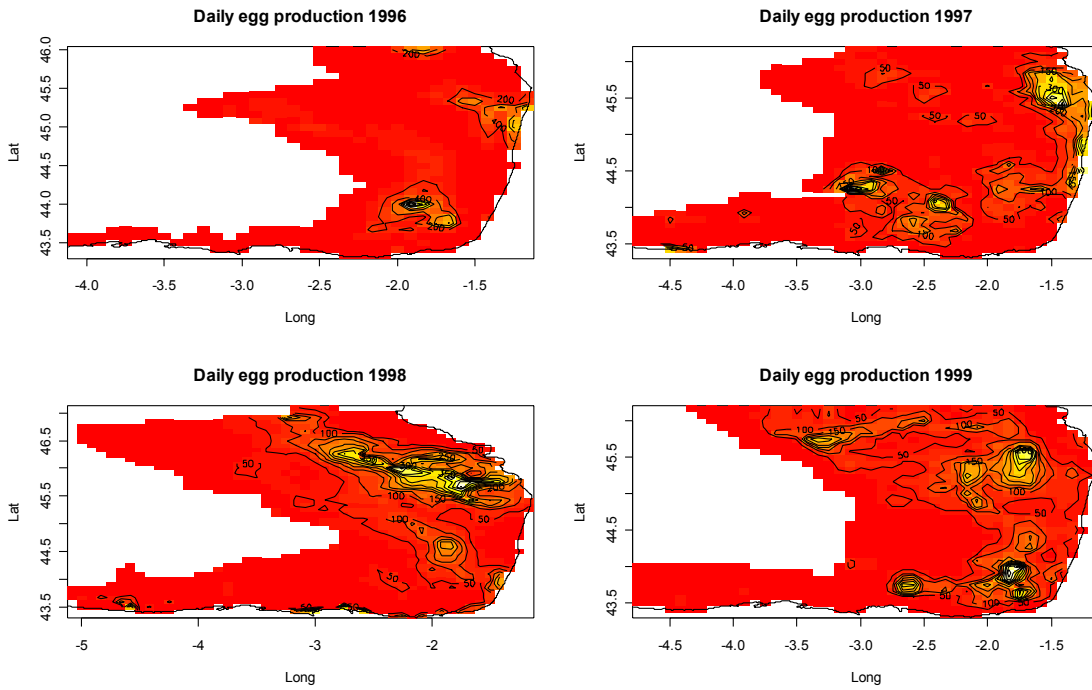


Figure 2.3.2.2 Fitted daily egg production surface image and contour plot for model m4 from 1996 to 1999 fitted to anchovy eggs.

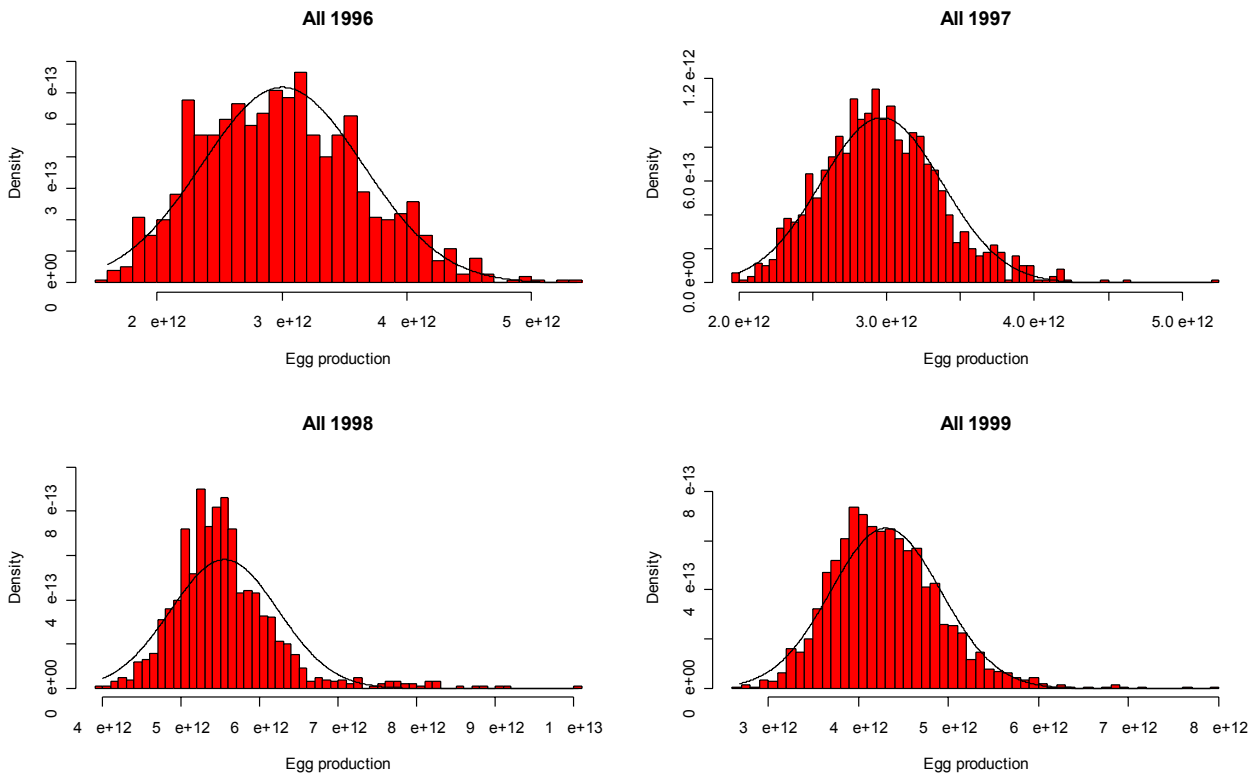


Figure 2.3.2.3 Histogram of the total egg production bootstrap estimates considering re-sampling on both the incubation experiment and survey data. Line represents the density function of a normal distribution with the observed bootstrap estimate mean and standard deviation.

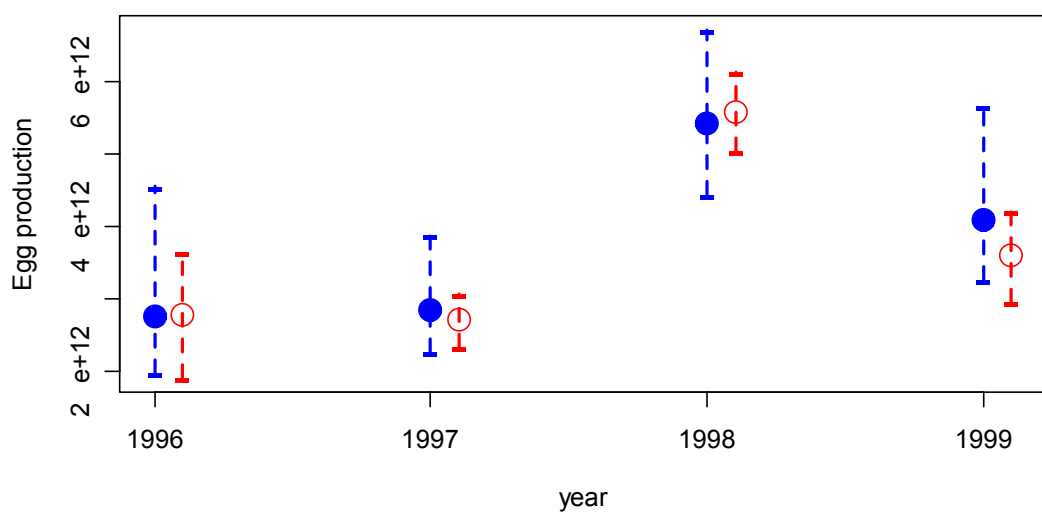


Figure 2.3.2.4 Time-series of estimates of anchovy daily egg production with estimated 95% confidence intervals (dashed line) based on the traditional method (red; assuming normality), the new GAM method (blue; assuming normality).

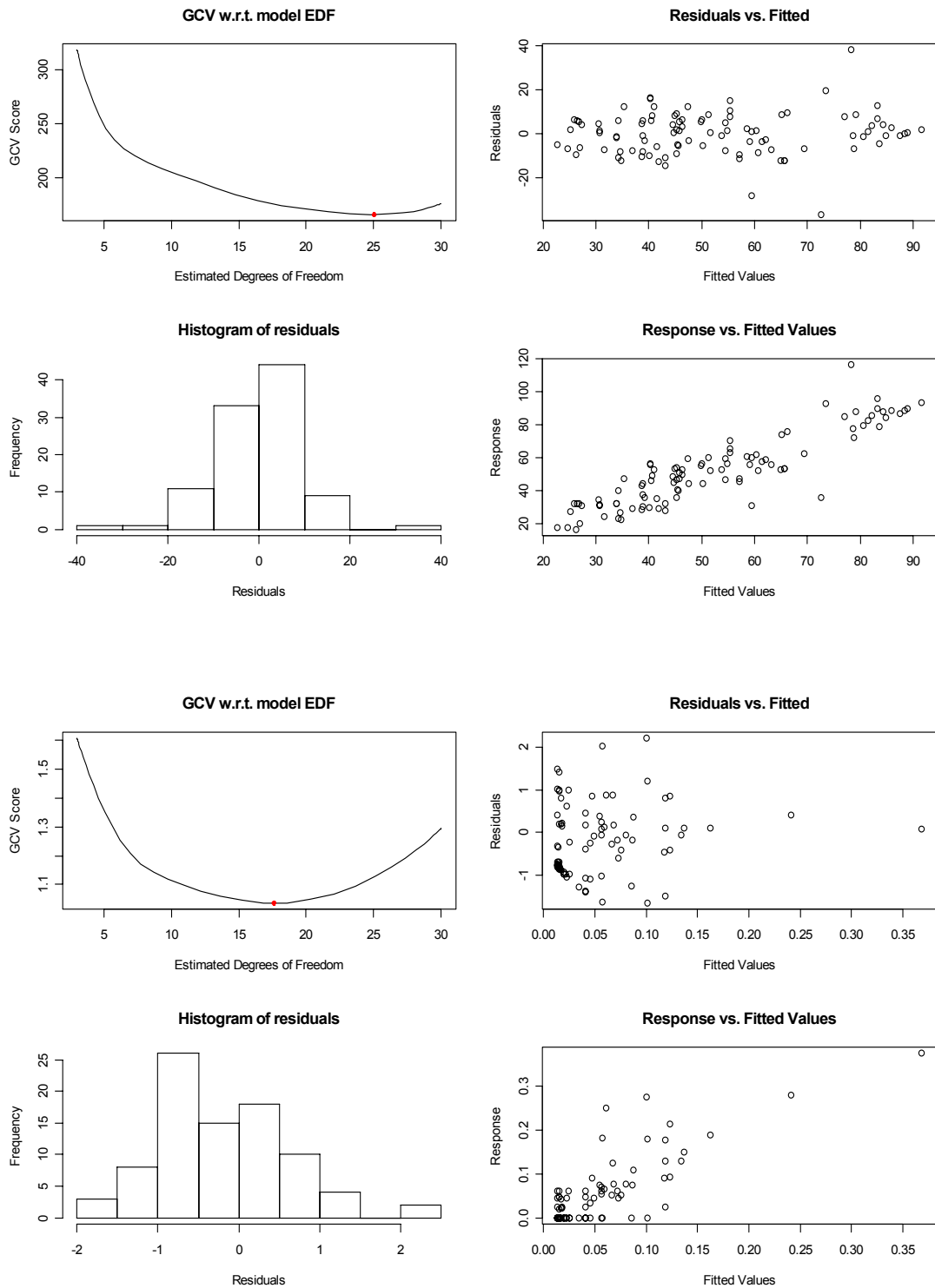


Figure 2.4.1.1 Inspection of residual plots testing the adequacy of the GAMs fitted to mean female weight (top) and spawning fraction (bottom) of sardine from the 2002 Iberian DEPM survey.

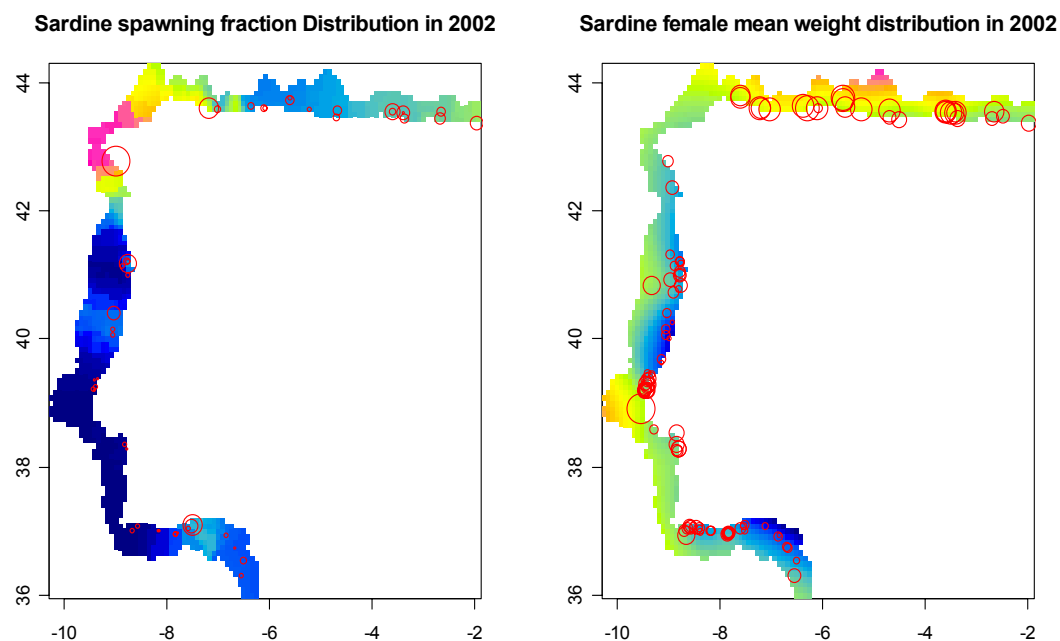


Figure 2.4.1.2 GAM-fitted (colour scale) and observed (circles) sardine spawning fraction (left) and female mean weight (right) from the 2002 Iberian DEPM surveys.

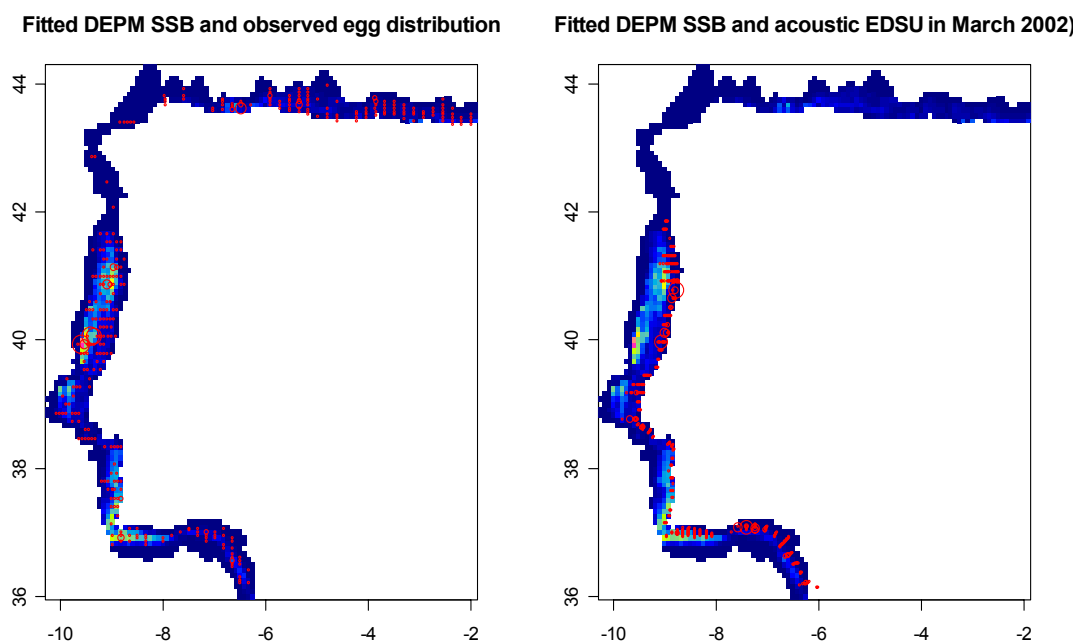


Figure 2.4.1.3 GAM-fitted (colour scale) spawning stock biomass against observed egg density (red circles, left) and acoustic energy attributed to sardine (red circles, right). SSB and egg densities refer to the 2002 Iberian DEPM survey, acoustic data refer to the March acoustic survey off Portugal.

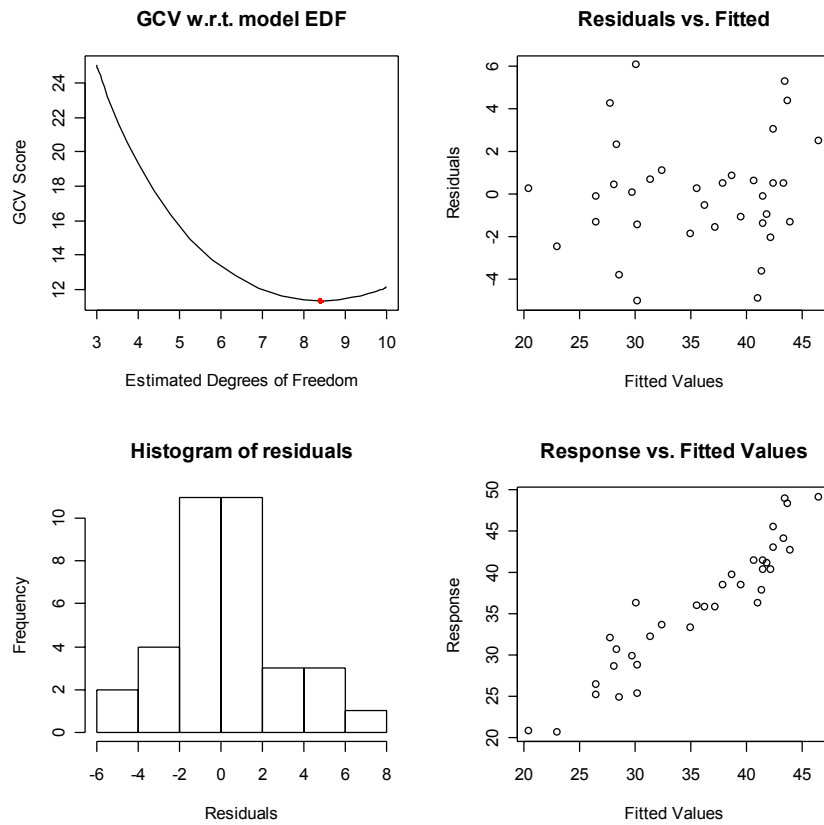


Figure 2.4.2.1 Standard checking plots for the fitted GAM model on anchovy female weight (allowing a maximum of 10 knots).

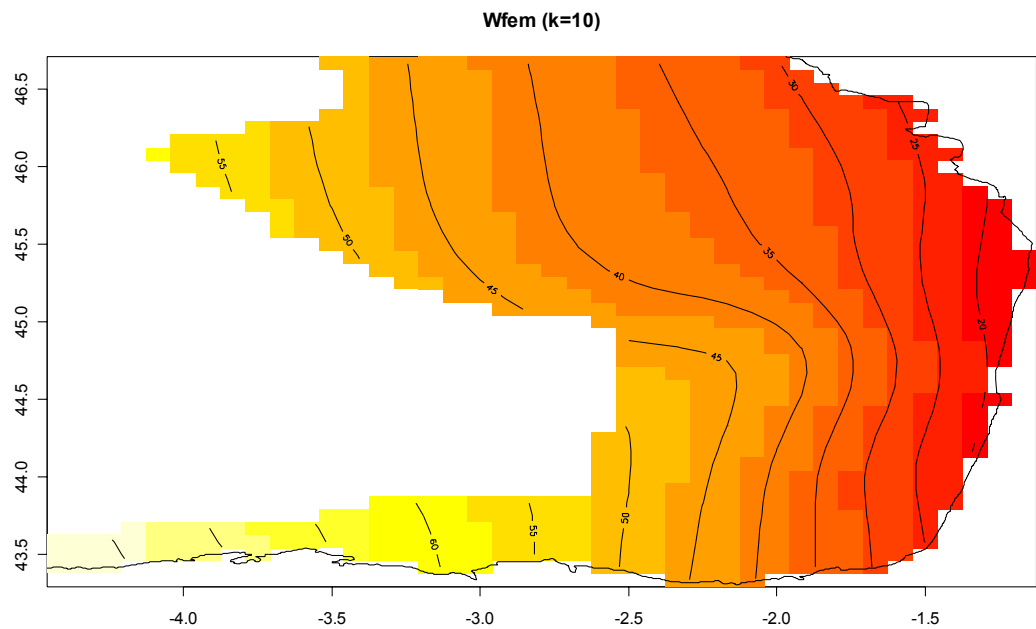
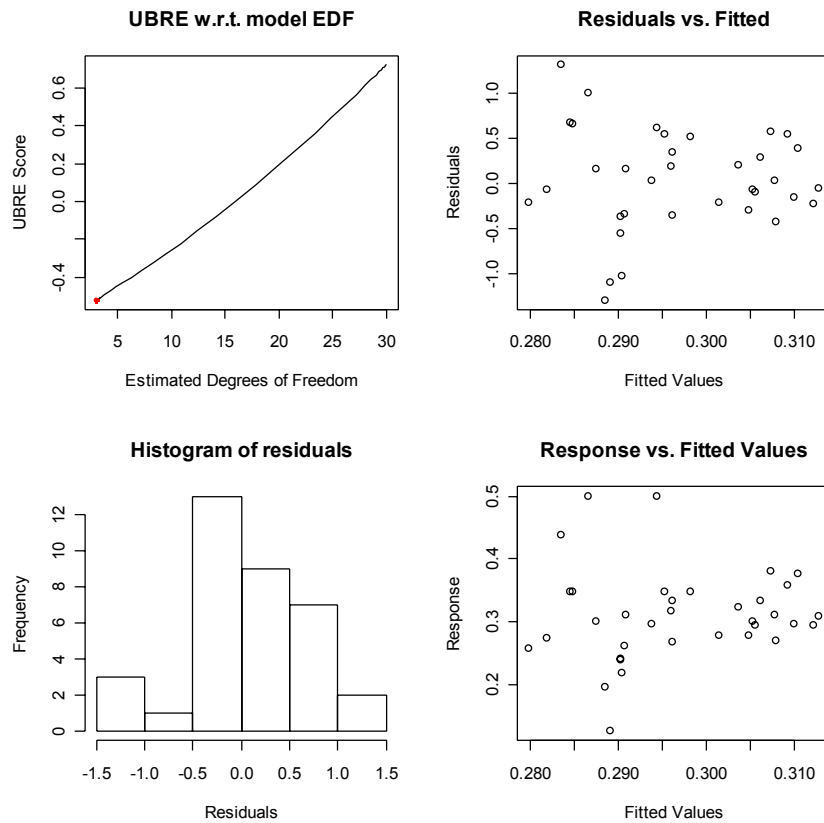


Figure 2.4.2.2 Fitted female mean weight surface for anchovy in 2002 (allowing a maximum of 10 knots).



Figures 2.4.2.3 Standard checking figures of the fitted model for anchovy spawning fraction in 2002.

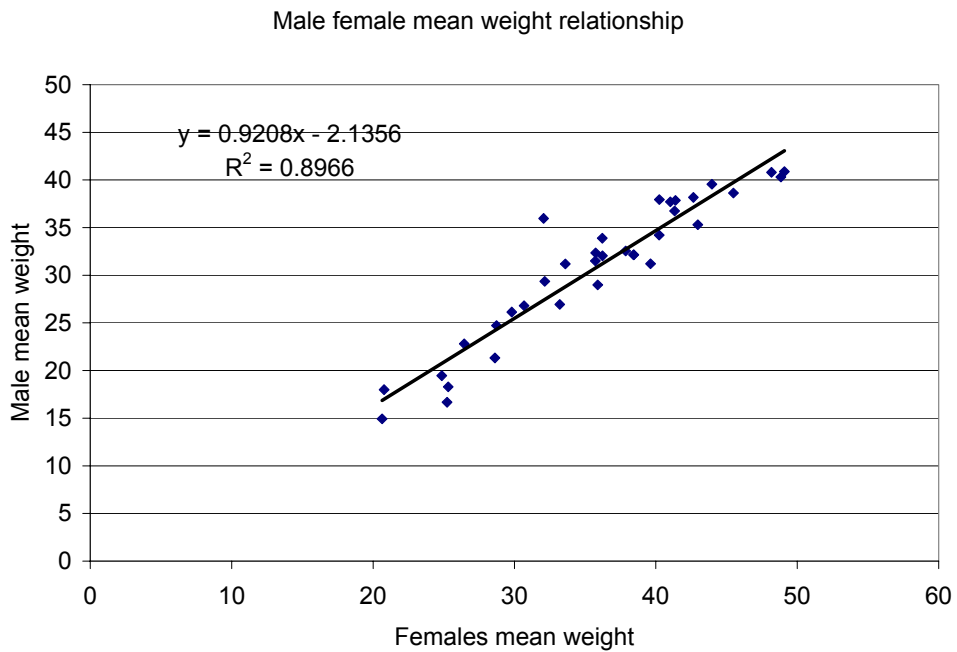


Figure 2.4.2.4 Fitted linear model of anchovy male weight as a function of female mean weight.

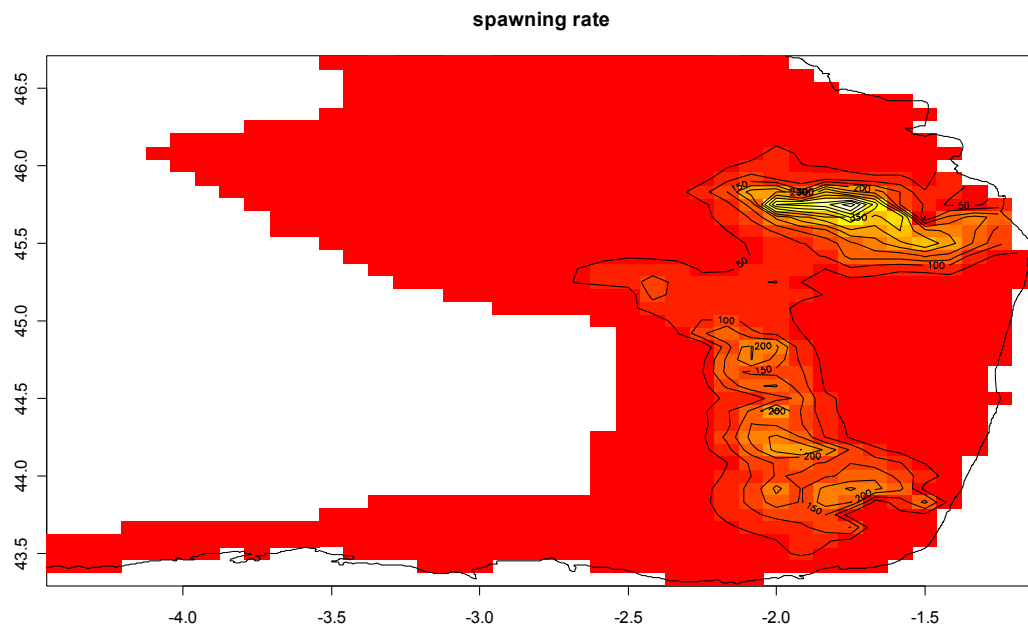


Figure 2.4.2.5 Fitted daily egg production rate for the Bay of Biscay anchovy in 2002.

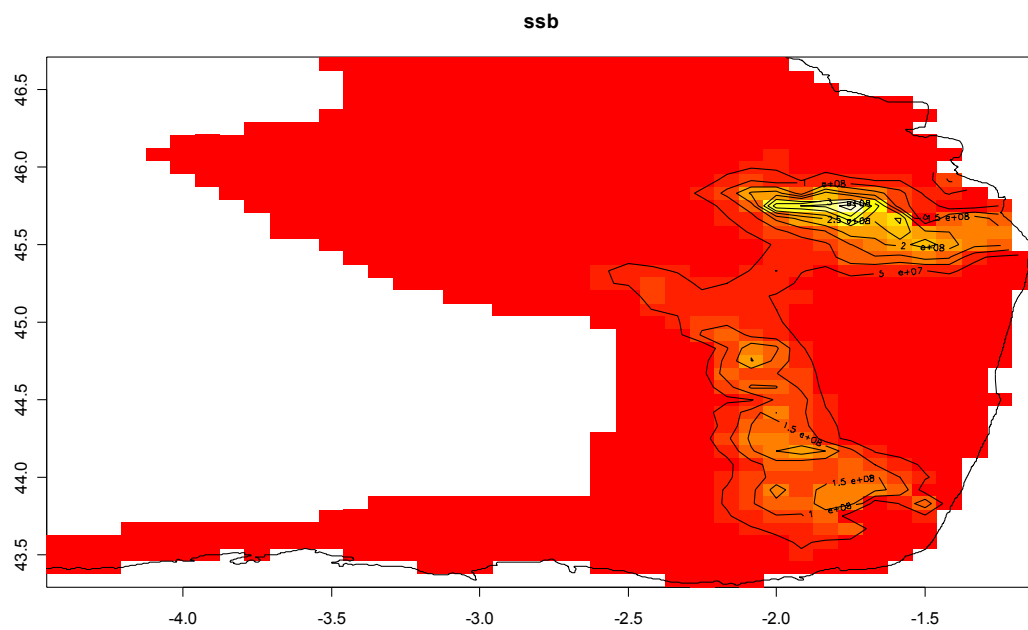


Figure 2.4.2.6 Fitted SSB surface for the Bay of Biscay anchovy in 2002.

3 OTHER ADVANCES IN DEPM METHODOLOGY

This chapter includes research topics related to DEPM (all forming part of the terms of reference for this meeting) that have advanced since the last SG report (ICES 2002), but for which the developments have not been that far-reaching to warrant entire chapters. Section 3.1 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 3.2 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 modeling such data (see section 2.2.1) and using them in the ageing of staged eggs. Section 3.3 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 3.4 is dedicated to spawning fraction estimation and the dating of post-ovulatory follicles, based on new data from sardine spawning populations in the northeastern Atlantic (Iberian peninsula) and the eastern Mediterranean (Aegean and Ionian Seas).

3.1 The Continuous Underway Fish Egg Sampler (CUFES)

3.1.1 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. 1999), 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). However, CUFES cannot yet be used as the main sampler for DEPM purposes, given that it is currently impossible to transform satisfactorily volumetric to aerial (i.e. vertically integrated in the water column) estimates of egg abundance. Section 3.1.2 describes recent advances in the comparison of CUFES with vertical plankton samplers, section 3.1.3 summarizes the use of CUFES in the most recent sardine DEPM survey, while section 3.1.4 highlights other potential uses for this sampler. Finally, section 3.1.5 synthesizes the opinion of the group on the potential use of CUFES in the DEPM for the near future.

3.1.2 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 3.1.2.1), 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 (section 3.1.2.2).

3.1.2.1 CUFES and CalVET/PAIROVET experimental sampling

Stratoudakis et al. (2001) presented the first 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 min 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 3.1.3). 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*).

However, CUFES gives a poor representation of egg abundance in the water column, 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 3.1.2.1.1). This is consistent with previous observations made on the same vessel for anchovy (Bez, 2000). This effect was also noted by Van der 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 3.1.2.1.1). 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 be mostly based on broad staging groups (but see also section 3.1.3).

3.1.2.2 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, William 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. *in press*) 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. (*in press*) 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 3.1.2.2.1 and 3.1.2.2.2). 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 relationships 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.*).

3.1.3 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 4.1.1). Table 3.1.3.1 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 25%, with 6% only indicating 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 3.1.3.1 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 sections perpendicular to the coast, where both samplers were used simultaneously. A total of 54 samples were obtained with CalVET and 54x3 with CUFES (following the sampling design used in PELASSES). Comparison of performance of CUFES at, before and after the CalVET stations (Table 3.1.3.2) show a high degree of within-CUFES consistency, with agreement ranging from 85 to 96%. Comparison between CalVET and CUFES (Table 3.1.3.3) 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 outperform 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 3.1.2.1 (no embryo, early embryo, late embryo and disintegrated) and another using the standard 11 stages described by Gamulin and Hure (1955). 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 3.1.3.2 and 3.1.3.3).

3.1.4 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 very 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 3.1.4.1). 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 3.1.4.1 would probably be covered by 6 – 9 CalVET stations, where (depending on the location) eggs from either or both cohorts would have been detected.

3.1.5 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 encountered in stratified environments, and further work is undergoing. In addition, CUFES shows a marked selectivity of anchovy eggs (see section 6.2), 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.

3.2 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 3.2.1 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 3.2.2 describe the application of new methods of analysis to old and new egg incubation data for sardine and anchovy, while section 3.2.3 identifies factors other than temperature that may also affect pelagic egg development. Finally, section 3.2.4 reviews existing information on the daily spawning activity of sardine and anchovy, highlighting its importance in egg ageing.

3.2.1 Staging workshop

During the previous meeting of the SG, an informal workshop was performed to assess the consistency in sardine and anchovy 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 3.2.1.1 to 3.2.1.3). 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 3.2.1.4).

3.2.2 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 2.2.1) and the traditionally used ageing method (Lo 1985) were both applied to analyse 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):

$$\bar{a}_{i,t} = \theta_0 e^{\theta_1 t + \theta_2 i} i^{\theta_3} \quad (1)$$

where t denotes temperature, i stage, $\bar{a}_{i,t}$ 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 $\bar{a}_{i,t}$ 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, 1992; 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 2.2.1 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 `depmodel` (section 2.2.5).

In the case of anchovy (section 3.2.2.1), 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 3.2.2.2).

3.2.2.1 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 different stations, were performed, each 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 3.2.2.1.1). 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 3.2.2.1.1), and assuming that there was no difference between incubations, data were gathered and analysed jointly. The observed (age, stage) data are shown in Figure 3.2.2.1.1. Expected values from Lo's model for the observed temperatures and for all stages are shown in Figure 3.2.2.1.2. 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 3.2.2.1.3).

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.

3.2.2.2 Sardine

A new incubation experiment for sardine was successfully carried out by IEO in 2002, following the recommendation of the previous SG meeting (ICES 2002). The incubation experiment was performed according to that described for anchovy (Motos 1994 and section 3.2.2.1), using the same incubator. Eggs were obtained from a survey onboard *RV Vizconde de Eza* in the Gulf of Cádiz (SW Spain) between 10-17/2/2002. 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 3.2.2.2.1). 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 3.2.2.2.1), 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 re-filled 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 3.2.2.2.1. Figure 3.2.2.2.2 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 3.2.2.2.3, 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 and 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 3.2.2.2.1), 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.

3.2.3 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 and southern calamary respectively. In the case of pelagic fish eggs, egg size is known to affect mortality rates (Pepin 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, Vereja 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 knowledge of this SG, no incubation experiments have so far 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 2.2.1, 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.

3.2.4 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 2.2.1) 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 1990-92, 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 3.2.4.1 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 maximums between 23 and 24 hours and particularly between 23.5 and 24 hours. The range of spawning time 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 to 5 in the morning.

Figure 3.2.4.2 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 $\sim N(23, 1.25)$.

In the case of sardine, existing DEPM applications have assumed a 19:00 GMT peak in spawning activity (Cunha et al. 1992, Garcia 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 3.2.4.3). 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 3.2.4.4) 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. 2003). Figure 3.2.4.2 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 $N\sim(19,1)$). 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 geographical differences (the data of Ganiyas et al. (2003) are from the Aegean Sea) may partly contribute to the observed discrepancies.

3.3 Sardine reproduction off Iberia

This section reviews information on the reproductive activity of sardine off Iberia. Section 3.3.1 describes sardine maturation, its classification and appropriate use for spawning biomass estimation, while section 3.3.2 identifies metrics of spawning activity and reports recent data on the seasonal dynamics of sardine spawning off Portugal.

3.3.1 Sardine maturity

In recent years, the macroscopic classification of sardine maturity for various research purposes at IEO and IPIMAR has been based on maturity scales adapted from Pinto and Andreu (1957 - described in Table 3.3.1.1 and Figure 3.3.1.1). 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 3.3.1.1) and macroscopic maturity stages are compared to the microscopic aspect of sardine gonads (section 3.3.1.2). Finally, section 3.3.1.3 synthesizes the findings from these comparisons to identify the best methodology currently available for the coherent estimation of SSB in surveys and stock assessment.

3.3.1.1 Clarification of definitions

It was realised during the meeting that 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: virgin and adult individuals that have clear signs of gonad development or reproductive activity (i.e. individuals within their spawning season).

The third set of definitions refers exclusively to mature individuals within the reproductive season, separating those that have ceased their activity (or even not succeeded in beginning) for the current season from others.

Criterion: spawning activity, refers to reproductive season (see also section 3.3.2.1);

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 mobilise sufficient energy resources to complete gonad development and release even the first batch of oocytes).

3.3.1.2 Comparison of macroscopic and microscopic maturity stages

In order to evaluate the criteria of macroscopic identification (Table 3.3.1.1), the macroscopic and microscopic stages of 177 sardine ovaries collected during an acoustic survey (Portuguese November 2000 survey - Silva et al. WD 2003) 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 3.3.1.2.1 and 3.3.1.2.2 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 3.3.1.2.2). 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 3 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 3.3.1.2.2, 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 3.3.1.1), in order to perform additional comparisons using also data collected in other periods of the annual reproductive cycle. However, it should be noted that in the current use of this scale, the “partial post-spawning” and “ultimate post-spawning” stages will continue to be numbered differently by the two institutes:

	IEO	IPIMAR
“partial post-spawning”	V	V-III
“ultimate post-spawning”	VI	V

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.

3.3.1.3 Maturity stages to be included in the estimation of SSB

According to the definitions in section 3.3.3.1 and the microscopic characteristics of maturation stage II (Table 3.3.1.1), 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 3.3.1.2 should be taken into account in future DEPM sampling.

3.3.2 Spawning season and dynamics

3.3.2.1 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 artesias (Pérez and Figueiredo 1992). For example, Hunter and Macewicz (1985) define a female anchovy (*Engraulis 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. (*submitted*) 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 3.3.2.1.1 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 3.3.2.1.2) 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.

3.3.2.2 Spawning seasonality and 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 4.1.3). 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 72 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 (section 4.1.3). Further,

unlike previous surveys, more than 10% of the macroscopically classified female gonads were at stage V (post-spawning), and this inactivity was confirmed by microscopic examination (see Table 3.3.1.2.2). 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 3.3.2.1), 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 3.3.1.1). Finally, the mean female gonadosomatic index (GSI) was uncharacteristically low in January 2002 and its evolution along the 2001/2002 spawning season (Figure 3.3.2.2.1) confirms a departure from the typical seasonal pattern described for previous years.

Cunha et al. (WD 2003) describe 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 (Figure 3.3.2.2.2). 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 3.3.2.2.3 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 3.3.2.2.4). 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 (see Figure 2.4.1.3). 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 northeastern Atlantic, Q5RS-2002-000818). Within this project, literature information and recent data on sardine spawning areas and seasons will be compiled from the northeastern 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. Advances in these areas will be reported to the next meeting of this Study Group.

3.4 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 & 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 4 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 apoptotic 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 in 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. A similar study was performed for sardine (Pérez et al. 1992), 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 2000) 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 fraction probably consist the only aspect of DEPM estimation where 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 3.4.1 considers the issue of sampling bias due to differences in the catchability of the spawning component of the population, while section 3.4.2 reviews the issue of POF staging and aging in the light of the methodology currently adopted for staging and aging of pelagic fish eggs.

3.4.1 Sampling bias

A common feature in the spawning behavior of most epipelagic 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. 2003) segregation of females with imminent and/or recent spawning activity (so called Day-0 females) surrounded by large numbers of males (Alheit 1984, Picquelle and Stauffer 1985, Ganias et al 2003). 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 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. (2003) 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. 2003). 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.

Taking into account the above considerations, it seems that accuracy and precision in spawning fraction estimation may be improved by setting some principles for sampling and laboratory analysis:

- ✓ Sampling should be conducted outside the hours of the spawning act in order to avoid overestimations of Day-0 spawners. Unbiased and thus reliable fractions of Day-0 females offer an extra estimator in the composite estimates of S .
- ✓ Sampling should be conducted in hours that females with POF-0 that spawned the previous night do not co-occur with hydrated females (which are supposed to spawn the night of sampling). At least for the Mediterranean sardine the lack of hydrated females in DEPM samples does not seem to create problems, because fecundity measurements may also be based on females at the tertiary and at the migratory nucleus stage of development (Ganias et al. *in press*).
- ✓ When the incidence of spawning is age/size dependent, samples used for the estimation of spawning frequency should have the same age/size structure with the assessed population

3.4.2 POF staging and aging

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 2.2.1) 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. Ganas et al. (2003) 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, Ganas et al. (2003) 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 3.2). 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 3.2.2.

The above information could then be used with the spawning probability density function (section 3.2.4) 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 2.2.1 and 3.2.2.

Table 3.1.2.1.1 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		CD (R2 adjusted)	Stand.Error of Reg.
	Common Value	P(b00=b01)	Difference	P(Dif=0)		
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 3.1.3.1 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 3.1.3.2 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 3.1.3.3 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 3.2.1.1 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
1 st reading	82.1	89.1	71.5	77.5
2 nd reading	92.5	96.8	86.4	85.7

Table 3.2.1.2 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 (%) Stages	Anchovy		Sardine	
	1 st staging	2 nd staging	1 st staging	2 nd 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 3.2.1.3

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	
	1 st staging	2 nd staging	1 st staging	2 nd staging
1	89	97	60	84
2	63	81	78	-
3	90	91	99	100

Table 3.2.1.4

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	1307	19	19
	S	516	0	0	3922	0	0
1990	S	475	0	0	1494	0	0
1997	P	373	50	2	1454	179	4
	S	515	0	0	1465	0	0
1999	P	413	57	2	5110	1191	7
	S	402	16	0	2340	28	0
2002	P	484	19	5	2585	72	6
	S	313	21	4	1939	33	4

Table 3.2.2.1.1

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 & 3	8.49	10.79	13.13	15.5	17.81	20	21.81
	(0.05)	(0.05)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)

Table 3.2.2.2.1

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 3.3.1.1 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 flacid 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 hemocytes present spread all over the ovary tissue (light hemocyte infiltration). Presence of all oocyte stages is possible.
VI	ULTIMATE POST-SPAWNING	Very deflated and flacid ovaries occupying from about 3/4 to 1/4 of body cavity; with many ruptured blood vessels that gives them a reddish color. Some small opaque oocytes can be visible (no hyaline oocytes are present)	Post-ovulatory Follicles may be present. Many ruptured blood vessels and free hemocytes invading the ovary tissue (generalised hemocyte infiltration). No yolked oocytes, or most of them in an atretic state.

Table 3.3.1.2.1 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 3.3.1.2.2 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

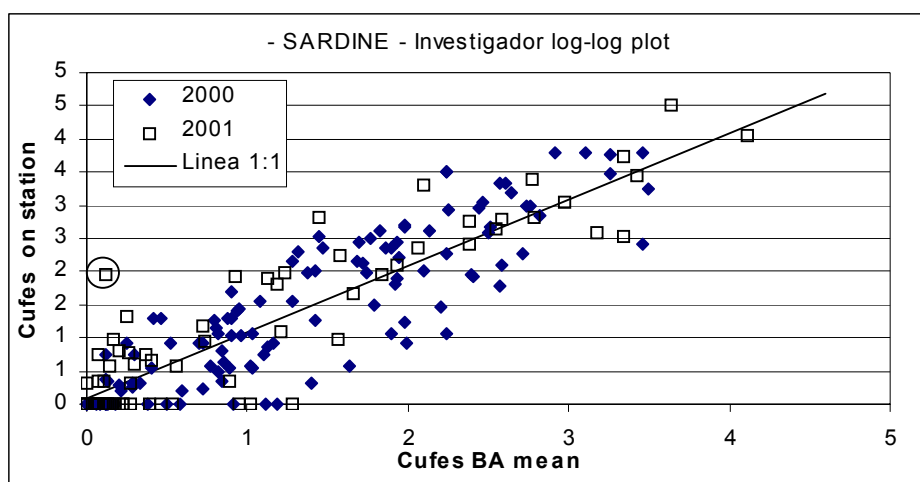


Figure 3.2.1.1.1 Comparison of CUFES sardine egg densities (eggs/m³, log-scale) in underway (before and after the PAIROVET haul - CUFES BA mean) and stationary (4 min during the PAIROVET haul (CUFES on station) samples from two years (2000 and 2001). Line is 1:1 relationship and encircled points are removed outliers (from Uriarte et al. 2003).

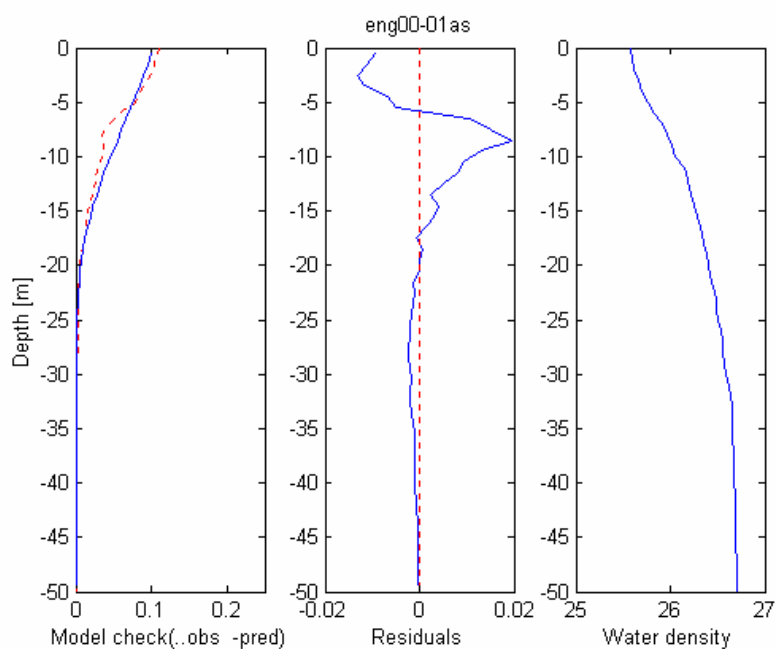


Figure 3.12.2.1 Example of vertical egg distribution fitting to the average of LHPR hauls from the sampling in 2000 and 2001 for anchovy in waters of high salinity at surface (Boyra et al. *in press*).

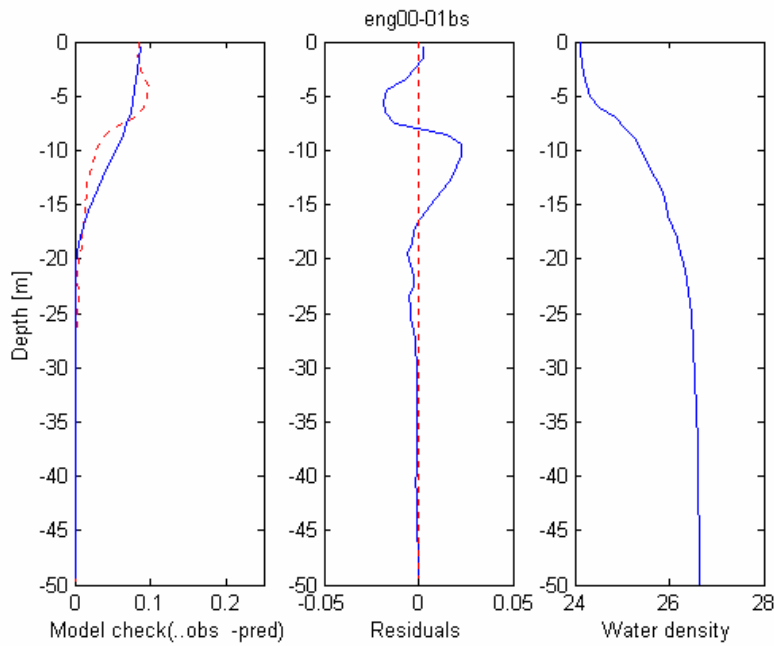


Figure 3.1.2.2.2 Example of vertical egg distribution fitting to the average of LHPR hauls from the sampling in 2000 and 2001 for anchovy in waters of Low salinity at surface (Boyra et al. *in press*).

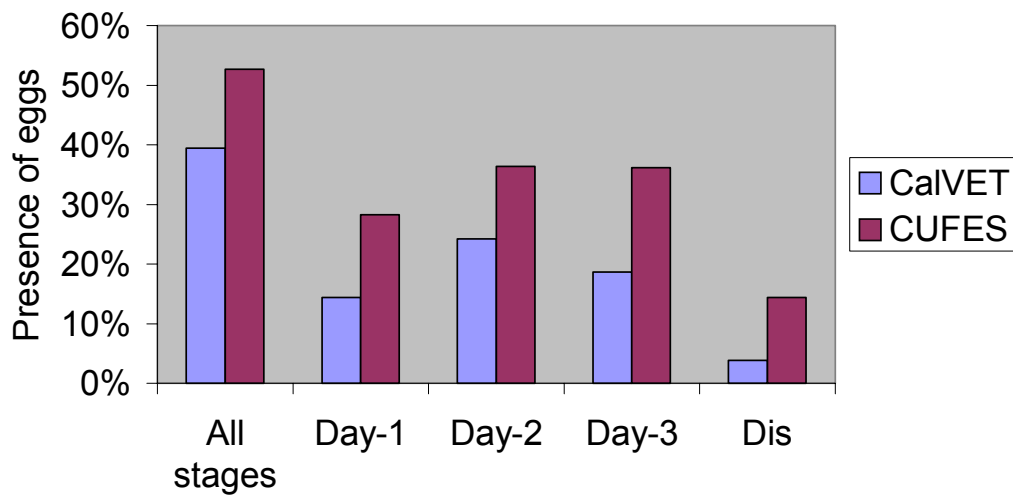


Figure 3.1.3.1 Comparison of the proportion of stations where sardine eggs (all stages and separately by broad categories of development) were detected by CalVET (blue columns) and CUFES (red columns) during the 2002 Portuguese DEPM survey.

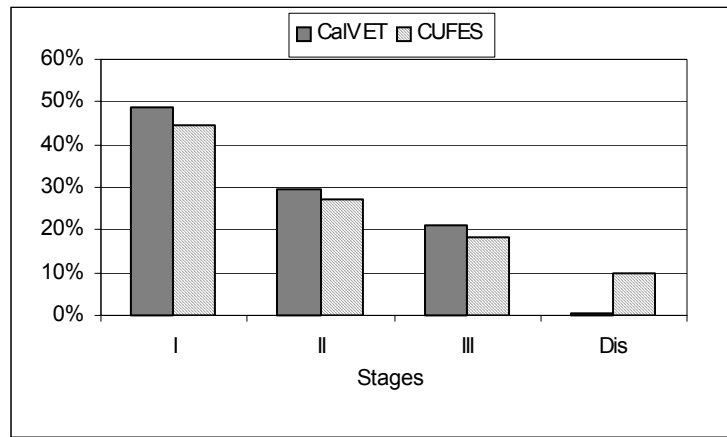


Figure 3.1.3.2 Percentage of sardine eggs at stage (broad stages for CUFES applications) in CalVET and CUFES in the calibration exercise performed during the Spanish 2002 DEPM survey.

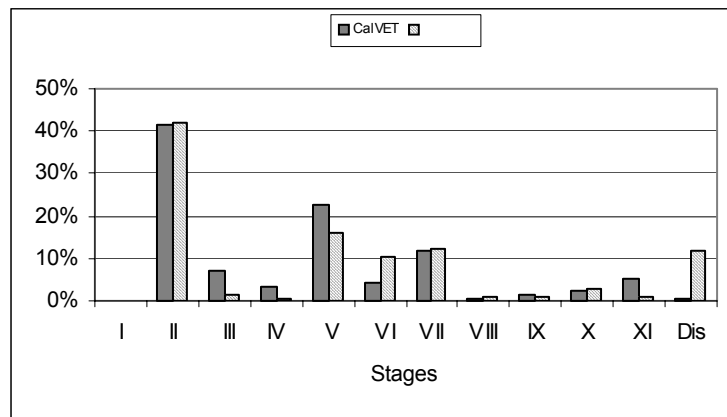


Figure 3.1.3.3 Percentage of sardine eggs at stage (standard stages for DEPM applications) in CalVET and CUFES in the calibration exercise performed during the Spanish 2002 DEPM survey.

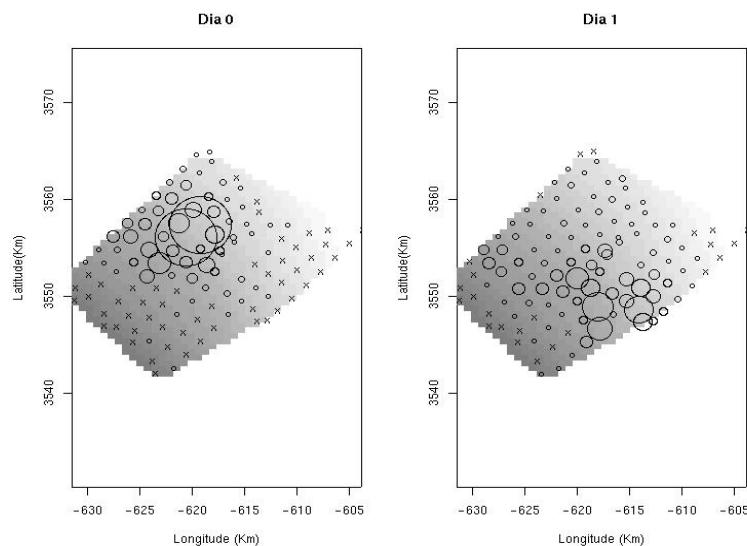


Figure 3.1.4.1 Distribution of two daily cohorts of sardine eggs (hatched during the night of sampling in the left and 1 night before in the right) within a restricted area (approx. 120 nm²) in the Gulf of Cadiz. Circles indicate egg presence (with radius proportional to egg density) and crosses egg absence.

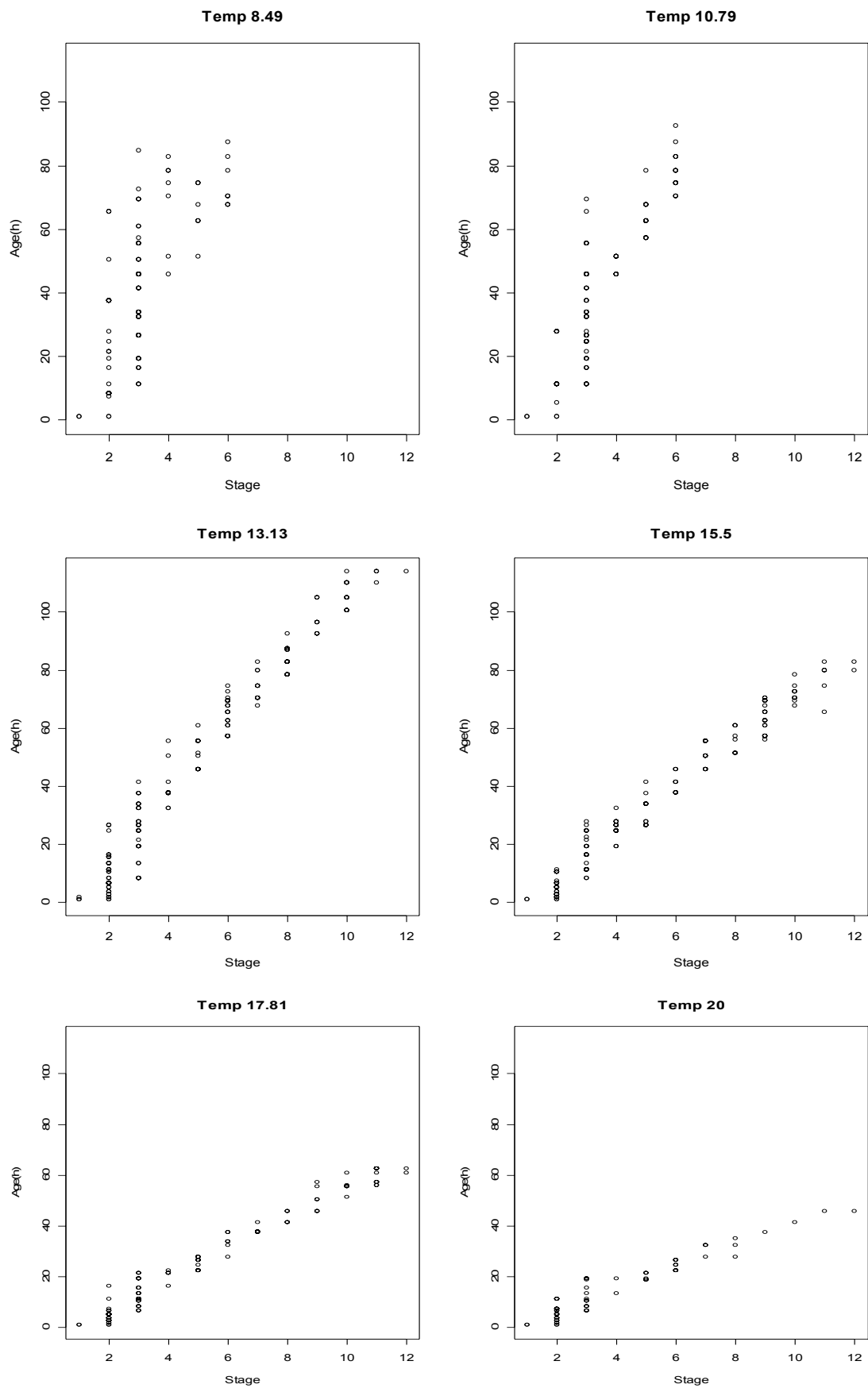


Figure 3.2.2.1.1 Observed age (in hours) for each stage and temperature (data from anchovy incubation experiment)

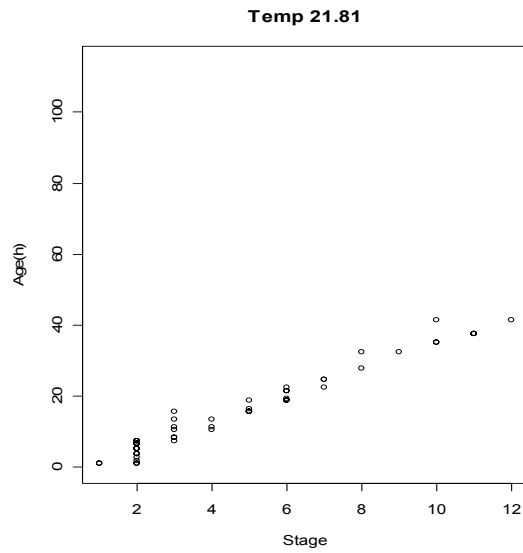


Figure 3.2.2.1.1 (continued) Observed age (in hours) for each stage and temperature (data from anchovy incubation experiment).

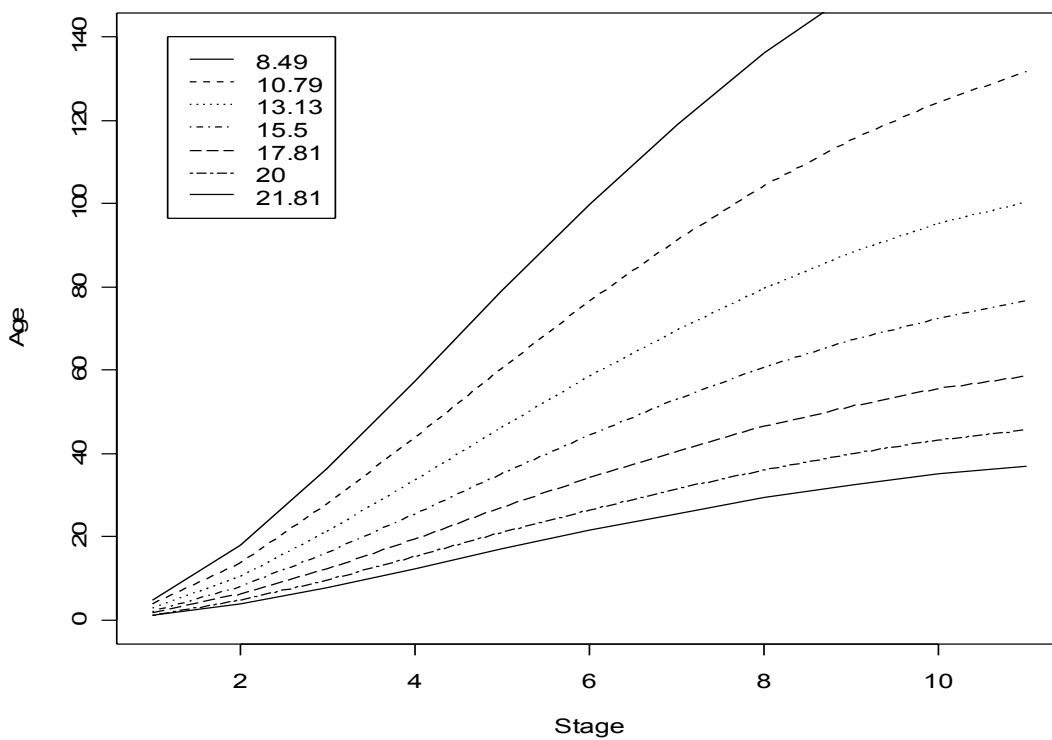


Figure 3.2.2.1.2 Mean age vs. stage from Lo's model fitted to anchovy incubation experiment data. Each line represents a different incubation temperature.

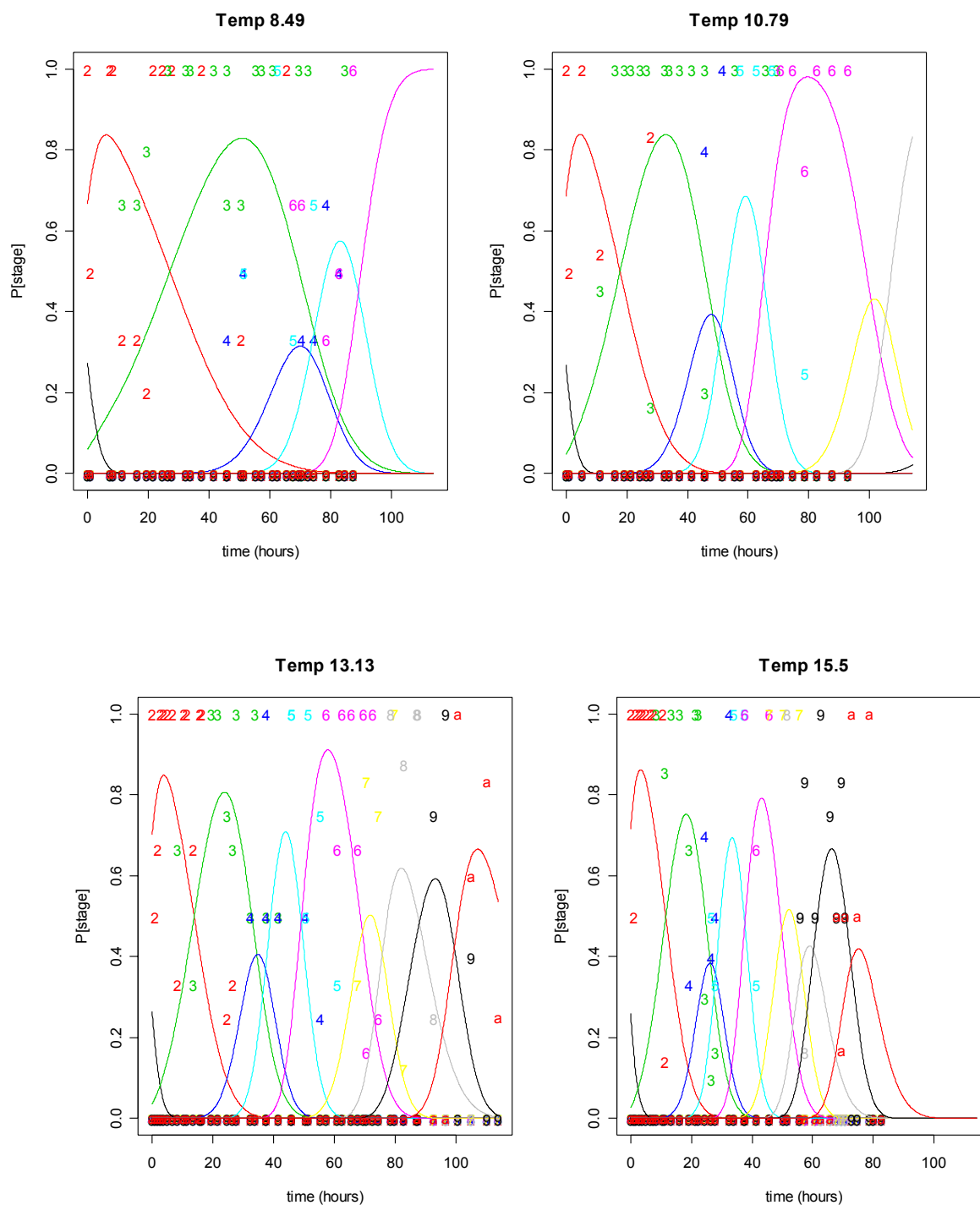


Figure 3.2.2.1.3 Probability of being at stage along observation time (age) from models fitted to anchovy incubation data (one incubation temperature in each panel).

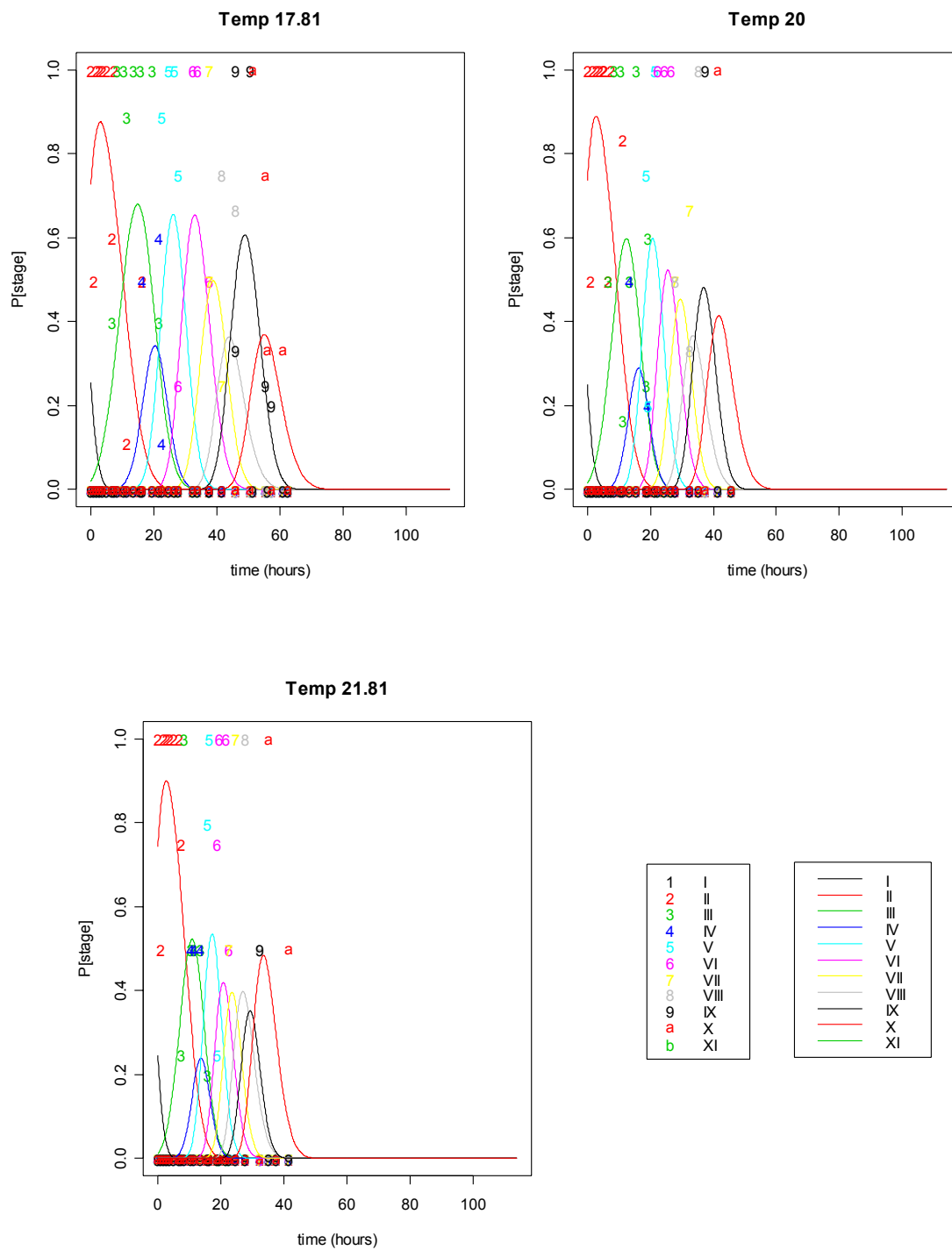


Figure 3.2.2.1.3 (continuation) Probability of being at stage along observation time (age) from models fitted to anchovy incubation data (one incubation temperature in each panel).

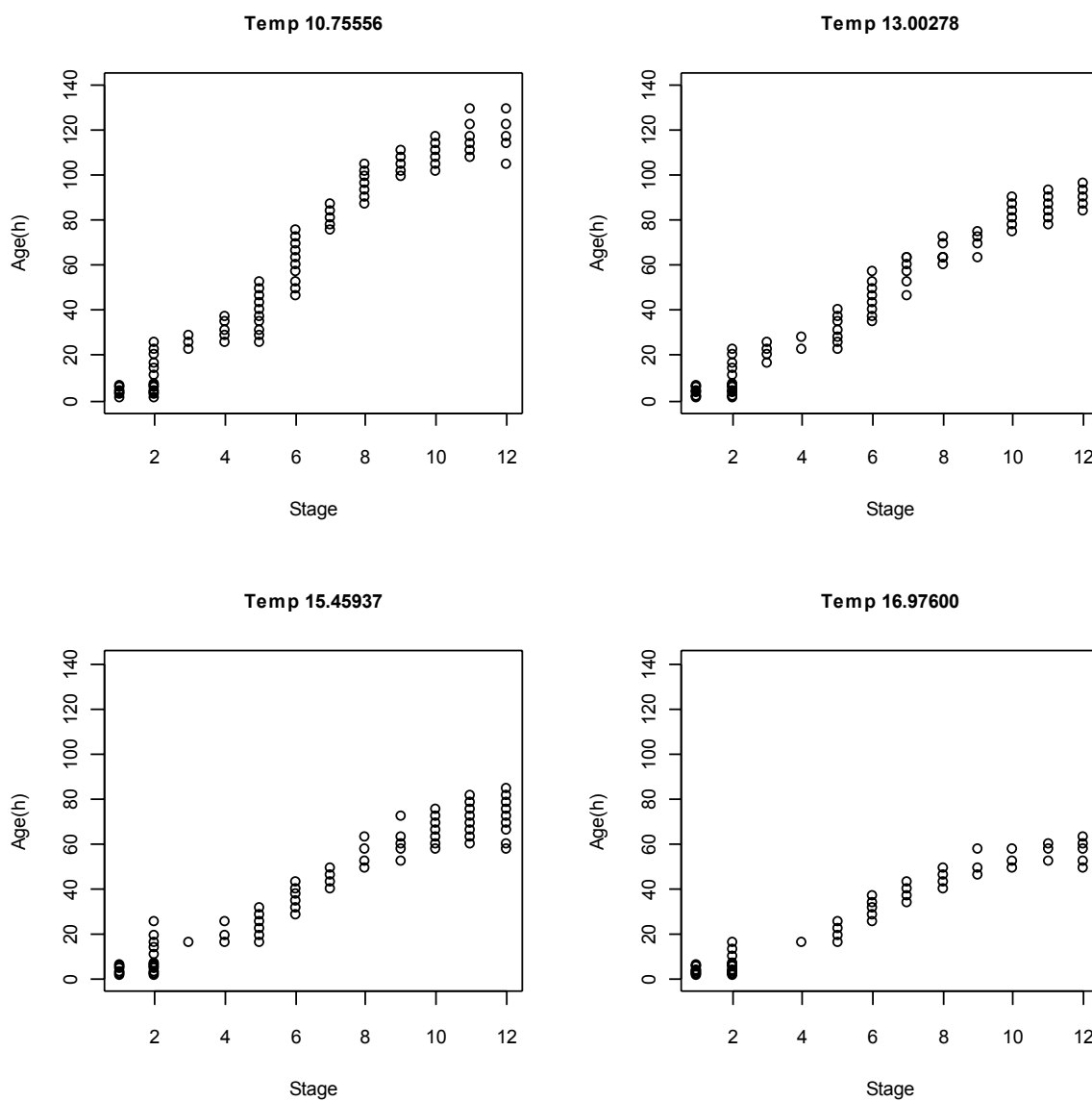


Figure 3.2.2.2.1 Observed age (in hours) for each stage and temperature (data from sardine new incubation experiment).

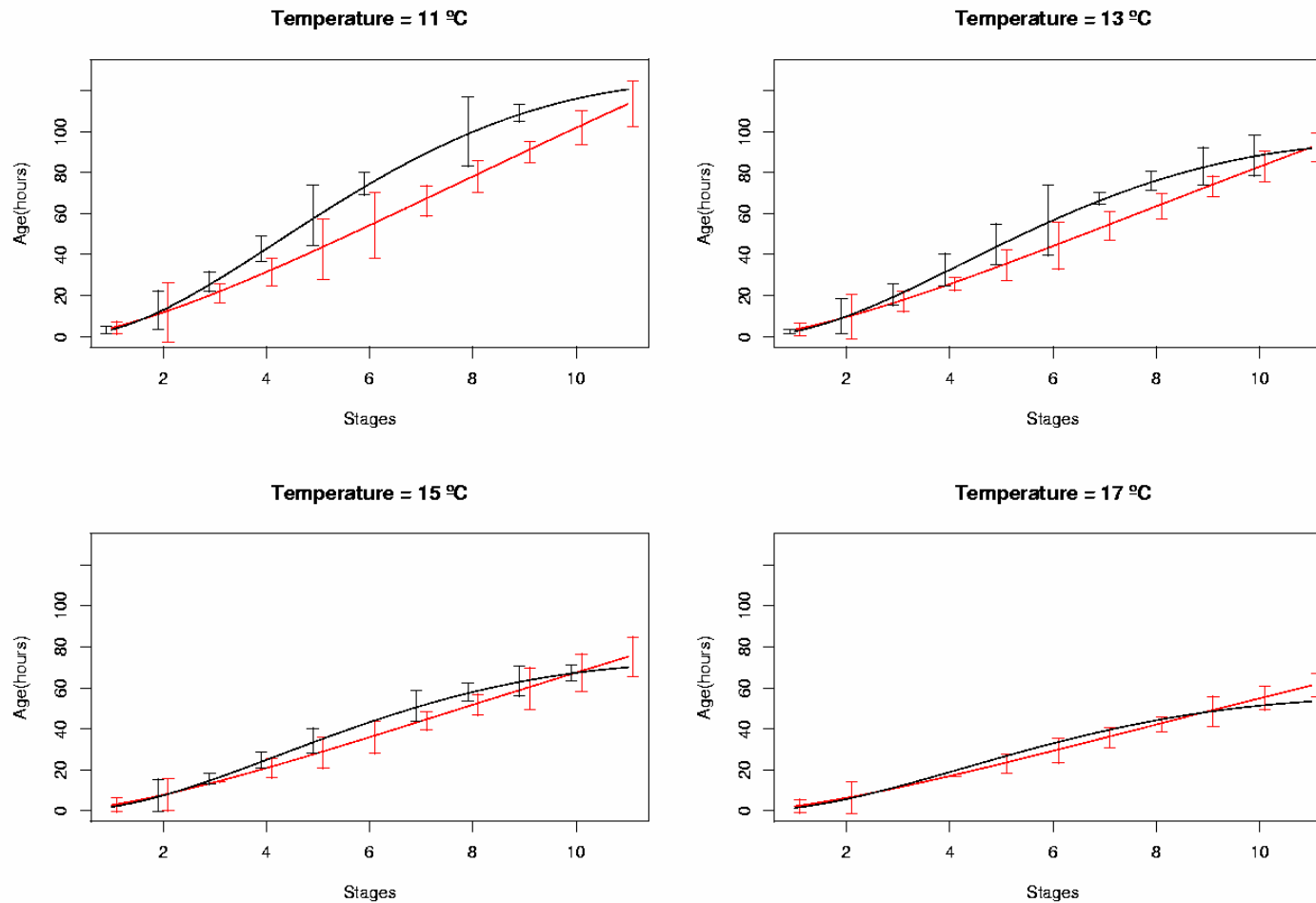


Figure 3.2.2.2.2 Lo's model applied to Cadiz (red line) and Cantabric area (black line) incubation data. Vertical lines indicate estimates of stage duration obtained directly from the data following Lo (1985). (No observations for temperature=17 °C available in the Cantabric area data set).

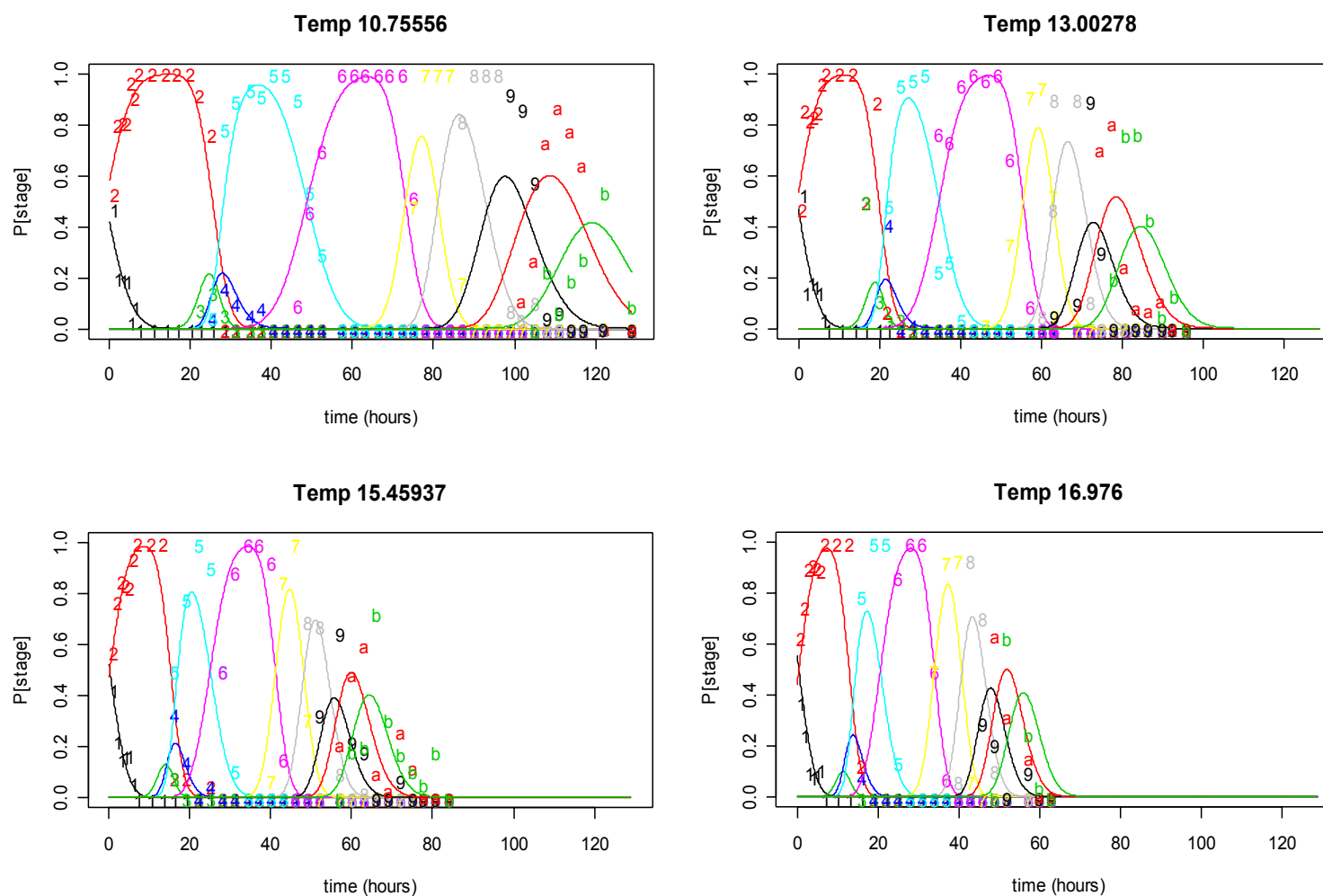


Figure 3.2.2.2.3 Multinomial model of probability of being in a given stage given age and temperature fitted to the sardine egg incubation data Cádiz data using the `depmode1` package.

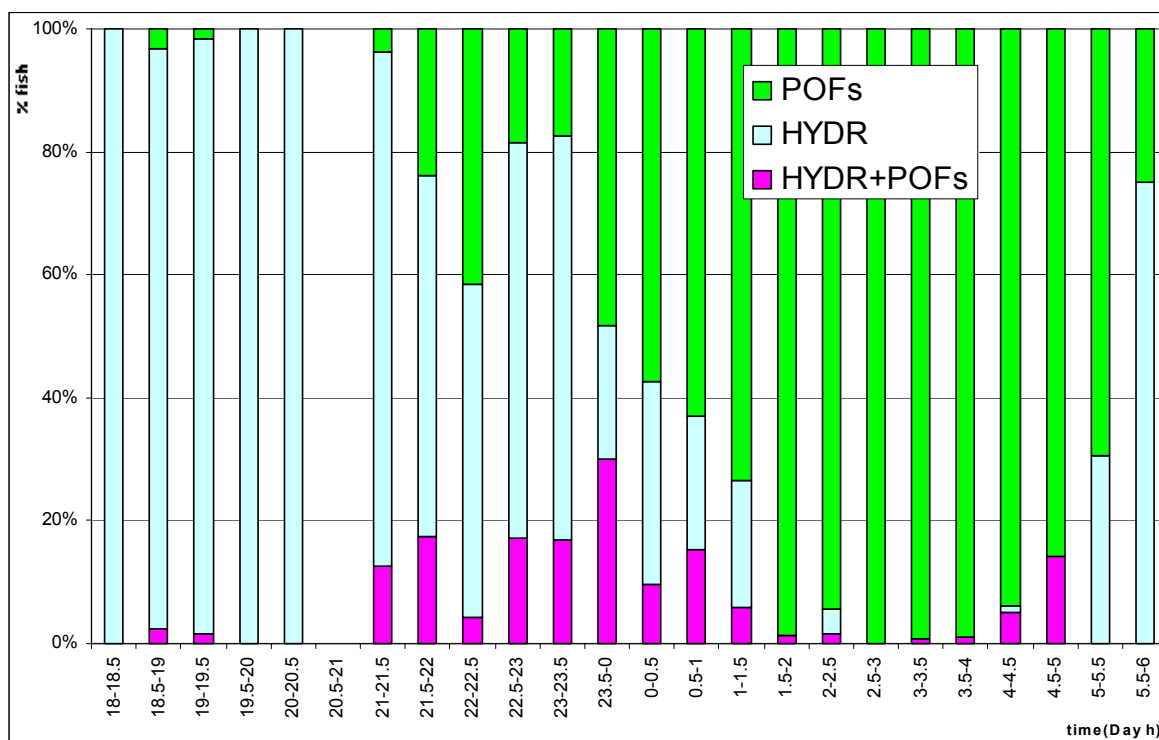


Figure 3.2.4.1 Half-hourly distribution of anchovy gonad states among the spawning females during the period 18:00 – 05:00 GMT (data from several DEPM surveys).

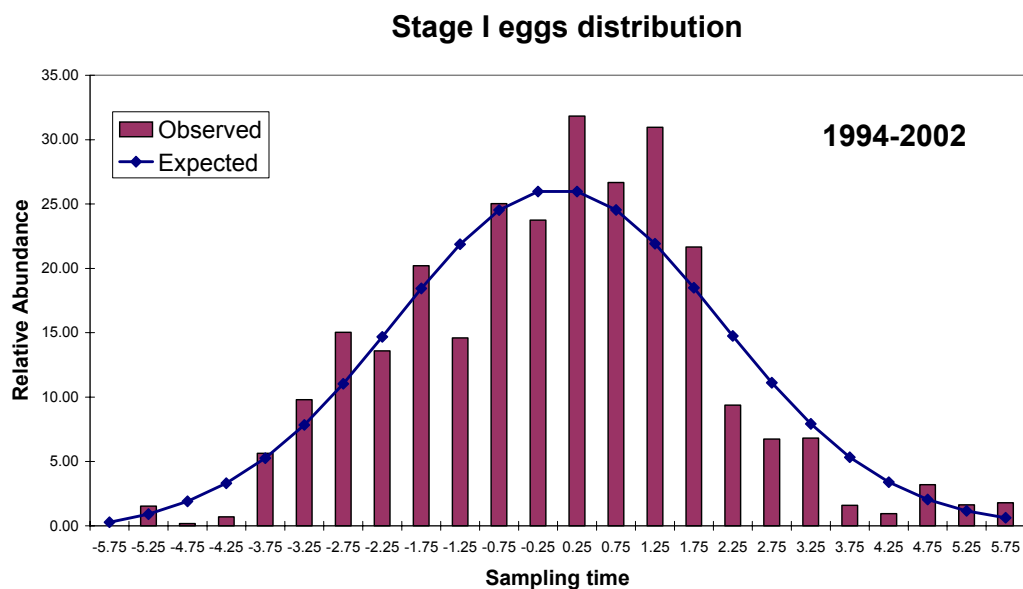


Figure 3.2.4.2 Distribution of Stage I anchovy eggs per surface unit versus sampling time in hours (in distance to midnight) and expected distribution according to the assumption of a normal curve to the distribution of spawning time (fitted parameters $N(-0.76, 2.04)$). Data from DEPM surveys 1994-2002.

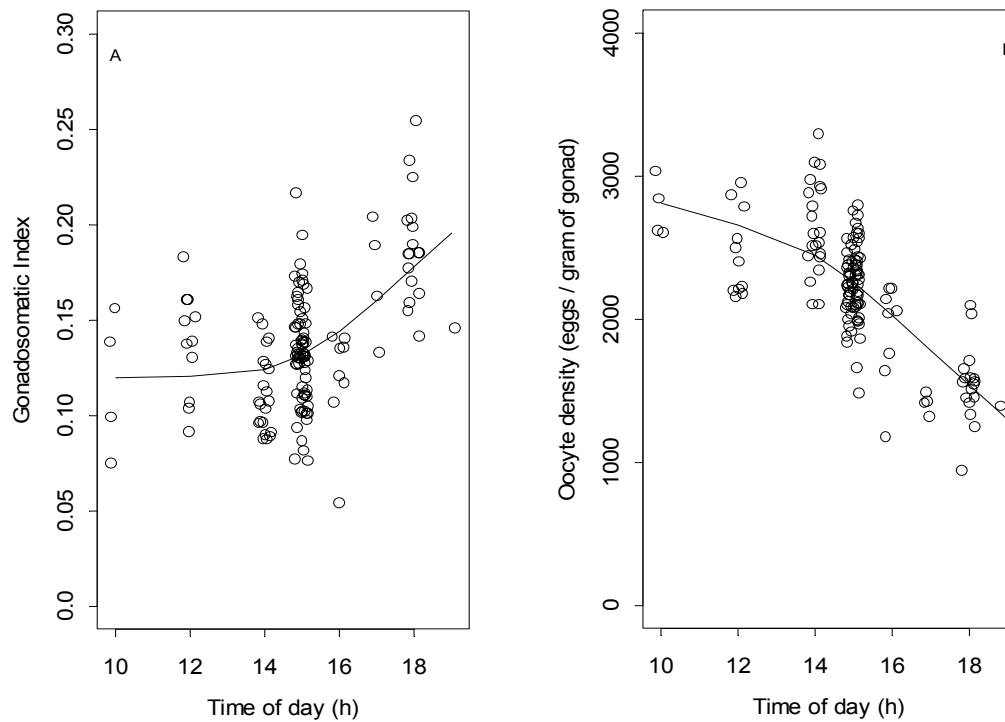


Figure 3.2.4.3 Diurnal development of gonadosomatic index (a) and hydrated oocyte density (b) for female sardines sampled during the day of anticipated spawning (from Zwolinski et al. 2001).

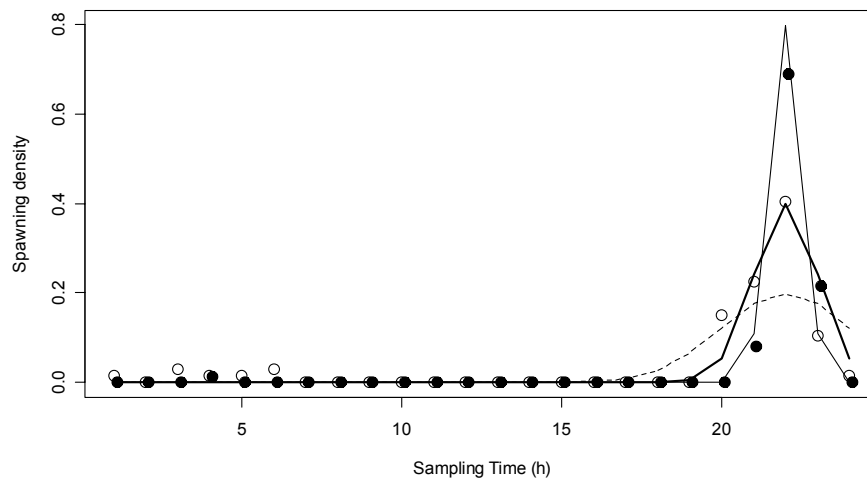


Figure 3.2.4.4 Proportion of stage I sardine eggs caught hourly during DEPM surveys (open circles) and during sampling in a fixed location (closed circles, data from Ré et al. 1988). Lines correspond to a normal density functions with a 22h mean and standard deviation of 0.5 (thin continuous), 1 h (thick continuous) and 2 h (broken) respectively.

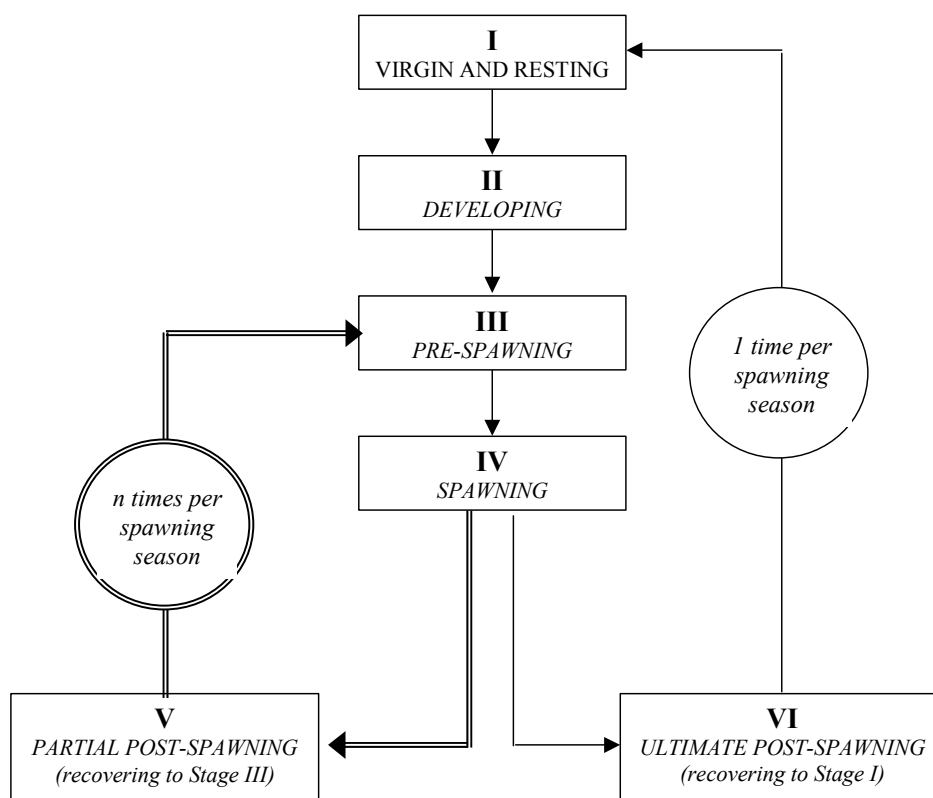


Figure 3.3.1.1 Female Sardine Maturity Cycle (based on the scale of Table 3.3.1.1).

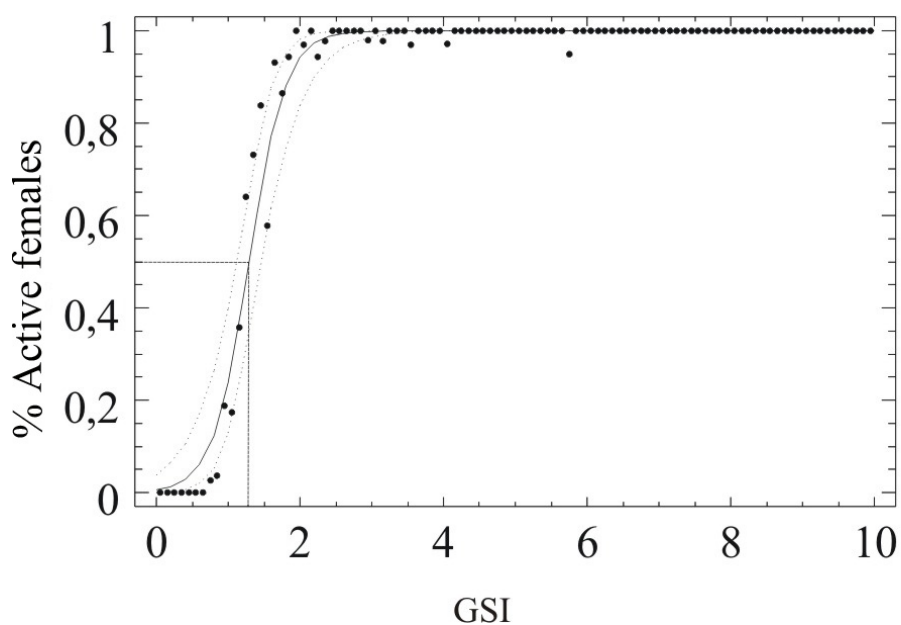


Figure 3.3.2.1.1 Proportion of active female sardines as a function of their gonadosomatic index (GSI) and respective ogive to identify the value of GSI at which half of the sardine females are active (from Ganas 2003).

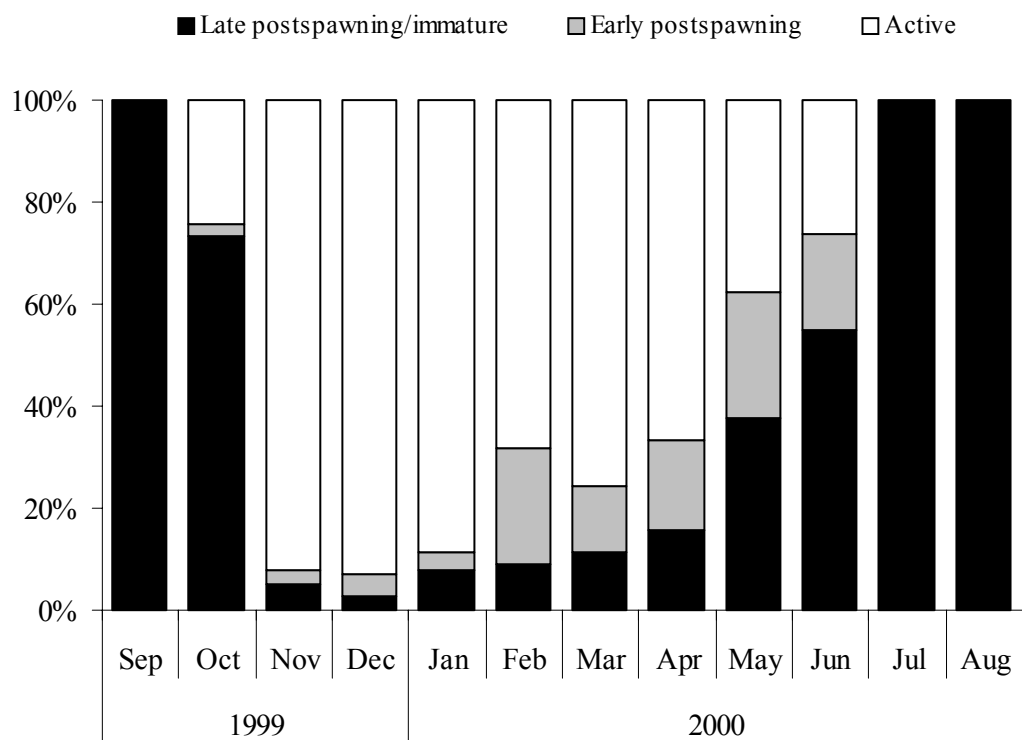


Figure 3.3.2.1.2 Evolution of the monthly fractions of active, early postspawning and late postspawning/immature female sardines in the Mediterranean (from Ganas et al. *submitted*).

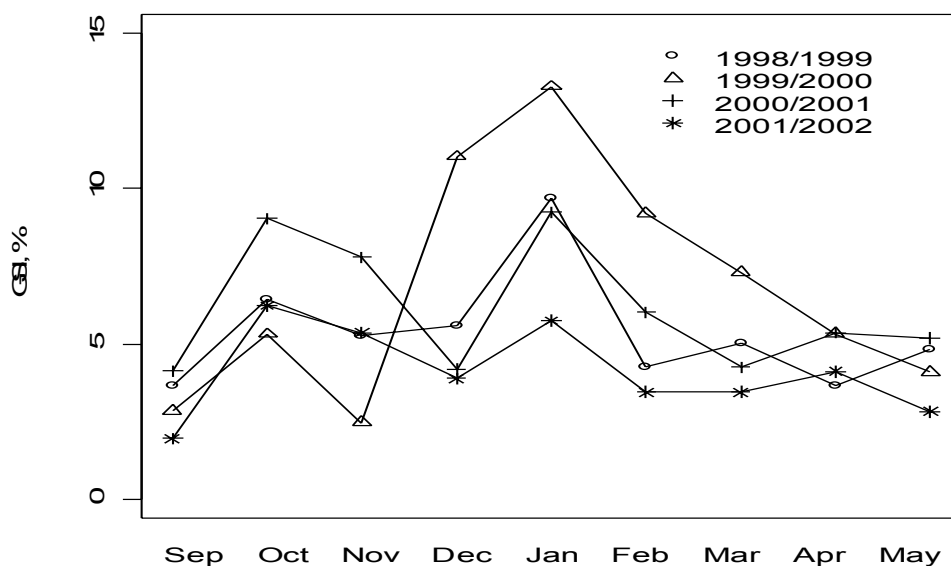


Figure 3.3.2.2.1 Average monthly female gonadosomatic index (GSI) along the sardine spawning season off Portugal in recent years (data from Portuguese market samples).

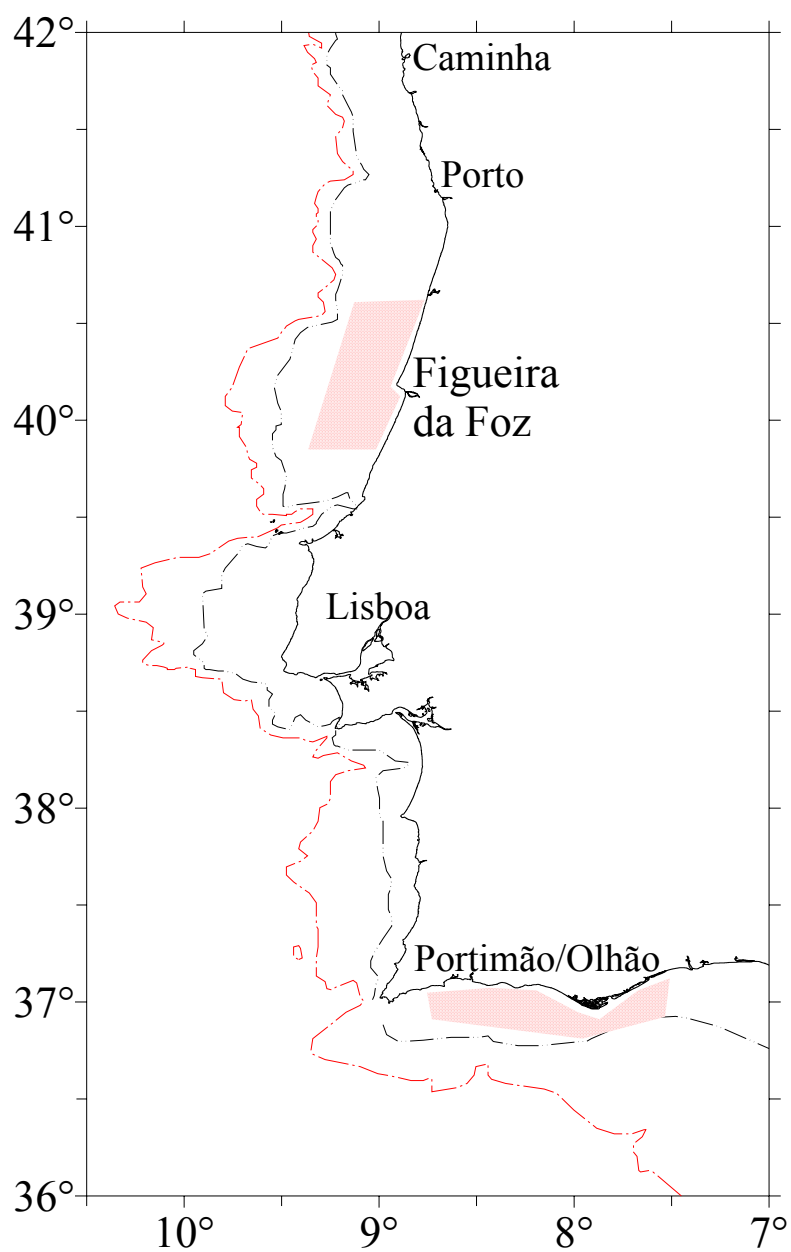


Figure 3.3.2.2.2: Geographic location of sampling along the northern and southern Portuguese coast use for the study of sardine spawning seasonality during 2001/2002 (Cunha et al. WD 2003).

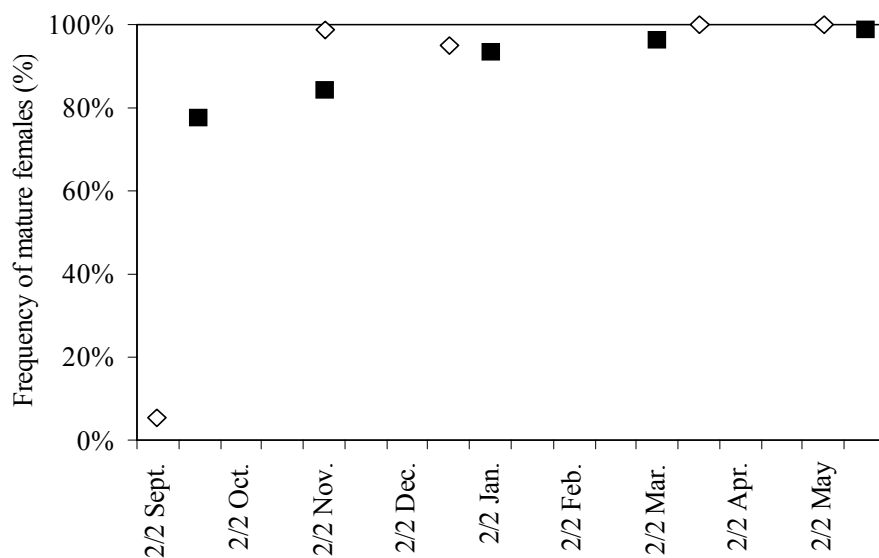


Figure 3.3.2.2.3 Evolution of female sexual maturation at the northern (diamonds) and southern (black squares) coasts during 2001/2002 spawning season.

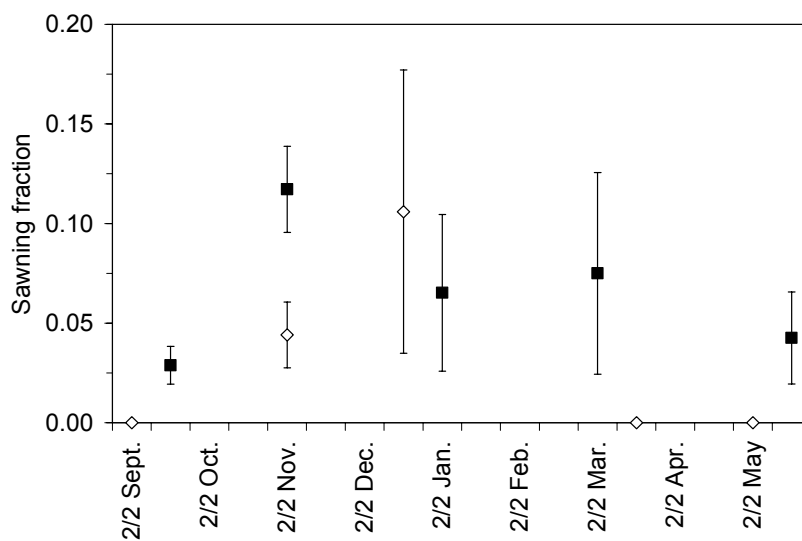


Figure 3.3.2.2.4 Temporal progression of spawning fraction along the northern (diamonds) and southern (squares) coasts during 2001/2002 spawning season.

This chapter describes the 2002 surveys and provides estimates of all DEPM parameters and spawning biomass for sardine (section 4.1) and anchovy (section 4.2). The section dedicated to each species starts with a brief summary of the surveys, followed by the estimation of egg production and adult parameters respectively. It should be noted that final estimates are based on the traditional methods (Lasker 1985), which continues, at present, to provide the standard estimates of spawning stock biomass for the purposes of analytical stock assessment. However, results should be compared to those obtained by the application of GAMs (sections 2.3 and 2.4), although GAM estimates of adult parameters and SSB are necessarily provisional (given that they were applied for the first time during the course of this 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 estimates and the acoustic results for 2002.

4.1 The Atlanto-Iberian sardine survey

4.1.1 Survey details

Following the recommendations of the previous Study Group report (ICES 2002), a sardine DEPM survey took place in 2002 covering the area from the Gulf of Cadiz to the inner part of the Bay of Biscay (Atlanto-Iberian stock). The region from the Gulf of Cadiz to the northern Portugal/Spain border (Miño river) was covered by IPIMAR, while IEO covered the north and north-western Iberian Peninsula and the Bay of Biscay (up to 45°N). The Portuguese survey (7/1 – 8/2/2002) was carried out onboard *R/V Noruega* (MPDO2002), while the Spanish survey (18/3 – 6/4/2003) was conducted onboard *R/V Cornide de Saavedra* (SAREVA0302) for the plankton component and *R/V Thalassa* (PELACUS 0302) for the adult component.

Both national surveys consisted of ichthyoplankton sampling on fixed (CalVET, Figure 4.1.1.1) and underway (CUFES, Figure 4.1.1.2) stations. 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 and salinity were determined using electrical 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 thermo-salinometer coupled to CUFES, while CTD profiles were obtained in each CalVET station.

Sardine eggs were identified and counted on board immediately after collection. 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 (773 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 onboard *R/V Cornide de Saavedra*) a calibration exercise was carried out in French waters (see section 3.1.3) 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 developed showing morphological characteristics of non-fertilized 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 were few and small quantities of sperm being collected in hauls with large number of hydrated females.

Adult fish samples were obtained from demersal and pelagic trawls (research vessels) and purse seining (commercial vessels). Figure 4.1.1.3 shows the location of adult sampling in the two surveys. Overall, 32 samples were obtained in the Spanish survey (4 in the French coast) and 74 in the Portuguese. Most samples were obtained in the inner shelf, with a mean fishing depth of 45 m. Random samples of 80 and 100 fish respectively were aimed in the Spanish and Portuguese survey respectively (most commercial Portuguese samples 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 and the elimination of gonads with POFs from the estimation of batch fecundity. Batch fecundity was estimated using the hydrated oocytes gravimetric method (MacGregor 1957) by counting the hydrated oocytes (Hunter *et al.* 1985).

4.1.2 Egg production estimation

Egg production estimates from the 2002 sardine surveys have already been reported (Cunha *et al.* WD 2002; Bernal *et al.* WD 2003) and tabulated at the WGMHMSA of 2002 (ICES 2003). 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 2.3.1). 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 group meeting. These results are reported in section 4.1.4 and are also used to obtain the 2002 estimate of spawning biomass.

4.1.3 Adult parameter and SSB estimation

In this section, sardine adult DEPM parameters and spawning biomass are separately estimated for the Portuguese and Spanish survey of 2002, without considering the 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 4.1.4). All estimates refer to mature fish (i.e. maturity stage II and above, according to the rationale describe in 3.3.1.4), 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 (Figure 4.1.3.1). 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 section 5.1.3). 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 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 no 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 4.1.3.1). 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 4 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 4.1.3.2 and 4.1.3.3). 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 14255 (CV = 6 %). Figure 4.1.3.4 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, in the Portuguese survey relative fecundity was the lowest ever reported for this population (see Table 5.4.2.1). For the Portuguese 2002 survey, this agrees with other indicators (see section 3.3.2.2) 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 that 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 4.1.3.2 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 (but also see chapter 5), 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 unsatisfactory, 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 2.3.1 showed 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 very 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 disappointing levels of precision, the information collected in that survey can contribute to improve the understanding on sardine spawning dynamics. For example, Figure 4.1.3.5 (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 4.1.3.5 (right) suggests that the smooth relation between chlorophyll fluorescence and observed spawning fraction (GAM with binomial error distribution) is significant.

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

In the absence of adequate model-based estimators, 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 judgement sampling, using acoustic density as an indicator of local fish densities (Cunha et al. 1992; Garcia et al. 1991; Garcia et al. 1992). 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 estimation is performed independently for each stratum. Post-stratification has been used in the Spanish DEPM surveys of 1988 and 1990 (Garcia et al. 1992; Garcia et al. 1991), where considerable differences in mean weight and spawning fraction were observed between Galician and Eastern 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 3 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 4.1.4.1). 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 Garcia et al. (1991 and 1992). Non-linear weighted least squares (nlm 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, following the methodology used by Cunha et al. (WD 2002). It should be noted that this estimator is not the one proposed by the SG (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 linear 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 4.1.4.1 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 4.1.4.2 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 4.1.4.3 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 4.1.3.2. 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 2.4.1). 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 4.1.4.4). 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 almost double), while the Spanish DEPM estimate is considerably lower than the Spanish acoustic one (about a third). In the future, such comparisons would be facilitated if estimates of spawning biomass would be routinely provided for acoustic surveys

4.2 The Biscay anchovy survey

4.2.1 Survey details

The 2002 anchovy DEPM survey in the Bay of Biscay took place in May using distinct research vessels for ichthyoplankton and adult sampling. The egg survey (BIOMAN 2002) was undertaken by AZTI (6/5 –21/5/2002) on board *R/V INVESTIGADOR* (Figure 4.2.1.1). 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.*, 1999), 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. In selected stations temperature, salinity and chlorophyll profiles were obtained 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 found 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 follow standard procedures (Lasker, ed., 1985) and are described in detail in previous papers (see for example, Motos *et al.* 1991 and 1994).

Adult anchovy samples for DEPM purposes were obtained from pelagic trawl hauls during the 2002 Bay of Biscay acoustic survey (IFREMER) onboard *R/V THALASSA*. Additional adult anchovy samples were collected onboard commercial purse-seiners in an opportunistic manner during the time of the egg survey (Figure 4.2.1.2). 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 3 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.

4.2.2 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 3 stations with eggs were encountered out of this area. (Figure 4.2.1.1) The spawning area was calculated as the sum of the representative area of those stations. The total sampling area was 56176 Km² and the spawning area was 35980 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 2.2.1. The egg mortality exponential curve was fitted to the daily cohort abundances and mean ages as a weighted non linear regression model (as traditionally has been done) and as a generalised linear model (GLM) with negative binomial error distribution and log link (as recommended by this SG 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 h) were removed to avoid possible bias on the final daily egg production and mortality rate estimates. Figures 4.2.2.1 and 4.2.2.2 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 4.2.2.1.

4.2.3 Adult parameters and SSB 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 2002 survey. 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:

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

Gonad-free-weight of hydrated anchovies was transformed to total weight using the above model. Figure 4.2.3.1 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 (from 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 strata) 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 strata) was the remaining area. The NE stratum had 11 adult samples and the RE had 24 samples, from which 2 samples (40 females) and 5 samples (62 females) respectively were selected for the analysis. After taken out 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 4.2.3.2). A unique area was considered for the final estimation of anchovy batch fecundity in 2002. The resulting linear regression model (Figure 4.2.3.3) was:

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

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 to 7 hours 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 nuclear migration, which evidence 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 (more than 55 hour 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 de 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 otolith was 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 4.2.3.1). 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 4.2.3.2). 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 30700 tonnes with a CV of 13% (Table 4.2.3.1).

Table 4.2.3.2 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 Northeastern Coastal stratum than in the rest. Table 4.2.3.3 gives the mean weight and length-at-age by regions and in total. 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 4.2.3.4. Table 4.2.3.5 shows the sensibility 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 sensible 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 4.2.3.2), 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 1 year old anchovies. Hence, sampling was considered to be unbalanced for the purposes of number-at-age estimation.

4.2.4 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. This SG (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 4.2.4.1 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 (section 2.3.2 and 2.4.2 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 (ICES 2003), 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 51000 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 30700 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. 1998). 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 30700 tonnes appoints to a strong decrease regarding the 2001 DEPM estimate (124000 tonnes, Figure 4.2.4.1 and Table 4.2.4.2). 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 was 2 years old anchovies and only 27% was 1 year old. This is the first time in the whole series of DEPM estimates since 1987 that 2 years old are more abundant than 1 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 downward 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 2 years 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 2 also largely predominate. 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 of about 25% those in 2001 and were largely sustained by big and old anchovies (68% were 2 year old anchovies). However, the acoustic and DEPM surveys diverge in the biomass estimates for 2002. The acoustic estimate of anchovy biomass is about 97000 t (Poisson & Massé 2002), much larger than the DEPM estimate of about 30000 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 4.1.3.1

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 4.1.3.2

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

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 4.1.4.1

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 (km ²)	P_{01} (eggs / 0.05 m ²) [cv]	Z (eggs per day) significance	Egg production
Spain, CANE	4621	2.22 [31]	-0.36 **	0.20 10^{12}
Spain, CANW	5348	6.12 [32]	0.14 **	0.66 10^{12}
Total Stratified (Spain)	9970	4.46 [32]	-0.09 --	0.86 10^{12}
Portugal, N	12280	5.36 [24]	-0.13 --	1.32 10^{12}
Portugal, S	4343	10.28 [36]	-0.83 **	0.89 10^{12}
Total Stratified (Portugal)	16623	7.97 [33]	-0.32 --	2.21 10^{12}
Unstratified (Spain)	9970	3.84 [23]	-0.11--	0.76 10^{12}
Unstratified (Portugal)	16640	6.23 [33]	-0.16 --	2.07 10^{12}
TOTAL Stratified				3.07 10^{12}
TOTAL Unstratified				2.83 10^{12}

Table 4.1.4.2

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 4.1.4.3 DEPM parameter and SSB estimates (with 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 4.1.4.3 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 4.2.2.1 Total daily egg production estimates for Bay of Biscay anchovy in 2002 (with the correspondent 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 4.2.3.1 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 4.2.3.2

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 4.2.3.3

Estimates of the anchovy mean weight (in g) and mean length (in cm) at age by strata and over the whole area (Total)

	COAST	OCEANIC	TOTAL	
Wage_1	21.34	24.23	22.66	g
Wage_2	29.34	35.60	33.81	g
Wage_3	30.69	38.64	36.57	g
Lage_1	14.69	15.30	14.97	cm
Lage_2	16.20	17.24	16.94	cm
Lage_3	16.43	17.70	17.37	cm

Table 4.2.3.4

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 calculating dividing the weighting factors for biomass (M_i') by 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 4.2.3.5 Sensitivity of the SSB and population at age estimates to the assumptions of balanced or unbalanced sampling. First column contains the adopted estimates with balanced sampling for the biomass estimate and unbalanced sampling for the population at age estimates. 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 Popage Proc Parameter	Unweighted Weighted Estimate	CV	Weighted Weighted Estimate	CV	Change %	Unweighted Unweighted (only by inverse of Wi) 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 4.2.4.1 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 4.2.4.2 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

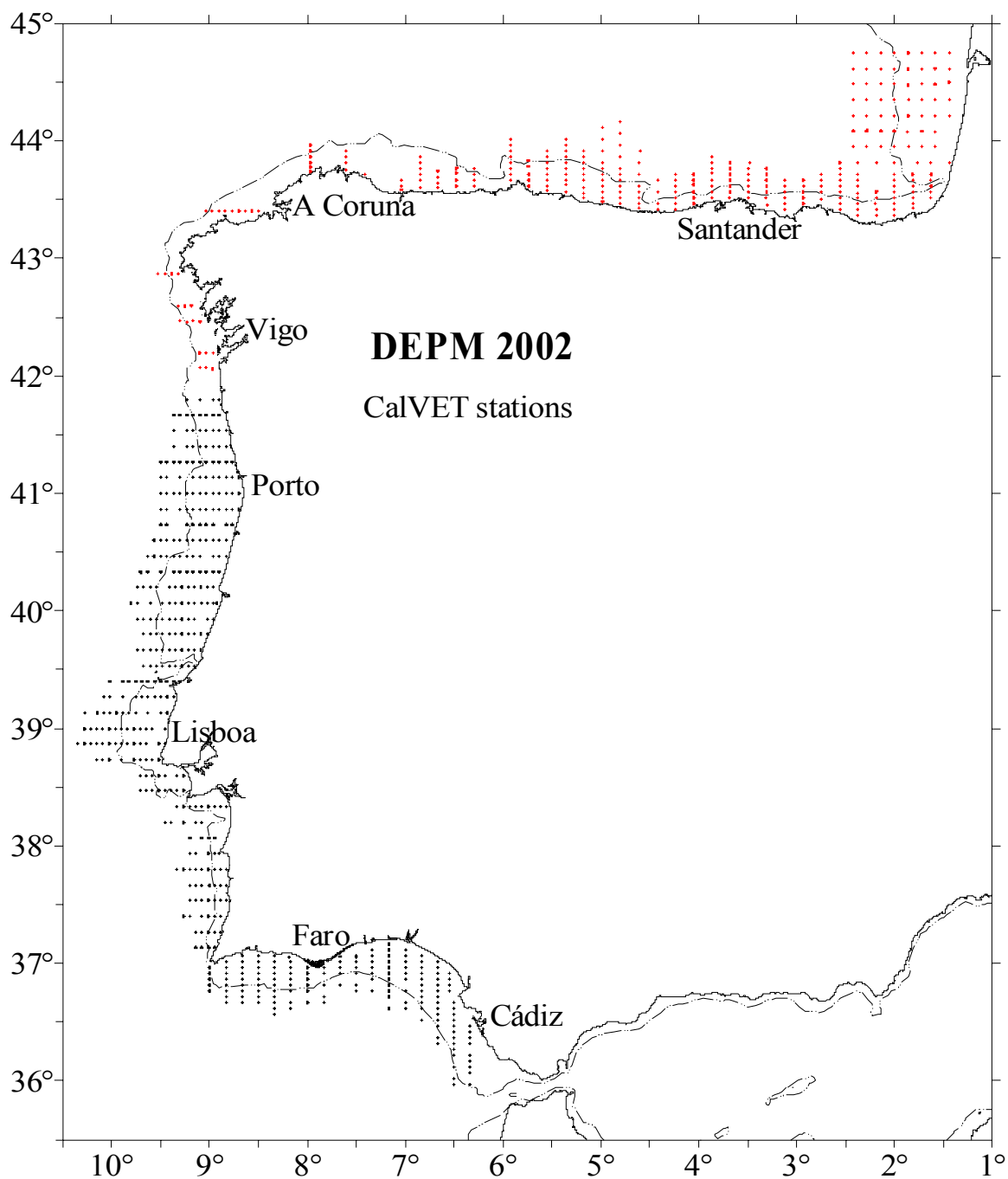


Figure 4.1.1.1 Location of CalVET stations during the 2002 DEPM survey for the Atlanto-Iberian stock of sardine (black and red points correspond to the Portuguese and Spanish surveys respectively).

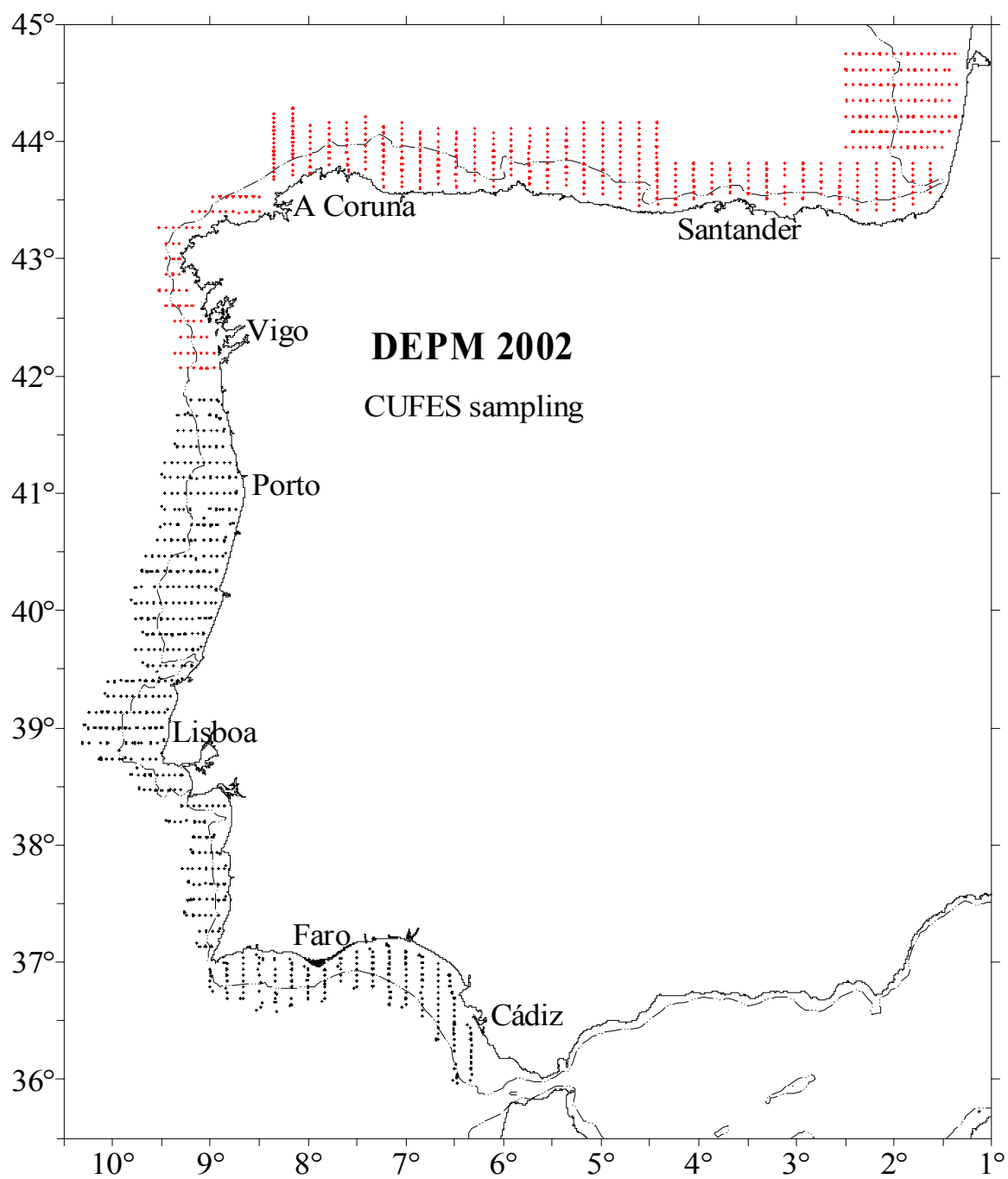


Figure 4.1.1.2 Location of CUFES stations during the 2002 DEPM survey for the Atlanto-Iberian stock of sardine (black and red points correspond to the Portuguese and Spanish surveys respectively).

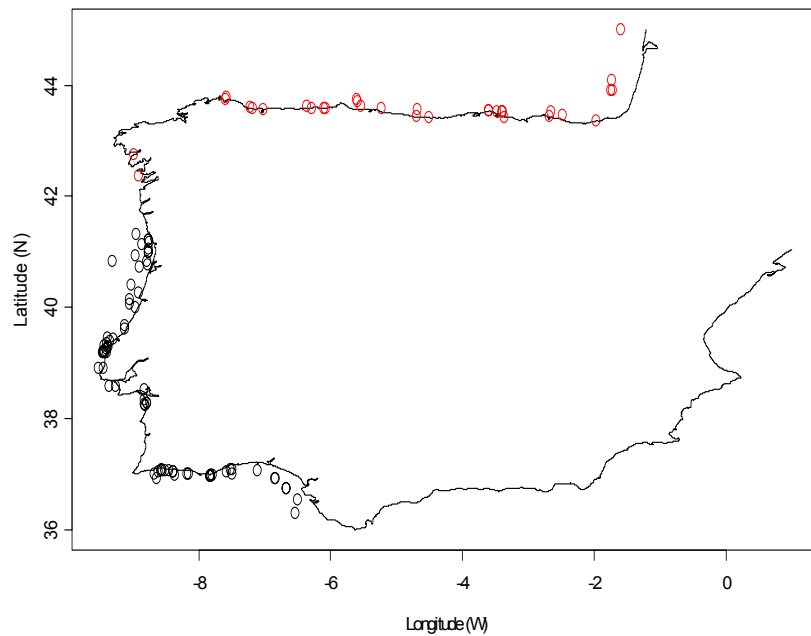


Figure 4.1.1.3 Location of fishing stations during the 2002 DEPM survey for the Atlanto-Iberian stock of sardine (black and red circles correspond to the Portuguese and Spanish surveys respectively).

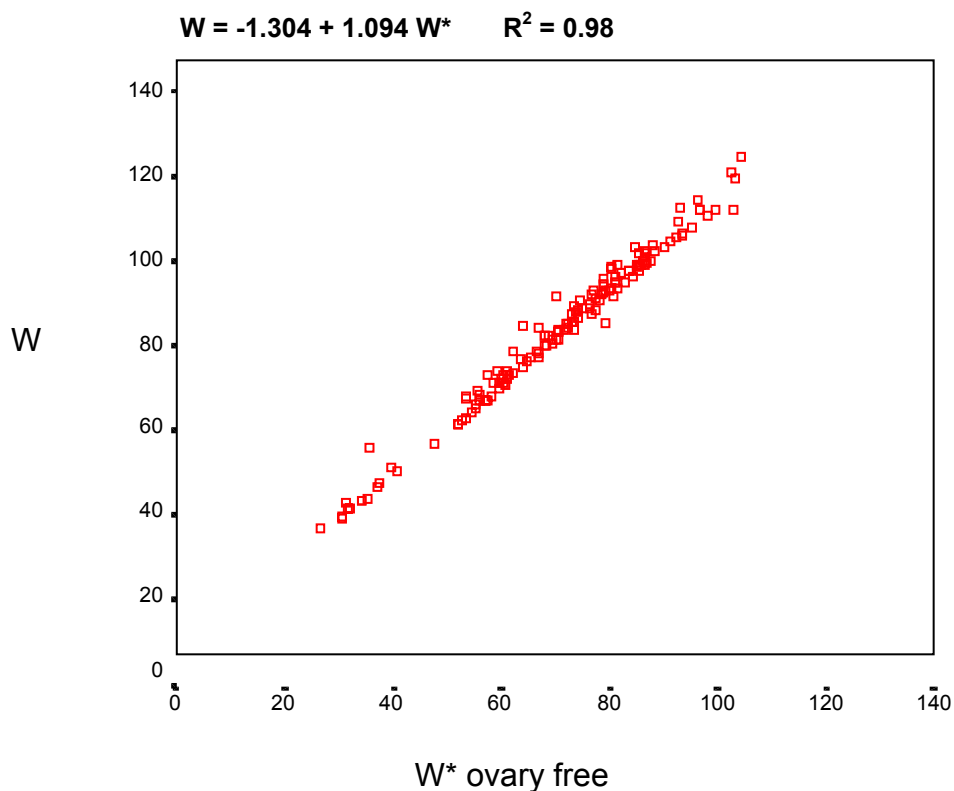


Figure 4.1.3.1 Relationship between total female fish weight (W) and ovary-free weight (W^*) from 520 non-hydrated female sardine caught during the 2002 DEPM survey in northern Spain.

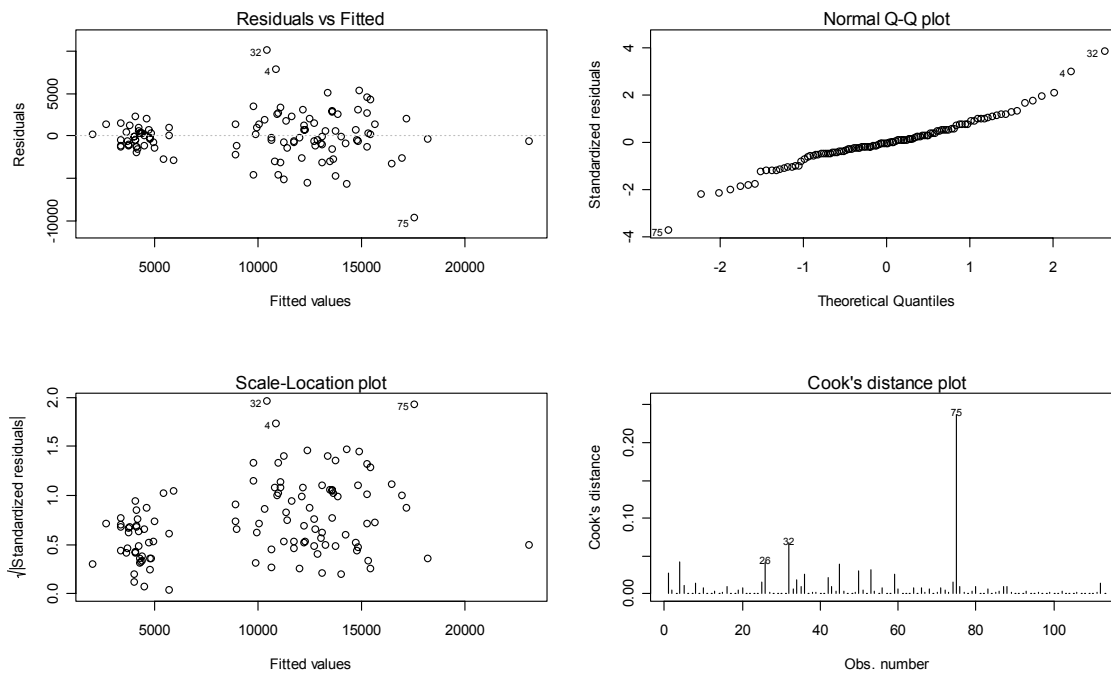


Figure 4.1.3.2 Residual inspection plots for unweighted linear model fitted to sardine batch fecundity data from the 2002 Portuguese survey (n = 113).

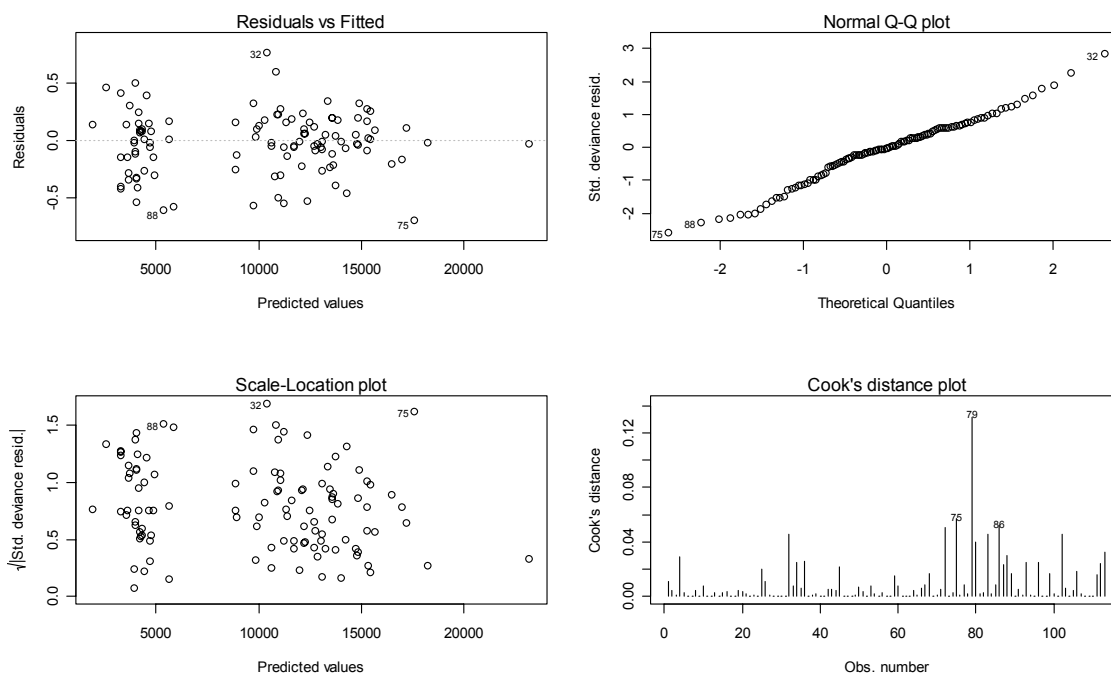


Figure 4.1.3.3 Residual inspection plots for generalized linear model (Gamma distribution with identity link) fitted to sardine batch fecundity data from the 2002 Portuguese survey (n = 113).

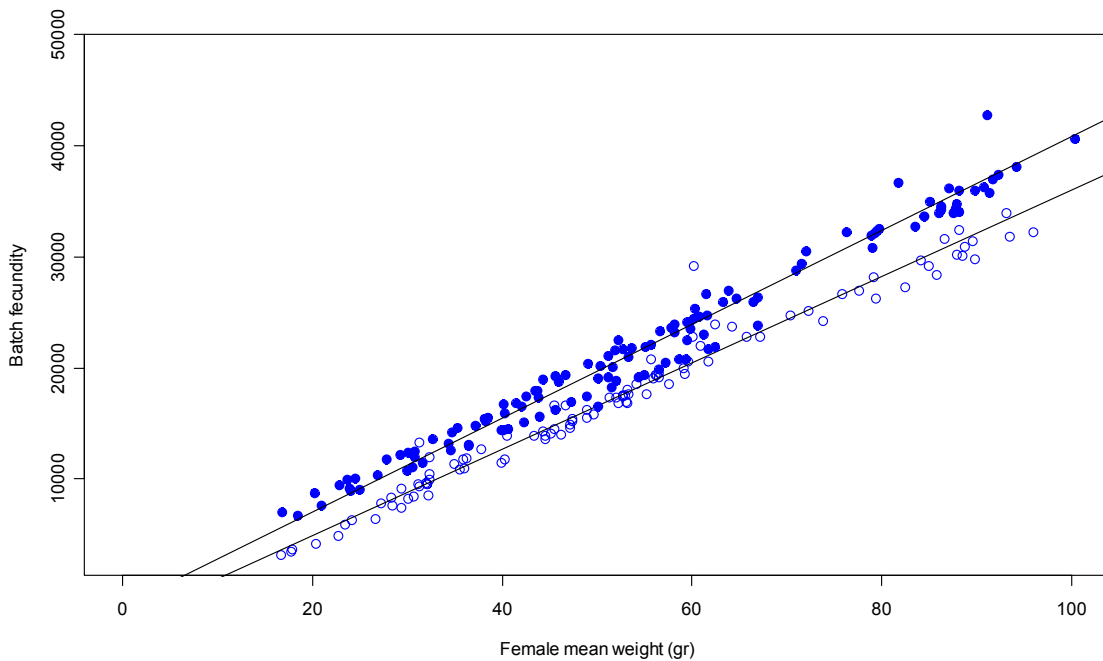


Figure 4.1.3.4 Relationship between mean female weight and batch fecundity in the 2002 (open circles) and all previous (closed circles) sardine DEPM surveys. Lines correspond to linear regression fits.

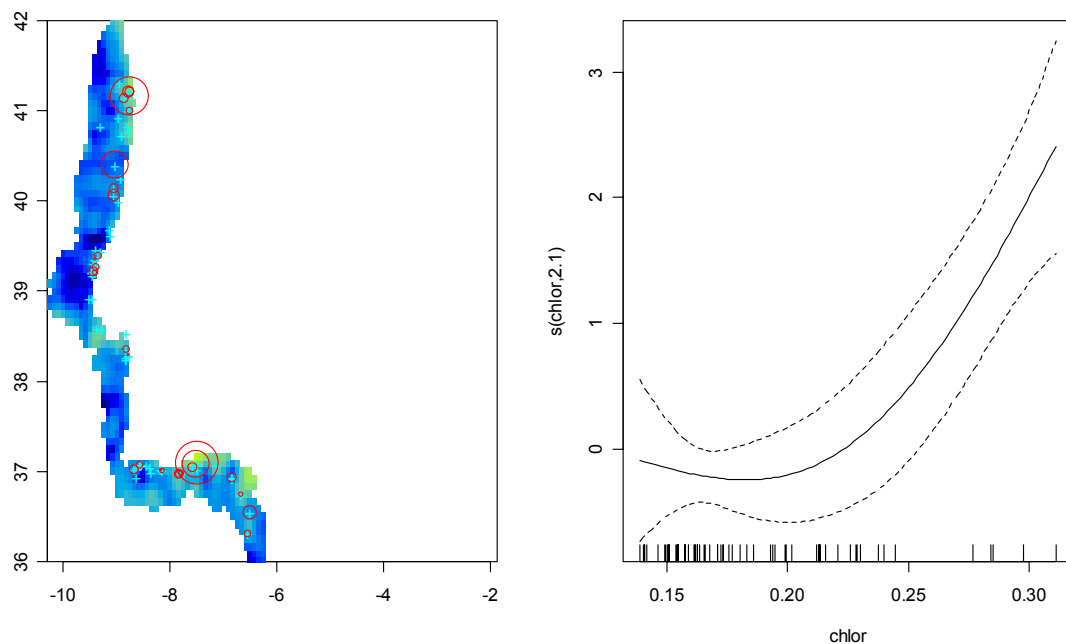


Figure 4.1.3.5 Distribution of modelled chlorophyll fluorescence (image plot: green-high, blue-low) and observed spawning fraction (red circles - non-zero S estimates, light blue crosses - location of adult sampling) during the 2002 Portuguese DEPM survey (left). Partial smooth effect of chlorophyll fluorescence to sardine spawning fraction from a GAM fitted to the same data (right).

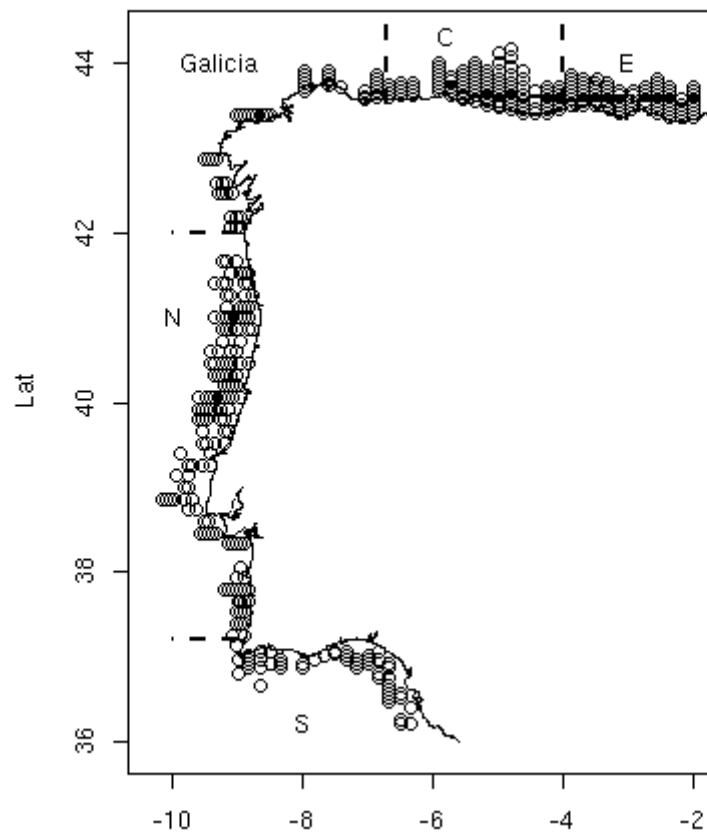


Figure 4.1.4.1 Ichthyoplankton observations and area stratification used for post-stratified estimates of the 2002 sardine DEPM survey.

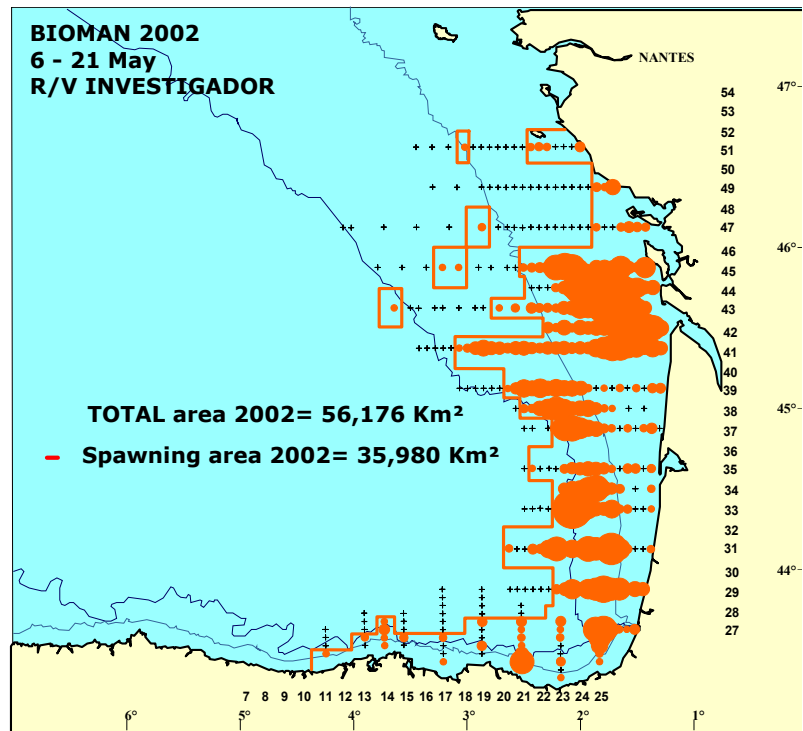


Figure 4.2.1.1 Anchovy egg/0.1m² distribution found during BIOMAN 2002. Solid line encloses the positive spawning area.

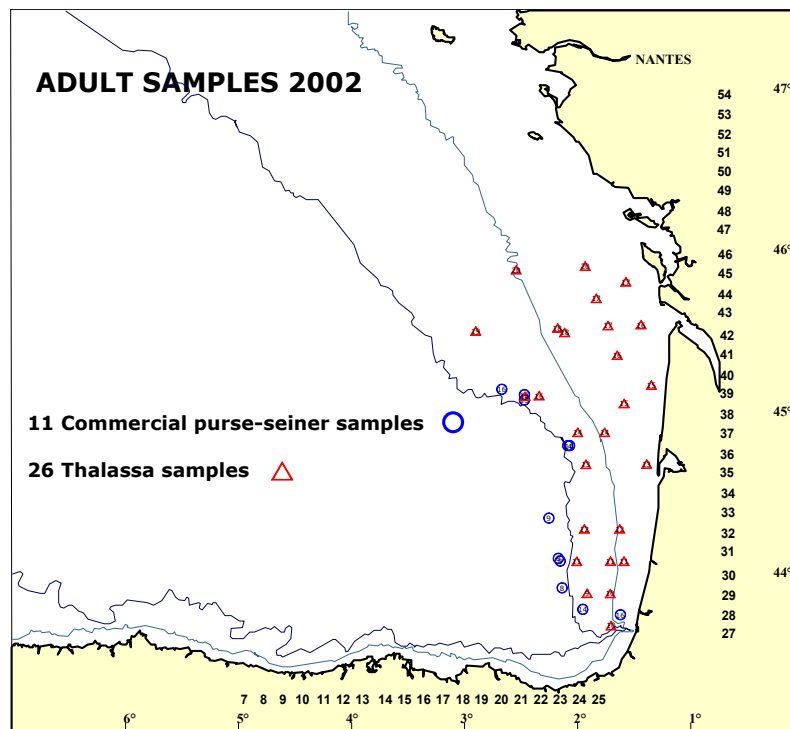


Figure 4.2.1.2 Adult samples obtained by commercial purse-seiners (in blue) and by R/V THALASSA (in red) for the estimation of anchovy adult parameters in 2002.

Exponential Mortality Model - All regions - 2002

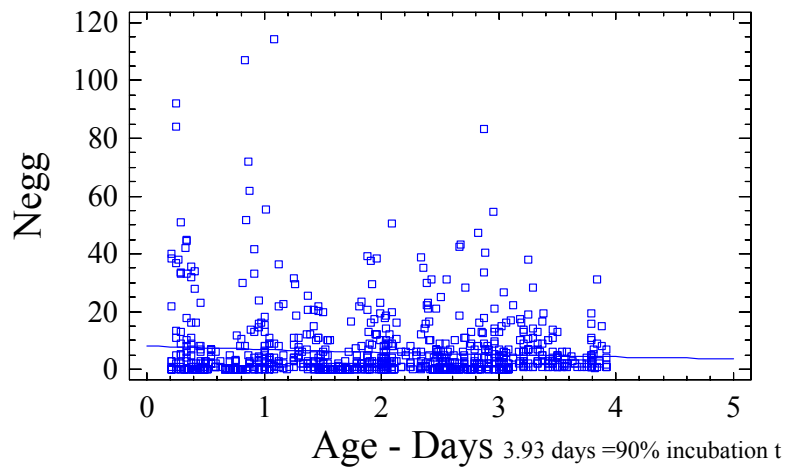


Figure 4.2.2.1 Egg mortality exponential curve fitted to daily cohort egg abundances and mean ages derived from Lo's ageing method using weighted non linear regression (as traditionally has been done).

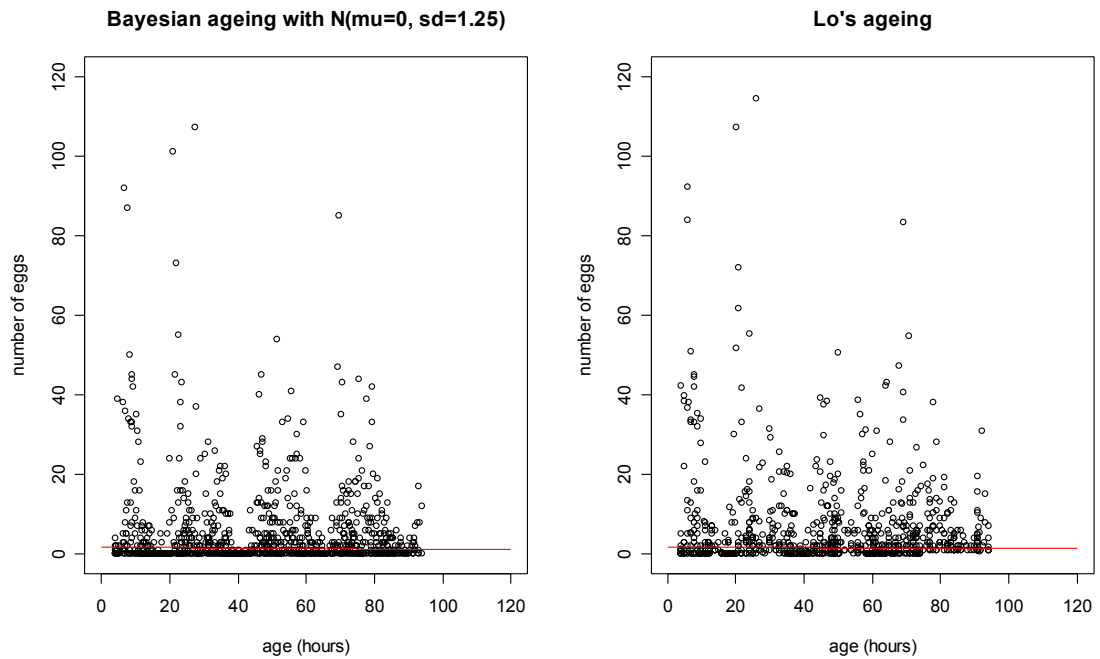


Figure 4.2.2.2 Egg mortality exponential curve fitted to the daily cohort egg abundances and mean ages derived from the new Bayesian ageing method (left panel) and from the Lo's ageing method (right panel) using GLMs with negative binomial error distribution and log link.

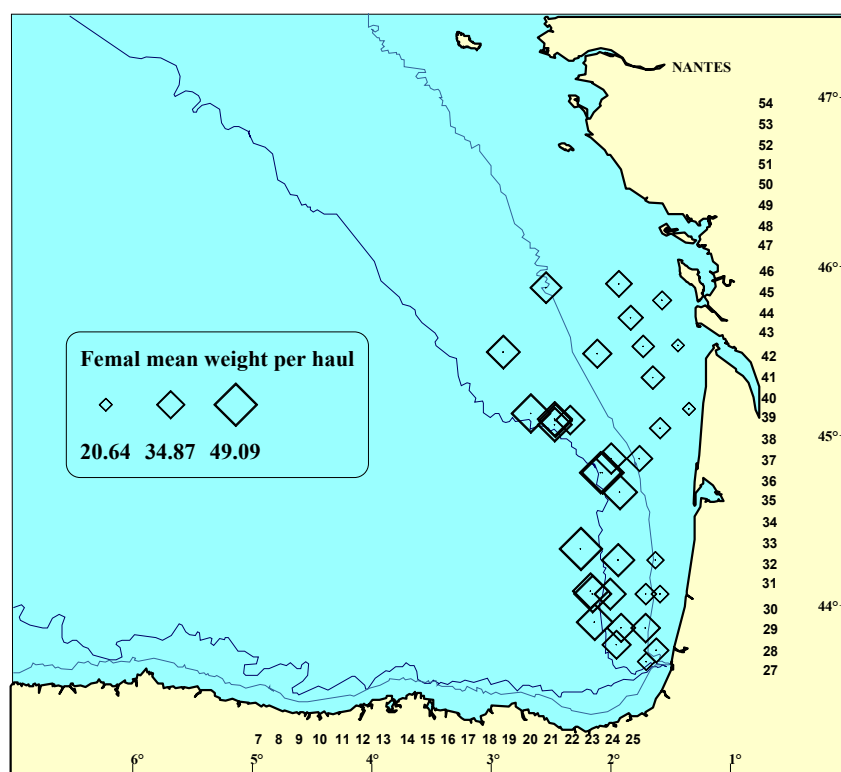


Figure 4.2.3.1 Mean female weight distribution in space for Bay of Biscay anchovy in 2002.

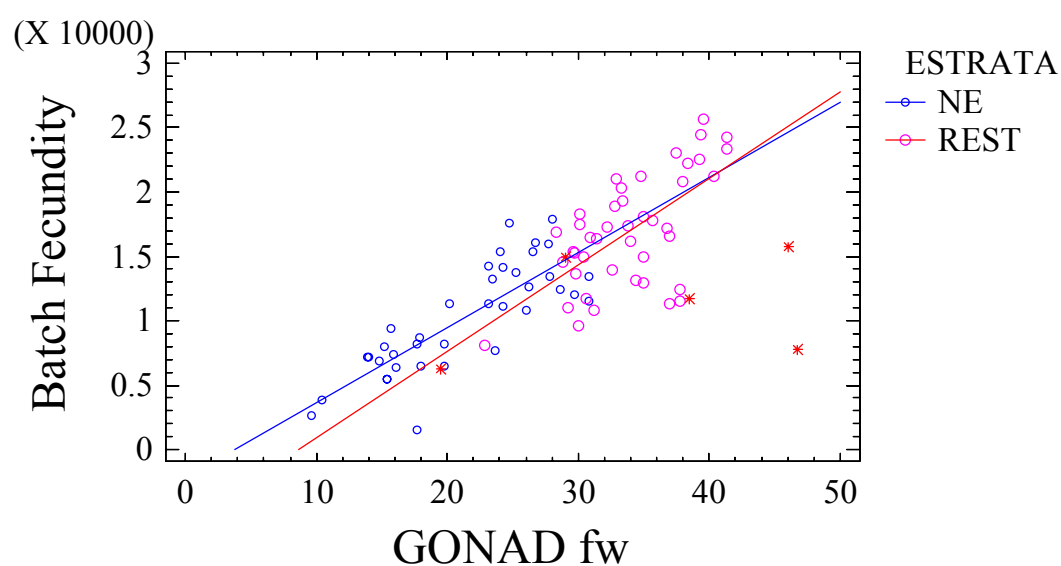


Figure 4.2.3.2 Batch fecundity vs. gonad free weight for the hydrated anchovy females. A regression line was fitted separately to each stratum (blue and red lines represent the model fitted to the NE and RE strata respectively). Red points represent the outliers, which were removed for the model fitting.

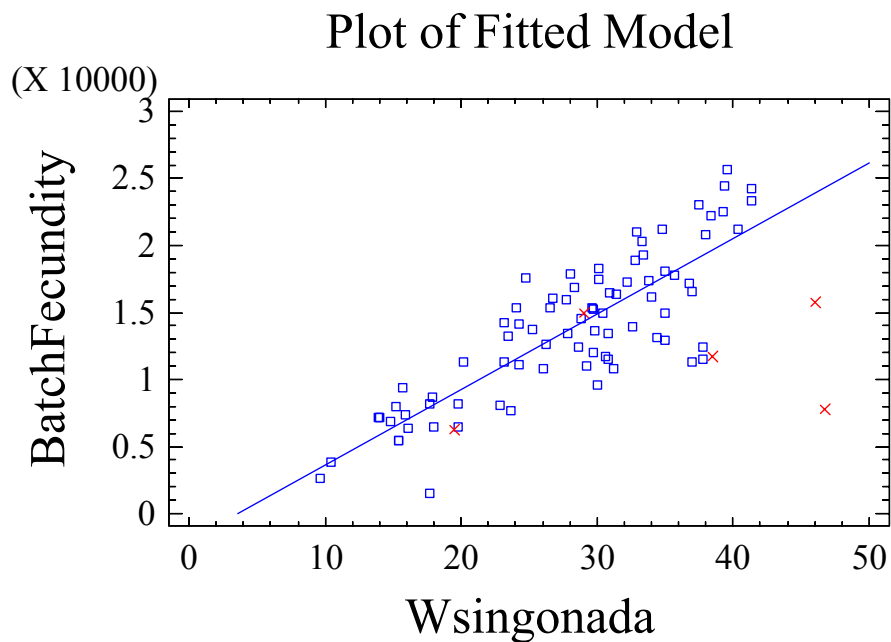


Figure 4.2.3.3 Batch fecundity vs. gonad free weight for the hydrated anchovy females. Regression line fitted to all the data, assuming that there was no difference between strata. Red points represent the outliers, which were removed for the model fitting.

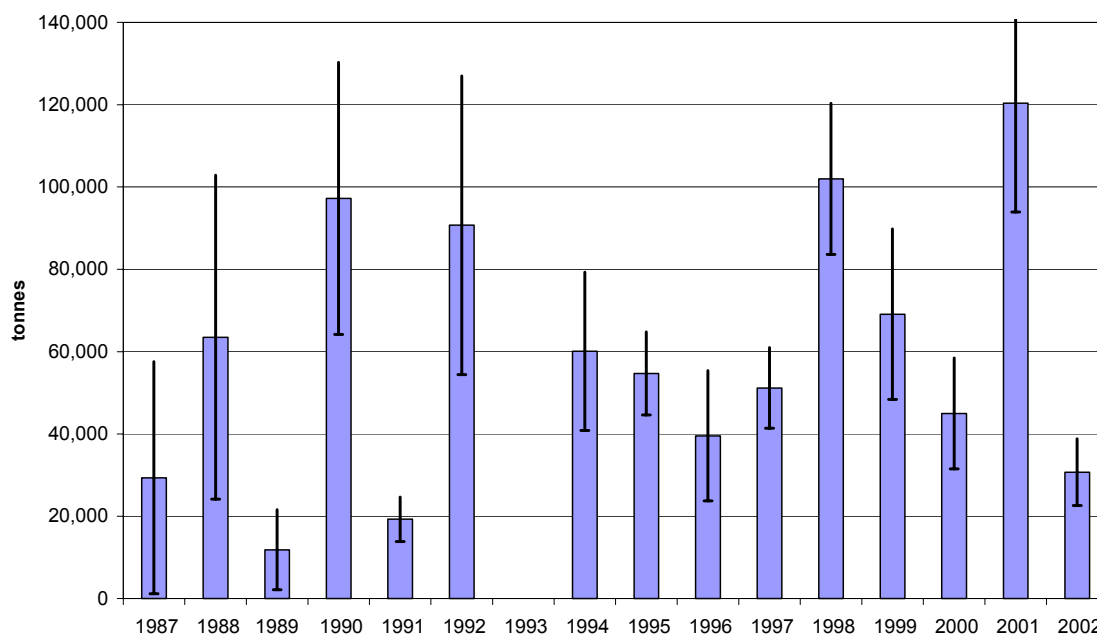


Figure 4.2.4.1 Series of biomass estimates (tonnes) for the Bay of Biscay anchovy since 1987. Most of them are full DEPM estimates, except in 1996, 1999 and 2000, which were deduced indirectly from the relationship of biomass with the spawning area and P_0 .

5 REVISION OF SARDINE DEPM SURVEYS

5.1 Introduction

Uncertainties in relation to the reliability of existing DEPM estimates for sardine and the need for clearer recommendations on their most appropriate use in assessment led the SG to recommend the revision of all existing estimates (ICES 2002). As part of the EU project on GAMs and of research activities inserted in national projects of IPIMAR and IEO, considerable effort was put in the past year to recover existing DEPM data, review and extend the histological observations for spawning fraction estimation (Portuguese surveys), revise the estimation methodologies for each DEPM parameter and compile all data in a database with a common format. The intention of the group was to provide finally revised estimates during this meeting. However, the rapid developments in GAM methodology (chapter 2) and some unplanned difficulties (incomplete recovery of Spanish raw data and questionable spawning fraction estimate for the 1997 Portuguese survey after revision) have altered this plan. The chapter summarizes the revision effort so far, highlights the remaining uncertainties associated to each estimate, and recommends a course of action for the short and the medium-term. Section 5.2 briefly summarizes the current situation with egg production estimates, highlighting some remaining inconsistencies in the existing traditional estimates. Section 5.3 provides details on all adult surveys and the sampling procedure followed, while section 5.4 describes the estimation methods followed for each adult parameter in each survey, as well as the (minor) modifications used in the revision. Finally, section 5.5 provides a synthetic description of the strengths and weaknesses associated to each estimate and section 5.6 states the opinion of this Group on the most appropriate course of action for the use of sardine DEPM estimates in assessment.

5.2 Ichthyoplankton surveys and egg production estimation

The Portuguese and Spanish ichthyoplankton surveys for DEPM estimation were described in detail in the past report of this Group, where the estimates of egg production were tabulated (ICES 2002). At the time though was also recommended that traditional estimation was performed using the more flexible GLM estimator, recommendation that has not yet been fully implemented. Further, methodological advances after the 2001 meeting in Lisbon now provide more reliable methods for sea area estimation and egg ageing, and these should be incorporated in traditional estimation. In the light of the above, all sardine egg production estimates should be recalculated using the methodology recommended by the Study Group. However, this revision is mainly necessary for consistency purposes, since a good agreement between existing traditional estimates of egg production and GAM-based estimates (that includes the recommended methodological modifications) has already been observed (see section 2.3.1).

5.3 Adult surveys and sampling

This section summarizes the details of all adult DEPM surveys for the Atlanto-Iberian stock of sardine up to 1999, separately for each country. Similar information for the 2002 survey is provided in section 4.1.1.

5.3.1 Portuguese surveys

In 1988 (27/2 – 1/4), only 11 of the planned 40 fishing stations were performed onboard *RV Noruega*, due to an unexpected interruption of the survey between 9/3 and 20/3 (Cunha et al. 1992). A random sample of 50 fish was collected in each haul, and the sex and maturity (macroscopic scale of Pinto and Andreu 1957) of each fish was immediately determined. In 3 hauls, non-random samples of hydrated females were obtained for the estimation of batch fecundity. Biological sampling was performed onboard and all fish (intact males and gonad-free females) were frozen for additional biological analysis in the laboratory (otoliths were removed and ages were read for all fish). To increase adult parameter information, fish from 6 commercial purse seine sets (all south of Lisbon) were also obtained, but these samples were not used in spawning fraction estimation because the gonads were not immediately separated and fixed. Overall, data from 17 fishing stations were used for the original estimation of mean weight, sex ratio and batch fecundity (Cunha et al. 1992), but only 9 for the estimation of spawning fraction (Pérez et al. 1992a). During the recent revision, only the original data from the research survey (11 hauls) were encountered. The histological data were recovered from Pérez et al. (1992a) for spawning fraction estimation and from computer files in old format for batch fecundity estimation, although minor inconsistencies in the reported numbers were detected. Cunha et al. (1992) provide estimates of all DEPM parameters and spawning biomass for the entire survey, and separately for two strata (western and southern), but the latter were never used due to the limited coverage in the southern stratum. Unlike the Spanish survey (see section 5.3.2), the DEPM estimates of spawning biomass in the 1988 Portuguese survey (total or by strata) were considerably lower (almost a third) than the acoustic estimates of biomass for ages 2 and above.

In 1997 (1/3 – 25/3), fish samples for DEPM were collected onboard *RV Noruega* as part of a routine acoustic survey for sardine (Cunha et al. 1997). Of the 49 fishing stations performed, sardine was only caught in sufficient numbers for biological sampling in 26 (in another 2 sardine was present but in small numbers). Sampling in each haul followed the

procedure of the 1988 survey. Cunha et al (1997) provide a preliminary estimate of SSB for 1997 based on F and S estimates from 1988 (the report was made soon after the completion of the survey). Gordo et al. (1998) revised the estimate of Cunha et al. (1997) after completion of part of the histological examination of gonads (using only data from 11 hauls). Gonads from the remaining 15 hauls were processed at the time, but the histological analysis was not completed. Laboratory work was resumed and concluded in 2002 and all histological preparations were reviewed to increase the sample size for spawning fraction estimation in that survey. Post-stratification was never considered for that survey and, once more the DEPM estimate of spawning biomass was almost a third of the acoustic estimate of biomass.

In 1999 (5/1 – 5/2), only 7 fish samples were collected onboard *RV Noruega*, all in the Spanish region of the Gulf of Cadiz. Fish sampling in Portugal took place onboard *RV Mestre Costeiro* (demersal trawling) and from commercial purse seine sets. Overall, sardine samples were made available from 40 stations, but only 35 were used for spawning fraction estimation (due to the long interval from sampling to fixation in the remaining 5). Stratoudakis et al. (2000) provide estimates of the adult parameters and SSB from this survey. Stratoudakis et al. (2000) also indicated that there was some evidence of spatial structure in the adult parameters during the 1999 survey (particularly for spawning fraction) which was confirmed by Stratoudakis and Fryer (2000) that also showed that spatial structure in combination with unbalanced, opportunistic sampling could have led to negative bias in this case (see section 4.1.4). Nevertheless, the spawning biomass estimate used in assessment did not consider post-stratification to maintain comparability with previous estimates.

During the revision, individual fish data from biological, histological and fecundity samples were recovered for all surveys. This permitted repetition of all calculations for adult parameter estimation and confirmation/revision of the original estimates, which were included in a common database with estimates by haul from all Spanish surveys. The only exceptions are for 1988, where data from the seven commercial samples were not found and where histological information is only available (per haul) from Pérez et al. (1992a). Table 5.3.1.1 summarizes adult sampling in each Portuguese DEPM survey for sardine performed so far and Figure 5.3.1.1 shows the location of adult fish sampling in the four surveys. Intensity of adult sampling has been improving over time, reaching a point in 2002 that sufficient information is gathered to explore spatial trends in adult parameters and fit flexible spatial models (see section 2.4.1). Clearly, the levels of sampling achieved in 2002 should be maintained in future surveys to permit better understanding of sardine spawning dynamics and more precise estimates of spawning biomass.

5.3.2 Spanish surveys

In 1988 (29/3 – 6/5), 30 sardine samples (from 44 fishing stations) were obtained with pelagic trawling onboard *RV Cosa*. Although some problems with rocky substrate and inaccessibility due to set gears were encountered, adult samples were obtained throughout the western Galicia and the Cantabrian Sea (Garcia et al. 1992). Otolith samples were obtained and spawning biomass was also estimated by age class. Adult parameter and spawning biomass estimation was separately performed in three strata (post-stratification into Galicia, western and eastern Cantabrian), following the stratification adopted in Spanish acoustic surveys (Garcia et al. 1992). The post-stratified estimate of mean female weight revealed a significant eastwards trend (bigger/older fish in the inner Bay of Biscay), in accordance with previous findings from the northern Spanish coast (Porteiro et al. 1986). The post-stratified estimates of spawning biomass were very close to the acoustic estimates of total biomass (Garcia et al. 1991, Garcia et al. 1992).

In 1990 (17/4-10/5) 14 sardine samples were obtained with pelagic trawling onboard *RV Igor Pvllyunchenkov*. Otoliths were again sampled and spawning biomass was also estimated by age class. Once more, adult sampling was often impeded in coastal zones with significant acoustic marks due to rocky bottoms and presence of set gears (Garcia et al. 1991, Garcia et al. 1993). Despite the lower sampling intensity, adult parameter and SSB estimation followed the same post-stratification criteria that were set in 1988, with natural consequences in the precision of the estimates provided. However, the DEPM estimates of spawning biomass were again very close to the acoustic estimates of total biomass, both overall and by stratum.

In 1997 (20/3-29/3) only 9 sardine samples were obtained with a pelagic trawl onboard *RV Thalassa*, despite the fact that 30 fishing stations were performed. The Galician coast was void of fish and sardine eggs in that year, while in the western Cantabrian large schools of sardine were detected near the coast, but could not be sampled due to rocky bottoms and set gears (Lago de Lanzós 1998, Quintanilla and Pérez 2000). As a result, adult samples were only collected in the eastern Cantabrian Sea. However, post-stratification was considered like in previous years, although, in practice, only egg production was separately estimated in each stratum (Lago de Lanzós et al. 1998). The 1997 estimate of sardine spawning biomass is considerably lower than those in 1988 and 1990. The survey also revealed considerable modifications in the distribution (no sardine in Galicia, inshore, dense schools in western Cantabria and inshore dispersed schools of sardine mixed with other species in eastern Cantabria) and demographic composition (dominance

of age 2 rather than age 5 and above in eastern Cantabria) with respect to the previous DEPM surveys of 1988 and 1990 (Lago de Lanzós et al. 1998).

In 1999 (6/3 – 23/3) 7 sardine samples were obtained with a pelagic trawl onboard *RV Thalassa* and 3 with a chartered commercial purse seiner (Bernal et al. 2000). Although sampling problems did not allow the estimation of spawning fraction in that survey (ICES 2000), histological data from 3 hauls are available (Pérez, pers. comm.) and this information can be used instead of assuming the 1997 estimate. Due to the problems in spawning fraction estimation and the limited number of adult samples, post-stratification was not considered in 1999. Overall, the 1999 survey confirms the marked decline in sardine abundance off northern Spain and the modification of its distribution in comparison to the surveys in 1988 and 1990.

Revision of the Spanish DEPM data has only permitted so far the creation of a common adult database with estimates by haul (i.e. the Spanish adult data are not still available for each sampled individual). Estimation for each survey was repeated based on this data set, but discrepancies with the reported estimates were found in several cases. Unfortunately, it was not possible to identify the reason for these discrepancies during the course of the meeting, making necessary the delay of final decision until the recovery of all individual data available. Table 5.3.2.1 summarizes the sampling effort for adult fish in the sardine DEPM surveys off northern Spain and Figure 5.3.2.1 shows the location of adult samples collected in the five surveys. Up to 1999, adult sampling effort has been gradually declining, partly due to the reduced abundance of sardine (in 1997 30 hauls were performed but sardine was only caught in 9) and the observed changes in its distribution (the inshore distribution in later years aggravated the sampling problems due to rocky bottoms and presence of set gears). However, in 2002 this tendency was inverted and for the first time 102 adult samples became available for adult parameter estimation of the Atlanto-Iberian stock of sardine (28 in Spain and 74 in Portugal).

5.4 Adult parameter estimation

This section describes, separately for each adult parameter, the method of estimation and the data used in each DEPM application for sardine in the two countries. Data and method revisions are highlighted.

Mean Female Weight (W): In 1988, W was calculated from the adjusted female weight, based on a regression between gonad-free female weight and total female weight (Cunha et al., 1992; Garcia et al. 1992). The same procedure has been adopted in all remaining Spanish surveys (Garcia et al. 1991, Lago de Lanzós et al. 1998, Bernal et al. 1999) and the 1997 Portuguese survey (Cunha et al. 1997, Gordo et al. 1998). This procedure aims to avoid positive bias introduced by the temporary hydration of female gonads near spawning (Picquelle and Stauffer 1985), by providing an estimate of total weight based on gonad-free female weight. Stratoudakis et al. (2000) abandoned the above method in the 1999 survey, when it was observed that estimates based on the regression of Cunha et al. (1992) were higher than when the raw data on total female weight were used. As a result, in 1999 total female weight was calculated from the sum of gonad-free and gonad weights, after correcting for the gonad weight gain during fixing and after eliminating fish that were macroscopically identified to be near spawning (macroscopic maturity stage IV). During the revision, it was confirmed that the regression model used by Cunha et al. (1992) led to higher estimates of W in all Portuguese DEPM surveys, so the method used by Stratoudakis et al. (2000) was applied to all years. Table 5.4.1 summarizes all available mean weight estimates for Portugal and the Spanish strata.

Batch fecundity (F): In 1988, a linear model was fitted to the combined batch fecundity data from Portugal (35 fish from 3 hauls) and Spain (89 fish from 7 hauls). Preliminary exploration of the data revealed non-significant country effects and, although the weight range in the samples from the two countries was very different (considerably larger fish in the Spanish survey), a common regression model was adjusted to individual batch fecundity as a function of female gonad-free weight (Pérez et al. 1992b). The same regression model was used during the preliminary estimation of the 1997 survey (Cunha et al. 1997), although the original estimate of F was later adjusted after treatment of the 1997 samples (Gordo et al. 1998). Nevertheless, the latter report does not provide sufficient details on the selected regression model. In all surveys up to 2002, the same methodology has been applied separately to the data from the two surveys and the two parameter linear model was maintained even in cases that the estimate of intercept was non-significant. Table 5.4.2 summarizes the regression models used to describe batch fecundity in each survey and the respective estimate of relative fecundity, while Table 5.4.3 provides all estimates of batch fecundity. It should be noted that for the Portuguese 2002 survey of 2002 an alternative estimator was used (GLM with Gamma distribution and identity link) but the difference with the traditionally used weighted linear regression was minimal (see section 4.1.3).

Sex Ratio (R): In 1988, R was estimated as the weight fraction of the females in each haul, using total fish weights (Cunha et al. 1992). In 1997 estimation was based on gutted weights during the preliminary estimation (Cunha et al. 1997), while Gordo et al. (1998) do not clarify on the method of estimation during their revision. In most other surveys it is also unclear whether total or gutted weight has been used in estimation, while in most Spanish surveys it is

indicated that all male fish are used in the calculation (in the Portuguese surveys only macroscopically mature fish are considered). Table 5.4.4 shows the existing estimates of sex ratio.

Spawning Fraction (S): Apart from the 1990 survey (only used Day-1 POFs), in all other surveys S has been estimated from the composite sample of females with Day-1 and Day-2 POF in their gonads. POF dating (with the imitations described in section 3.4) has been mainly based on the classification of Pérez et al. (1992a), which is composed of four day classes: Day-0 (0-6 hours old POFs); Day-1 (18-30 hours old); Day-2 (42-54 hours old); Day-3 (POFs older than 60 hours). In cases that evidence of under or oversampling for the Day-0 class were evident, a bias-correction factor was introduced. All Portuguese data from 1997 onwards have been recently revised, and for 1997 the number of histologically examined gonads has been extended to consider samples from another 15 hauls that were originally not used due to time limitations. During this revision, two elements differed from the previous work:

- ✓ In 1997 and 1999, the density of POFs was also counted (number of POFs per area of the histological slide). A significant decrease in the number of POFs per unit of histologically examined area (Figure 5.4.1) was observed between Day-0 and Day-2. Given that routine POF dating is performed based exclusively on morphological criteria, this difference in density provides some external confirmation on the validity of datings: old POFs are known to be smaller, thus reducing the probability of detection per unit of gonad area.
- ✓ A different classification for POFs dating was used, composed only of three day classes (Day-0, Day-1, Day-2). This dating was based not only on the morphological characteristics of the POFs, but also on the information of the sampling time (and choosing arbitrarily 19.00 as the peak spawning time): Day-0 POFs correspond to the oocytes released between 19.00 of the spawning day and 19.00 of the following day; Day-1 to those released 25 to 48 hours before; Day-2 to those released more than 48 hours before. This classification was adopted for the DEPM sardine revision because no morphological differences were observed to justify a longer POF deterioration period.

POF dating revision was performed by two readers. In 1999 the new reader performed an independent estimation and the results were very satisfactory. Based on this experience, the slides of 1997 and 2002 were read jointly by the two readers with the common criteria established, leading to S estimates that are more than 3 times lower than for 1999. As it has already been described in sections 3.3 and 4.1.3, the very low S estimate for 2002 is plausible due to the series of indications that spawning activity in January 2002 was atypically low. However, this is not the case for 1997, where the revised estimate is four times smaller than the previous one, leading to an unrealistically high estimate of spawning biomass. This strange result was discussed during the group meeting and three hypotheses were put forward:

- ✓ Material deterioration: Histological analysis was completed 5 years after sampling. Although the gonads were immediately fixed and processed in paraffin blocks, it is possible that deterioration occurred within this long period, preventing the correct identification of all POFs. However, this is not considered very likely, since the experience of the two readers indicated that the general aspect of the 1997 slides was not distinct from that in other years
- ✓ Inadequate definition of POFs: Unlike the other two years, in 1997 some structures that resembled very old follicles were excluded from the revised estimates because of doubts on whether these structures were real follicles; despite the fact that only Day-1 and Day-2 POFs are considered for S estimation, it is likely (in combination with the third hypothesis) that S was underestimated due to the exclusion of these structures;
- ✓ Temperature-dependent POF degradation: In March 1997, seawater temperature was uncharacteristically high (more than 1.5° C higher than in all other DEPM surveys). Given that the rate and duration of follicles resorption is likely to be temperature-dependent (Fitzhugh and Hettler 1995 and section 3.4.2), higher temperatures would have led to a faster deterioration of POFs leading to an erroneous dating. This, together with the exclusion of the atypically old POF-like structures (hypothesis II above) is a very plausible reason for the very low S estimate in 1997.

Table 5.4.5 shows the estimates of spawning fraction in all surveys after the revision. Clearly, additional research is needed for the 1997 Portuguese estimate, for which the recommendation in section 3.4.2 is particularly pertinent.

5.5 Uncertainties in available DEPM estimates

In summary, the main modifications introduced in the revision of the Portuguese surveys are:

- ✓ The exclusive use of research vessel samples in 1988 (slightly reducing the precision of all adult parameters other than spawning fraction)
- ✓ The modification of the estimation methodology for mean weight (providing slightly lower estimates of mean weight and batch fecundity in 1988)
- ✓ The revision and addition of previously unobserved histological slides for spawning fraction estimation in 1997.
- ✓ The repetition of all calculations using an identical methodology for all surveys.

Figure 5.5.1 shows the distribution of all adult parameter estimates within each Portuguese DEPM survey after the revision. Mean weight and sex ratio have been very stable in the last three surveys, but had lower values in 1988. Batch fecundity and spawning fraction were very low in 2002. Batch fecundity was also low in 1988, but this was mainly due to the smaller size of females and not due to a reduction in relative fecundity. The main modification from this revision is the extremely low estimate of spawning fraction for 1997 (see section 5.4), which leads to an unrealistic estimate of spawning biomass. In the case of the Spanish surveys, revision has provided the raw data from all ichthyoplankton surveys, but only the estimate of each adult parameter in each sampling station. Based on these data, estimation was repeated for each parameter, stratum and survey, but in many cases the results were diverging from the reported ones. This indicated that fully revised estimates from the Spanish surveys can only be provided after the recovery of raw data from adult sampling has been concluded. In the meanwhile, the only revision possible is for 1999, where an estimate of spawning fraction based on a small number of hauls is preferable to using the 1997 estimate that has been assumed so far (ICES 2002).

Table 5.5.1 summarizes the revised estimates of spawning biomass in all surveys after the revision. To maintain Portuguese estimates comparable, the unstratified estimate of 2002 is tabulated, despite the fact that the stratified estimate is considered more reliable (section 4.1.4). Revision had a minor impact in the Portuguese survey of 1988 and the Spanish survey of 1999 (in both cases increasing the biomass from 115.1 to 129.1 and from 10.4 to 13.4 respectively) and a major impact in the Portuguese 1997 survey (increasing the biomass from 127.2 to 590.3). However, this Table is only presented to illustrate the impact of the revision performed so far and is not to be considered for assessment purposes (see section 5.6).

Table 5.5.2 summarizes the main coverage, sampling and estimation characteristics of the 5 DEPM surveys dedicated to the Atlanto-Iberian stock of sardine. This summary also considers the 1990 Spanish survey, which, albeit never used in assessment, provides useful information for the northern Spanish coast at a period of an apparent transition in sardine spawning. An important message from this Table is that the first two surveys (1998 and 1990) did not cover adequately the stock area, thus spawning biomass cannot be estimated for the entire stock. In 1988, the problem is not only related to the lack of sampling in the Gulf of Cadiz, but also to the very limited adult sampling in the area south of Lisbon (see Figure 5.3.1.1). Given that more recent Portuguese surveys provide evidence of spatial patterns in adult parameters, reliable adult parameter and spawning biomass estimates can only be provided for the western Portuguese coast in that year. In 1990, only Spain performed a DEPM survey, and this information has never been used in assessment. Nevertheless, the 1990 survey followed the same methodology applied in 1988 and, although adult sampling was more limited, the survey provides useful information on sardine egg distribution and a reliable estimate of egg production and spawning biomass for northern Spain. From 1997 onwards, coverage is adequate and in the two most recent surveys (1999 and 2002) additional information on sardine egg distribution and production can be obtained for the Bay of Biscay. This extra information will be analyzed within the remit of the EU project SADRYN and will provide additional insight into the spawning dynamics at the northern boundary of the Atlanto-Iberian stock. However, in 1997 and 1999 the distribution of sardine in northern Spain was very reduced in comparison to the late 80s, resulting in a limited number of adult samples for these years. Finally, additional revision is required for the 1997 Portuguese survey (verification of low spawning fraction estimate) and the 1999 Portuguese (use post-stratification) and the 1999 Spanish survey (clarify the most appropriate estimate of egg production based on the two coverages of western Cantabria).

5.6 Recommended use of sardine DEPM estimates in assessment

Up to the last sardine assessment (ICES 2003), sardine SSB estimates from the 1998, 1997 and 1999 DEPM surveys were used, despite the known problems of limited coverage in 1988, few samples in 1997, and the potential bias due to the lack of post-stratification in Portugal in 1999. Since then, the estimate from the 2002 survey has become available (section 4.1), where it was confirmed that post-stratification provides more reliable estimates for both national surveys. Overall, the intensified adult sampling in 2002, the accumulated experience from the revision of sardine DEPM data and their comparison with the data from the Bay of Biscay for anchovy, lead to the conclusion that there is considerable spatial structure in both adult and egg sardine parameters, and their distribution and magnitude can vary among years. To obtain an unbiased estimate of sardine spawning biomass, these spatial patterns need to be adequately captured,

particularly in years that there is a very heterogeneous fish distribution within the survey area (Stratoudakis and Fryer 2000). As a result, reliable estimates for assessment can only be provided for areas/years that adequate adult data exist either to post-stratify or to fit a GAM.

Within the past year, considerable laboratory effort was invested by IPIMAR and IEO (histology for 2002, additional histology and data revision for previous years), which was just concluded within weeks from the group meeting in Malaga (in time to provide an estimate for the 2002 survey). This, together with the considerable effort invested by most members of this Study Group for the successful completion of the GAMs project (concluded in May 2003), did not leave sufficient time to complete the revision and assimilate sufficiently the implications from GAM-based estimation. For example, the application of adult GAMs was only developed just prior and during the study group meeting, where it was demonstrated that model selection is of crucial importance. Also, variance estimation in GAMs is very computer intensive (in the case of egg production, it required more than a week of computing time for one estimate), while software is not yet adapted to include adult bootstrap. As a result, the SG is not currently in a position to provide final estimates of SSB for all years, for that it recommends a distinct course of action for the use of sardine DEPM estimates in assessment in the short and the medium-term.

Based on the work performed up to this study group and the uncertainties described in section 5.5, we recommend that for sardine assessment in 2003 the following information becomes available to the WGMHMSA:

- ✓ a series with 5 points (1988, 1990, 1997, 1999 and 2002) for northern Spain.
- ✓ a series of 3 points (1988, 1999, 2002) for western Portugal.
- ✓ a series of 2 points (1999 and 2002) for the stock area.

This information will become available through a Working Document that will be prepared by the members of this study group, focusing on the following:

- ✓ Repeat estimation for Portugal in 1988 using only data from the western stratum.
- ✓ Post-stratify the 1999 Portuguese survey.
- ✓ Review the reported Spanish estimates up to 1999.
- ✓ Fit an adult GAM for 1999 and refine the 2002 GAM.

With this approach, the Group will provide spawning biomass estimates for the stock only in years that it considers estimation to be reliable (1999 and 2002). These two estimates could be considered to provide an absolute estimate of stock biomass at the time of the surveys. However, simple observation of the egg distribution across surveys demonstrates that considerable changes in the spawning dynamics of sardine have occurred in the start of the 1990s. To allow some of this information to be used in assessment, the Group has also decided to provide the two series of spawning biomass estimates for subareas of the stock with contrasting temporal evolution: the series of 5 points in northern Spain (where the 1990 estimate will also be included for the first time) and a series of 3 points for western Portugal (where a reliable estimate for 1998 can be obtained). These series would inevitably have to be used as relative indices of abundance in routine assessment, but could be used as absolute in corresponding area-based assessment exercises.

For the reasons that have been described in the previous sections, the provision of finally revised estimates of sardine spawning stock biomass from the DEPM had to be postponed until the next meeting of the Group (probably in November 2004). Until then, the group suggests that the following action is taken:

- ✓ Recovery of all Spanish adult data is completed.
- ✓ Additional information to evaluate the reliability of the 1997 Portuguese estimate of spawning fraction is collected.
- ✓ Post-stratified estimates of egg production are repeated for all surveys following identical criteria of area delimitation, egg ageing and sea area calculation (using depmodel).

- ✓ A final series of sardine DEPM estimates based on GAMs is provided by a sub-group with experience in GAM fitting for anchovy and sardine.

Based on the outcome of these actions, will be possible during the next SG meeting to provide a final series of sardine DEPM spawning biomass estimates. In addition, evaluation of the performance of GAMs in spawning biomass estimation for both species and for several surveys (including variance estimation) will help the group to take a final decision on whether GAM estimation of spawning biomass can become the adopted standard method for DEPM. In the case of sardine, the GAMs will include the Spanish data from the French coast (eggs and adults) and distributions for the whole area will be provided (although SSBs will be provide only within the stock area). These distributions will provide additional information on the situation at the northern boundary of the stock. With this approach, this SG will be in a position to provide a definite series of reliable DEPM estimates for sardine and auxiliary information for the species dynamics to the assessment group meeting of 2005.

Table 5.3.1.1 Summary of adult sampling in Portuguese DEPM surveys for sardine.

Variable	March 1988	March 1997	January 1999	January 2002
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	1300	3824	5302
Histology stations	9	26	35	67
Fish histology (mature)	186	420	660	1350
Fish fecundity	37	31	75	113

Table 5.3.2.1 Summary of adult sampling in Spanish DEPM surveys for sardine. The 2002 survey includes 4 adult samples from the French coast.

Variable	April 1988	March 1990	March 1997	March 1999	April 2002
Fishing stations (total)	30	14	9	10	32
Fishing stations (commercial)	-	-	-	3	13
Gear	MT	MT	MT	MT, PS	MT, PS
Fish sampled (total)	1119	587	491	524	2222
Histology stations	30	9	9	3	21
Fish histology (mature)	611	333	314	60	389
Fish fecundity	89	68	155	116	73

Table 5.4.1 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 5.4.2 Regression models (intercept and slope) for batch fecundity estimation and mean relative fecundity (F_R) in the DEPM surveys for the Atlanto-Iberian stock of sardine.

Year	Country	Intercept	Slope	F_R	Reference
1988	P + S	-1184.7	443.3	427	Pérez et al. (1992b)
1990	S	370.7	390.0	NA	Garcia et al. (1991)
1997	P	NA	NA	NA	-
	S	-17192.5	705.7	408	Quintanilla and Pérez (2000b)
1999	P	-89.3	437.1	435	Stratoudakis et al. (2000)
	S	9046	244.3	415	Bernal et al. (2000)
2002	P	-4286	464.3	346	Stratoudakis et al. (2003)
	S	-3255	436.3	394	Pérez (pers. comm.)

Table 5.4.3 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 5.4.4

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 5.4.5

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 5.5.1

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 5.5.2

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 (very 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 appropriate 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

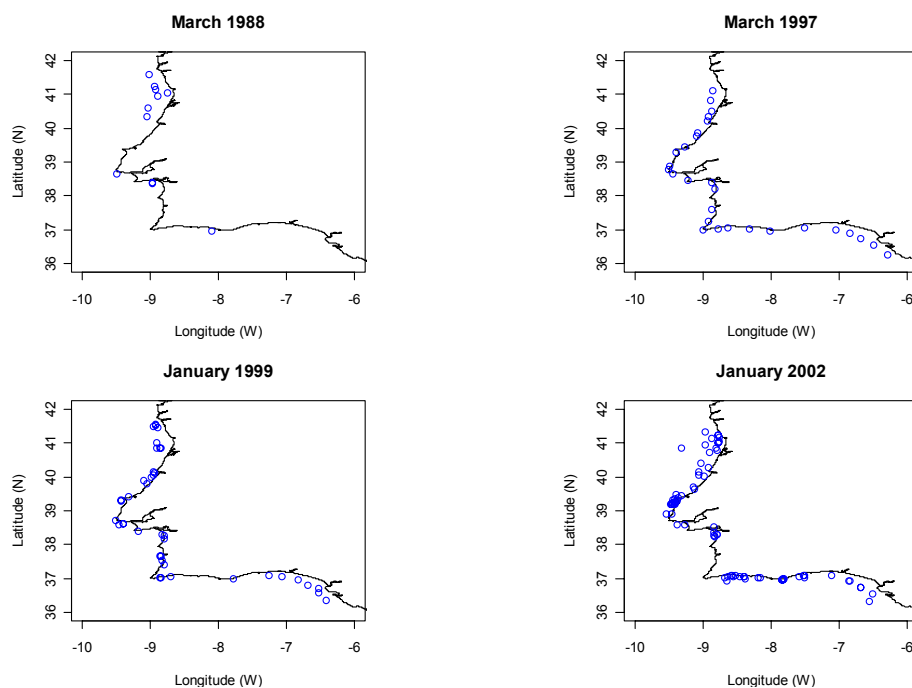


Figure 5.3.1.1 Location of adult sardine sampling during the Portuguese DEPM surveys.

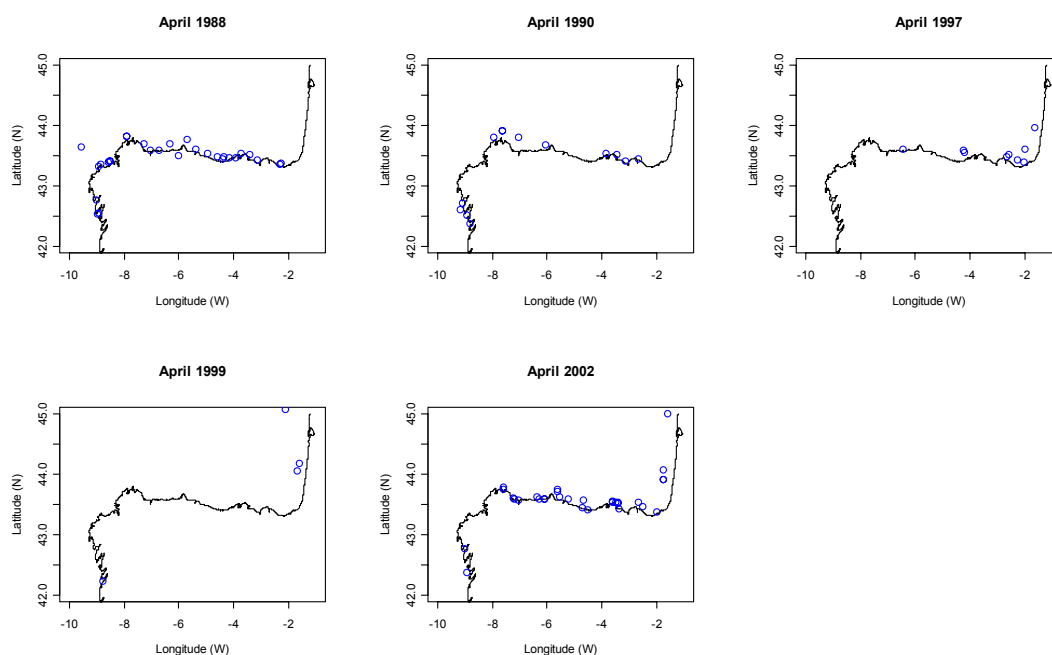


Figure 5.3.2.1 Location of adult sardine sampling during the Spanish DEPM surveys.

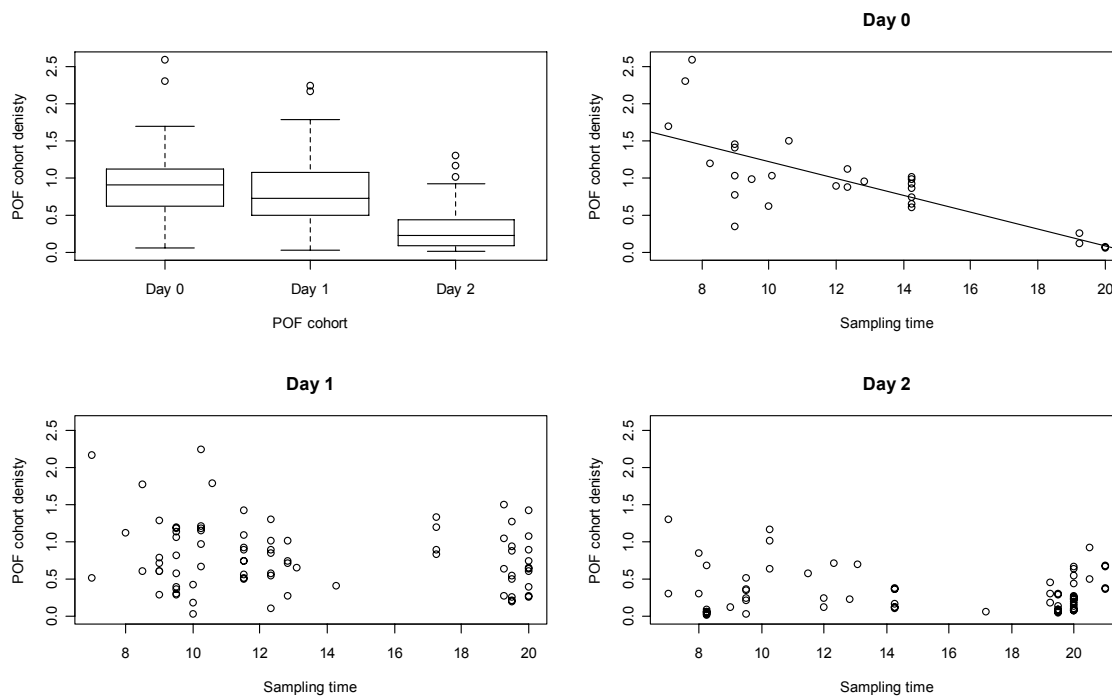


Figure 5.4.1 Mean density of POFs (number per unit of histologically examined area) for each daily cohort in sardine gonads from the 1999 survey (upper left) and evolution of density over sampling time, separately for each daily cohort.

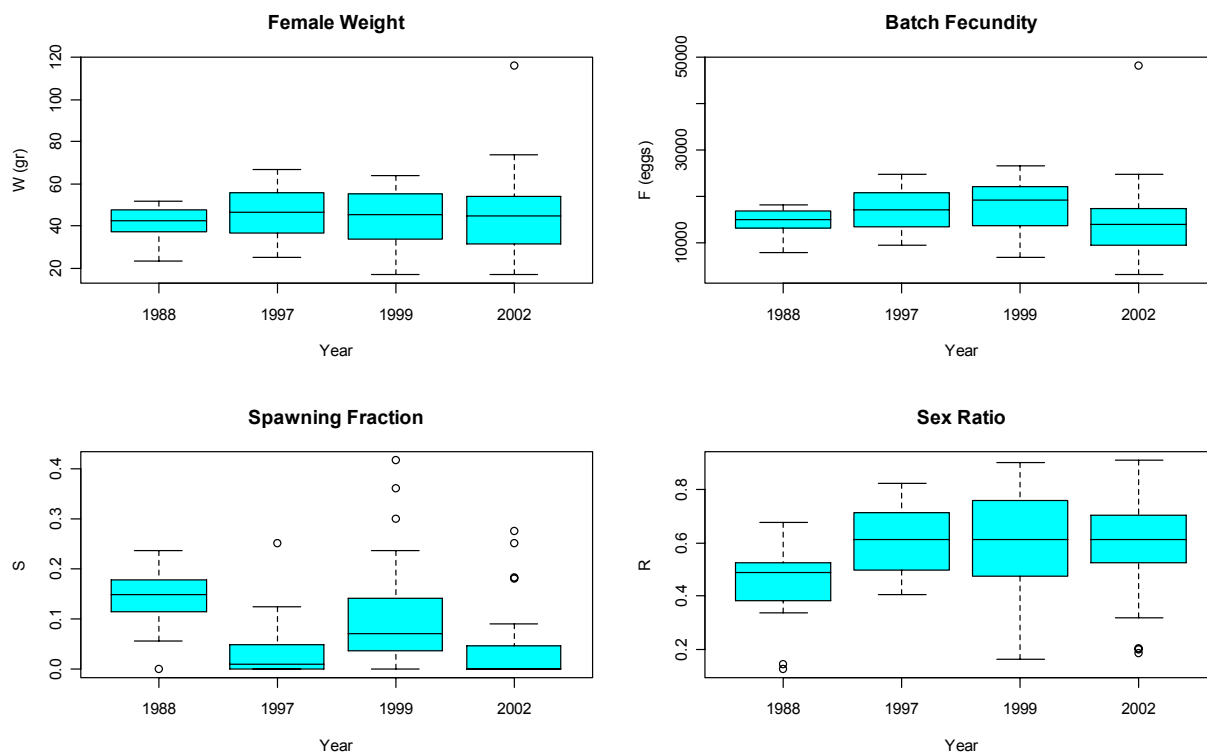


Figure 5.5.1 Boxplot of Portuguese estimates of adult parameters in each DEPM survey after the revision.

6 PROTOCOL AND CRITERIA OF STANDARDIZATION FOR SURVEY GEARS

6.1 The CalCOFI Vertical Egg Tow (CalVET)

6.1.1 The sampler and its deployment

CalVET is a double ring (\varnothing 30 cm \Rightarrow 0.05 m² of surface) net sampler used in high-speed (70m/min) vertical hauls with a motionless vessel (Smith et al. 1985). PAIROVET is the term used when samples from both CalVET nets are considered (for example, the egg sample from one net - CalVET - is used in the Iberian sardine surveys, the egg sample from both nets - PAIROVET - is used in the Bay of Biscay anchovy surveys). It was devised to reduce sorting time for abundant pelagic fish eggs, for which normal oblique tows with larger samplers (e.g. Bongo) would produce unnecessarily large samples per tow (Smith et al. 1985). In DEPM applications for sardine and anchovy in the northeastern Atlantic, a net with mesh aperture of 150 μ m is used to avoid extrusion of early anchovy and sardine eggs. For the estimation of total daily egg production the whole depth range of the egg distribution has to be covered. Given that sardine and anchovy eggs are known to be mainly concentrated in the upper part of the water column (Lopez-Jamar et al. 1991, Olivar et al. 2001) the net is usually deployed until the bottom or a maximum depth of 150m/100m for sardine and anchovy respectively, left at maximum depth during 10 seconds for stabilization and then retrieved to the surface at a rate of approximately 1 m/sec. To achieve verticality of the hauls a depressor of at least 25 kg is attached to the end of the cable and a inclinometer is used to determine the departure of the cable from vertical (i.e. a 0° angle corresponds to a perfectly vertical tow). Hauls whose angles are higher than 30° should be discarded and the haul repeated. Angles of accepted hauls should always be registered. Recently calibrated flowmeters should be used in each of the CalVET/PAIROVET nets and the amount of released cable must always be registered. After hauling, nets should be washed from the outside with salt water under pressure. Besides preventing clogging, flushing concentrates the sample into the cod-end and facilitates retrieval. It is also important to determine the temperature of the water column in each station, since egg development is dependent on temperature (see section 3.2.2). To facilitate the work onboard, as well as to save ship time, it is advisable to use a CTD coupled to the egg sampler. After collection, plankton samples should be preserved in 4% saline formalin solution according to the following procedure: plankton is washed from the net cod-end into a jar with salt-water until reaching a known volume after which the correct amount of 38 % formaldehyde solution is added to obtained a final concentration of 4%.

6.1.2 Estimation of sampled volume and area

The amount of water filtered by the CalVET/PAIROVET can be determined based on the length of the released cable times the mouth aperture of the net, when clogging is not considered a problem. Alternatively, readings from recently calibrated flowmeters may be used to determine the length of the haul. Comparison of flowmeter readings with the length of the cable released and the towing angle can provide useful indications on whether problems related to underwater currents, slight motion of the vessel or clogging (Smith et al. 1985, see section 6.1.5).

6.1.3 Selectivity

Selectivity problems arise when the target organism (eggs in this case) can pass through the meshes of the sampling gear. No selectivity problems have been reported for sardine and anchovy eggs targeted by CalVET (which are more than an order of magnitude larger than the mesh opening used). In the case of anchovy (whose egg is oblong), Smith et al. (1985) calculated that any net with less than 400 μ m would be adequate to retain all anchovy eggs.

6.1.4 Catchability

Catchability problems arise when the target organism can avoid the sampling gear. In the case of eggs this is not an issue, and CalVET is an efficient sampler for abundant pelagic fish eggs. However, the small size of the sampler and its application with a motionless vessel suggest that catchability problems may be encountered when fish larvae are also targeted. This only becomes important for DEPM applications when early larval stages are included in estimation (e.g. Hunter and Lo 1997).

6.1.5 Sampling efficiency

The sampling efficiency of CalVET can also be affected by factors other than those related to selectivity and catchability of gears for mobile organisms. The design objective of the CalVET was to filter a uniform and measured volume of water from the deployment depth to the surface (Smith et al. 1985). Ship drift and undersea currents, net clogging and cyclic vertical motion of the towing cable due to the heave and roll of the ship can all affect the efficiency of the sampler. Ship drift and undersea currents distort the distribution of water filtered per unit of surface area to a

given depth. This is best resolved by repeating hauls that have a towing angle more than 30° (Smith et al. 1985). Net clogging leads to the relative undersampling of the shallow vs the deep water layer in a tow. Although the design of CalVET makes clogging unlikely, careful washing of the net after each haul and comparison of the flowmeter reading to the length of released cable can provide additional guarantees. Finally, excessive heave and roll can at times impart velocities to the net below or in excess of design expectations. When downward velocity exceeds the recovery rate of the net, the net can collapse and expel the eggs from the mouth of the net. On the contrary, increase of recovery speed by roll and upward heave can lead to egg damage or extrusion. To diminish these effects, the net should be lowered at 1-1.2 m/s, left for 10 s at maximum depth and retrieved with the same constant speed. Tows that vary substantially from this regimen should be repeated.

6.2 The Continuous Underway Fish Egg Sampler (CUFES)

6.2.1 The sampler and its deployment

The Continuous Underway Fish Egg Sampler (CUFES) was introduced in the 1990s (Checkley et al. 1997) to sample the highly contagious distribution of pelagic fish eggs. It consists of a submersible pump, concentrator, electronics and sample collector. Water is continuously pumped at approximately 600-700 liters per minute from 3 m depth to the concentrator, where particles are gathered by an oscillating net in about 3% of the flow. For sardine and anchovy eggs a mesh size of 500 µm is used to both maximizing the efficiency of retention of eggs and minimizing retention of smaller particles and clogging. However, occasionally the concentrator becomes clogged. Then the CUFES needs to be turned off and the concentrator mesh has to be cleaned with water under pressure. The concentrated material passes to the sample collector, where the particles are retained.

CUFES operates continuously at nearly all sea conditions. In the case of sardine and anchovy plankton surveys, CUFES is incorporated to the sampling design according to the procedure agreed for the PELASSES project. Stationary CUFES samples are taken simultaneously to vertical plankton tows (CalVET/PAIROVET) for a minimum period of 4 minutes whereas underway CUFES samples are taken for 1.5 nautical miles (10 knots) before and after each station. The samples are checked immediately for sorting out and counting anchovy and sardine eggs. Then, they are fixed in a 4% buffered formaldehyde solution in seawater and kept on 50 ml bottles.

In order to transform number of eggs collected per unit time to egg concentration (egg/m³) it is necessary to estimate the flow pumped by the CUFES and a calibrated flowmeter is located just before the concentrator. Simultaneously with pumping, complementary data such as date, time (GMT), position, speed and course of the vessel can be registered. These can be taken from a GPS. In addition, other environmental variables, as temperature, salinity and chlorophyll a fluorescence, can be measured by means of a CTD sensor and a fluorimeter, both plugged to the water circuit. These data allow to obtain a description of the hydrographic conditions in the spawning grounds.

6.2.2 Estimation of sampled volume and area

Sampled volume of water can be obtained either from the calibrated readings of a flowmeter placed in the entrance of the CUFES concentrator or manually, by measuring the time needed to fill a tank of known volume. In the project PELASSES it was found that the volume of water filtered per minute by CUFES varied according to the vessel, speed and year (Table 6.2.2.1). Hence, it becomes clear the need of calibrating the flow pumped by CUFES every time for each vessel at different speeds. Estimation of CUFES egg density by sampling area is still not possible, given that CUFES does not yet provide adequate estimate of egg density for the entire water column (see section 6.2.5).

6.2.3 Selectivity

The use of a 500 µm net in the concentrator suggests that some selectivity problems may be encountered with CUFES, particularly in the case of anchovy eggs (see section 6.1.3). A preliminary experiment recently performed by AZTI demonstrated that selectivity problems indeed exist for anchovy eggs. In a small number of stations, anchovy eggs were also found in the outward water flow (which should be void of eggs if the mesh of the concentrator would be fully selective). In some cases, anchovy eggs in the outward flow were more than those found in the collector (Santos, pers. comm.). Additional work is underway to assess the magnitude of the problem (both for sardine and anchovy) and to propose solutions.

6.2.4 Catchability

Once more, catchability is not an issue for fish eggs sampled by CUFES, while very little is known on the potential of CUFES as a sampler for fish larvae. Relatively small sardine and anchovy larvae and post-larvae are often found in

CUFES samples, but in most cases damaged. However, in a recent Portuguese survey, many intact large sardine post-larvae (ca 3 – 4 cm) were collected by underway CUFES samples in several neighbouring stations in the Gulf of Cadiz (Stratoudakis, pers. comm.). A plausible explanation is that the high suction pressure of the CUFES pump reduces the capacity of gear avoidance in fast-swimming post-larvae. Given that post-larvae of this size have rarely been caught by traditional plankton samplers in Portuguese waters (Cunha, pers. comm.), it is interesting to explore further whether CUFES is an appropriate sampler for late sardine and anchovy post-larvae.

6.2.5 Sampling efficiency

CUFES permits cheap, underway fish egg sampling at the expense of providing estimates of egg density only for the upper few meters of the water column. Research so far has shown that the vertical distribution of sardine and anchovy depends on egg buoyancy (which changes through development) and local oceanographic conditions (which affect the degree of mixing in the water column, see section 3.1.2.2). As a result, the relation of egg density at the upper layer of the water column to the local egg density in the entire water column will vary in relation to the above factors. Further, experiments during the PELASSES project have demonstrated that sampling efficiency can differ between vessels, years and steaming speeds (section 3.1). The above problems condition the use of CUFES as a quantitative sampler of fish egg abundance in the water column, and for that CUFES is used as an auxiliary sampler in DEPM surveys (see sections 6.3 and 6.4).

6.3 Adaptive criteria for DEPM ichthyoplankton surveys

A more precise estimate of egg production and mortality is achieved by increasing the percentage of samples within the area with presence of sardine eggs (positive stratum). Since the area of spawning varies from year to year, the offshore limit of spawning is only determined during the egg survey using *in-situ* evaluation with CUFES to adapt the sampling design to a denser grid of CalVET/PAIROVET stations along transects inside the positive stratum. CalVET/PAIROVET stations are usually performed along transects 8 nm apart. Within the same transect the distance between CUFES sampling should always be 3 nm. CalVET hauls should be 3 nm within the stratum with eggs (positive stratum) and 6 nm in the stratum with no eggs (negative stratum). The decision on the distance between CalVET stations can be based on information from the previous CalVET and/or CUFES stations. The procedure is as follows:

1. When starting a transect from the shore the distance between stations should always be of 3 nm;
2. The decision to change the distance between stations will be based on the results of two consecutive zero eggs stations (negative station);
3. After the third negative station change the course into the next transect;
4. When starting the transect from the offshore, if the first station is negative perform the next CalVET/PAIROVET 6 nm apart (use the information from intermediate CUFES to control the decision);
5. When an intermediate CUFES is positive, perform a CalVET/PAIROVET station in that location and from then on start with CalVET/PAIROVET stations every 3 nm within the positive stratum. In case of 2 consecutive negative CalVET/PAIROVET stations change to 6 nm distance even when close to the coast (the presence of eggs should be controlled by the results from the intermediate CUFES).

6.4 Application in future DEPM surveys

The use of CUFES as a quantitative estimator of egg abundance for sardine is very appealing since it can reduce enormously the cost of the Daily Egg Production estimate. Comparative performance of CalVET and CUFES to capture sardine eggs showed good agreement (see section 3.1.3) but we are still on a stage of qualitative approach. The model that will enable for conversion of CUFES surface sampling of sardine eggs into a quantitative estimator for sardine and anchovy egg abundance on the water column is still in a developing phase (see section 3.1). Therefore the egg surveys in 2004 (anchovy) and 2005 (sardine and anchovy) will continue to be CalVET-based, with CUFES providing auxiliary information to allocate stations along transects and/or for decision to define the offshore limit of a transect (Table 6.4.1).

Table 6.2.2.1 Flow (m³/min) estimate stationary and underway (10 knots) for different vessels and years.

Vessel	2000		2001	
	underway	stationary	underway	stationary
R/V INVESTIGADOR	0.729	0.646	0.709	0.625
R/V THALASSA	0.602	0.507	0.455	0.428

Table 6.4.1 Details of the main characteristics in future (2004 and 2005) DEPM surveys to be carried out by Portugal and Spain for sardine and anchovy.

2004 /2005 survey	Portugal (IPIMAR)	Spain (IEO)	Spain (AZTI)
Date of survey	January 2005	March-April 2005	May-June 2004 & 2005
Area of survey	Portugal+Gulf of Cadiz	NW and N of Spain	SE Bay of Biscay
Type of haul	Vertical	Vertical	Vertical
Maximum sampling depth	150	100	100
Flowmeter	To know clogging	To know the amount of water filtered	To know the amount of water filtered
Sampler	CalVET	CalVET	PAIROVET
Mesh size	150	150	150
Sampling grid (miles)	8x3	8x3	15x3

In relation to the terms of reference for the present meeting (section 1.1), the following summarizes the main conclusions that were reached during the meeting:

- 2002 estimates for sardine and anchovy: The 2002 surveys for Atlanto-Iberian sardine and Bay of Biscay anchovy were described and DEPM parameters and spawning biomass were estimated (441.6 Kt for sardine and 30.7 Kt for anchovy). Sardine SSB for 2002 is the highest estimate of the series, despite the low estimate of egg production. This is because daily fecundity was exceptionally low in 2002 (particularly in Portugal), contributing also to a relatively low precision in the estimated SSB (despite the considerable increase of sampling effort). Anchovy SSB is one of the lowest recorded in the relatively long DEPM series in Biscay. This is due to the poor recruitment of 2001 which led to the lowest ever estimate of age 1 fish and the first time that age 2 fish were the most abundant cohort in the spawning population.
- Revision of sardine DEPM series: Most egg and adult data from sardine DEPM surveys were compiled, survey details and methods of estimation for each parameter were reviewed and the main uncertainties in existing DEPM estimates were identified. A working document produced by the SG will provide revised estimates for the assessment Working Group in September. However, finally revised estimates will only become available after the next meeting of this group, when a decision on the routine use of GAMs for DEPM estimation will also be taken.
- Timing of future sardine surveys: Analysis of the 2001/2002 spawning season off Portugal demonstrated that sardine spawning is protracted in that area and, although a biologically strange regression in spawning activity was detected in January 2002, January remains a sensible choice for the DEPM survey off western and southern Iberia. Extensive work on sardine spawning seasonality in the northeastern Atlantic is anticipated under the EU project SARDYN, so the group recommends that the timing of future sardine DEPM surveys is only altered (if necessary) in the light of the SARDYN results.
- Use of GAMs in DEPM estimation: Based on report of the EU Study Project on GAMs and the work performed during the meeting, it was concluded that GAMs are in a position to eventually substitute the traditional estimator in DEPM, both for the estimation of egg production and SSB. However, the latter can only be achieved when adult sampling is sufficiently dense in space, covers the entire survey area and takes place sufficiently close in time to plankton sampling. In addition, the first application of GAMs to adult parameter estimation revealed that results are sensitive to model selection criteria, providing a clear warning that routine use of these powerful tools in DEPM estimation will require a blend of modelling skills, familiarity with the survey data and good knowledge of the species biology and dynamics.
- Traditional DEPM estimator: The Group believes that traditional estimation should be maintained in the future for comparative purposes or for situations that GAMs cannot be applied (very sparse data sets, lack of spatial structure, etc.). However, it recommends that sea area estimation and egg ageing are only performed with the new software, while post-stratification is always considered when strong spatial patterns are detected in the survey data.
- Use of CUFES in future surveys: Recent advances in the use of CUFES as a quantitative sampler (comparisons with CalVET/PAIROVET, year and vessel effects, models of vertical egg distribution, etc.), its potential in the testing of DEPM assumptions and its application in the 2002 DEPM surveys are reported. On the light of this evidence, the Group believes that CUFES should be maintained in future DEPM surveys as the secondary plankton sampler, mainly used to delimit adaptively the length of transects and the intensity of vertical sampling within them. The most appropriate use of CUFES in the DEPM will be reviewed once more data on its performance as a quantitative sampler become available (work currently in progress).
- Stage-age models: The methodology and software described, provide a statistically rigorous and efficient solution for routine ageing of staged eggs. The methods were tested with existing and new data from incubation experiments leading to satisfactory results. The Group believes that the new stage-age models should be used in all DEPM applications.
- POF dating and S estimation: POF classification and grouping in daily cohorts remain imprecise and subjective, thus affecting the quality of spawning fraction estimates. However, the recent methodological advances in egg stage-age models provide a sensible way to overcome these problems in the near future.

- Sardine maturity ogive: The calibration of the macroscopic maturity scale used for sardine revealed problems in its application, while the uncharacteristic regression of spawning activity in January 2002 showed that the dynamics of sardine spawning are more complicated than previously thought. Additional work is currently underway to provide a better understanding of sardine spawning dynamics and to identify the most reliable sets of data to be used in routine estimates of maturity-at-age for assessment purposes.
- Standardization of sampling tools: The main characteristics of the plankton samplers used in DEPM surveys and the protocol followed in their deployment were described. The Group believes that the sampling tools and their deployment in the ichthyoplankton surveys of DEPM are satisfactorily standardized, permitting uniform application and comparable results across surveys, countries and species.

8 RECOMMENDATIONS FOR FUTURE RESEARCH

The SG recommends that:

- The next meeting of the Group takes place in San Sebastian (3 days) during the autumn of 2004 (October/November), immediately after the annual meeting of the EU research project SARDYN. The following terms of reference are proposed:
 - Plan 2005 DEPM surveys for anchovy and sardine;
 - Compare traditional with GAM-based estimates of SSB to decide whether GAMs can be recommended as the standard methodology for routine DEPM estimation of sardine and anchovy spawning stock biomass;
 - Map anchovy and sardine egg production, female weight and spawning fraction to describe inter-annual changes in spatial distribution, explore their relation to environmental conditions and describe the dynamics in the northern border of the sardine stock;
 - Create an objective list of POF stages for anchovy and sardine and describe the biological properties of mature/immature and active/inactive fish within the sardine spawning season;
 - Refine models of vertical egg distribution and resolve selectivity problems with CUFES to assess its performance as a quantitative sampler.
- New research is performed to develop POF age-stage models and POF dating procedures, along the lines of egg stage-age models and ageing of staged eggs through the Bayesian methodology.
- The depmodel software is extended to include a function for traditional egg production estimation, as well as for the calculation of the variance of adult parameters and SSB through non-parametric bootstrap.
- A specialist meeting takes place in San Sebastian during the winter/spring of 2004 to fit reliable GAMs to adult parameters of sardine and anchovy for a series of years, providing model-based estimates of SSB.
- Sardine macroscopic maturity data from DEPM and acoustic surveys (autumn and spring) are further analysed to compare the correspondence between ogives and chose the most appropriate sets of data for routine use in sardine assessment.
- The area limits used to provide estimates of catch, acoustic and DEPM abundance at fine spatial scales (below the ICES Subarea level) become uniform to facilitate comparisons for the Atlanto-Iberian stock of sardine. Also, acoustic estimates in the future provide estimates of spawning biomass (in addition to total biomass) to permit direct comparisons with DEPM estimates.
- A summer DEPM survey is performed for anchovy in the southern Iberia (Algarve and Gulf of Cadiz) to improve the biological knowledge on this population (i.e. reproductive parameters during peak spawning) and combine this information with data from Portuguese autumn and spring acoustic surveys to explore whether the recent decline in commercial catches reflects diversification of fishing targets or a population decline.

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10 WORKING DOCUMENTS

Cunha ME, Grilo C, Silveira S (2003) Sardine spawning seasonality off Portugal. This work describes the seasonality of spawning of sardine, *Sardina pilchardus*, in two spawning regions off Portugal, one located in the northern (Figueira da Foz) and the other in southern (Olhão/Portimão) parts of the coast. The period of study was September 2001 to May 2002 and the objective was to clarify differences in spawning behavior off the two regions found in previous years. Results of gonadosomatic indices and spawning fraction show an earlier and longer spawning season in the south when compared to the north. This was also the region with a larger proportion of females spawning per day. During the spawning season the proportion of females presenting atretic structures was very high (almost 100%) suggesting that atresia may be also an indicator of spawning. Based on the presence of atresia on the ovaries, massive spawning seemed to have started in the southern region, in November, while in the north, it occurred in January. These were also the periods when recruitment to spawning in each region occurred. Percent of atresia in the female population also indicate that the period of spawning was longer in the south. In both regions, batch fecundity was higher during January.

Cunha ME, Varela F, Vendrell C, Stratoudakis Y (2002): Sardine (*Sardina pilchardus*) daily egg production off Portugal during January/February 2002 (WD originally presented at the ICES 2002 WGMHMSA, Copenhagen 8-17/9/2002). Spatial distribution and abundance estimates of sardine eggs off the Portuguese coast and Gulf of Cádiz during January/February 2002 were obtained during a cruise of the R/V "Noruega". The Daily Egg Production Method (DEPM) was used to evaluate the sardine biomass in the area. The estimate for Daily Egg Production in the area was 2.07×10^{12} eggs.

Ganias K, Somarakis S, Koutsikopoulos C, Machias A, Theodorou A (2003): Ovarian atresia in the Mediterranean sardine, *Sardina pilchardus sardina*. (MS submitted for publication to *J Mar Biol Ass UK*). Histological analysis was used to describe and analyse the process of ovarian atresia in sardine (*Sardina pilchardus sardina*) from the central Aegean Sea (eastern Mediterranean). The spawning potential of females in relation to intensity of atresia was evaluated and the rate that the ovary passed from the active to inactive condition was followed in fish collected over an annual cycle. Early postbreeding females, defined as those with 100% *alpha*-atretic oocytes, occurred throughout the spawning period; they had lower gonadosomatic and hepatosomatic index, but similar total length compared to reproductively active females.

Silva A, Afonso-Dias I, Amorim P, Nunes C, Soares E (2003): Calibration of the six-stages macroscopic scale used to classify sardine (*Sardina pilchardus*, Walb.) ovaries. Ovaries from sardines collected during acoustic research surveys (177 in November 2000 and 52 additional in other surveys up to March 2002) along the Portuguese coast and data from the DEPM database (number of gonads: 460 in 1997, 636 in 1999 and 574 in 2002) were analysed to evaluate the agreement between macroscopic and microscopic staging of maturity. Overall agreement between macroscopic and microscopic classifications of female sardine maturity is low (about 56%), mainly due to poor consistency within initial development stages (I, II) and post-spawning stages (V, V-III). Using a more generalist classification system, with just five stages would greatly improve the accuracy of ovary classification. However, the macroscopic characteristics of the maturity stages should be carefully reviewed based on a more extensive histological study encompassing the annual reproductive cycle. The presence of atretic oocytes was observed in all stages of development; even immature females had atretic oocytes during the spawning season. The ovarian seasonal changes and evidence of gonad regression in young females were also assessed to try to find out the boundary between immature and mature individuals.

Stratoudakis Y, Nunes C, Soares E, Grillo C (2002): Spawning fraction in sardine DEPM estimation. (WD originally presented at the EU GAMs project meeting, San Sebastian 26-27/9/2002). Spawning fraction (the proportion of mature females spawning at any given day) is the most important adult parameter in DEPM estimation. Unlike fish weight and sex that can be easily and cheaply determined for a large number of individuals, spawning fraction involves costly histological preparations and subjective decisions in the microscopic identification and ageing of post-ovulatory follicles (POFs). Unlike batch fecundity estimation that is model-based (usually a linear model provides an adequate fit

for a reasonable (50-100) number of observations), spawning fraction estimation is sample-based and is therefore affected by inadequate sampling design and small numbers of samples. Thus, spawning fraction is the adult parameter estimated with least precision and with a high potential of bias that is directly reflected on the biomass estimation (as a result of the multiplicative nature of the DEPM estimator). Here, we review the most important aspects in spawning fraction estimation, exploring revised data from the Portuguese DEPM surveys of 1997 and 1999.

Stratoudakis Y, Bernal M, Quintanilla L, Dixon C, Borchers D, Lonergan M, Wood S (2003): Daily egg production in the Atlanto-Iberian stock of sardine: traditional vs GAM-based estimators. Four ichthyoplankton surveys (1988, 1997, 1999 and 2002) were used to estimate the daily egg production and mortality in the Atlanto-Iberian stock of sardine. Eggs at stage of development were transformed into daily cohort densities using a novel procedure for age assignment that relies on data from an incubation experiment and an assumed daily probability density function of spawning. Daily egg production and hourly mortality rate were estimated through a Generalised Additive Model (GAM), where both parameters could be estimated as smooth functions of spatial and environmental variables. Egg production was estimated by the summation of predicted values on a dense regular grid over the explanatory variables space, while its variance was estimated by non-parametric bootstrap. Variation associated to egg ageing, was incorporated by including the original incubation data and the respective stage-age model in the bootstrapping. Iberian sardine egg production estimates based on GAMs were close to the traditional estimates, when mortality was assumed fixed within the survey. In these cases, GAMs offered a 5-12% reduction in the estimated coefficient of variation (CV) with variation due to ageing contributing around 1% in the CV. Maps of the fitted egg production demonstrate a major change in the distribution of sardine spawning off Iberia between 1988 and the late 1990s, with the epicentre of spawning shifting from the northwestern to the southern Iberia. Relaxing the assumption of constant mortality and modifying the spawning PDF led, in some cases, to substantially higher estimates of total production, and additional research is required in that area. Overall, these results suggest that GAMs clearly outperform the traditional estimator for routine daily egg production estimation of sardine and the associated software permits an easy implementation of new biological information that can allow to relax or modify the existing assumptions of the method.

Stratoudakis Y, Nunes C, Soares E, Garção M, Silveira S, Grillo C, Mota F, Morais D, Cunha E, Silva A, Pestana G (2003): Sardine adult parameters and SSB estimation in Portuguese DEPM surveys: an update and revision. This document revises (1988, 1997 and 1999) and updates (2002) adult parameters and spawning biomass (SSB) estimates from the Portuguese DEPM surveys for sardine using the traditional estimators. All existing data (biology, histology and fecundity) were recovered and stored under a common format. Additional histological preparations were made for the 1997 survey (from gonads that had been fixed in paraffin but not treated further at the time) and all slides from 1997 and 1999 were reviewed. Biological data from the concurrent acoustic surveys in 1988 and 1997 were retrieved and compared to the biological data used in DEPM. Minor modifications were introduced in the estimation of mean weight and batch fecundity, and all adult parameters were re-estimated for all years using the data recovered. In all cases an average estimate was provided for each adult parameter, without considering sample weighting or post-stratification. Data revision had a minor impact on the 1988 estimate (whose main weaknesses remain the small number and limited geographical representation of the samples used for spawning fraction estimation) and the 1999 estimate (whose main weakness is the unknown impact of the geographical gradient in S to SSB estimation). On the other hand, data revision had a major impact on the 1997 estimate (new estimate 4 times higher) due to the downwards revision of S. A similarly low S estimate was obtained for 2002, but in the latter case several indicators (low batch fecundity estimate, high prevalence of atresia and post-spawning females, low GSI) appoint to an abnormal for the period reduction in sardine spawning activity off Portugal. Overall, despite the revision effort for older years and the considerably higher sampling effort in 2002, the persisting uncertainties in relation to the impact of spatial structure in adult parameters and fish distribution indicate that the existing series of Portuguese estimates is unreliable for assessment purposes. Alternatives, including GAMs or post-stratification should be considered within the meeting and evaluated.