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Report of the Study Group on the Estimation of Spawning Stock Biomass of Sardine and Anchovy (SGSBSA)

11–13 November 2004
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1 Introduction

1.1 Terms of Reference

During the ICES Annual Conference in Tallin, Estonia (90th Statutory meeting in September 2003) was decided that the third meeting of the Study Group on the Estimation of Spawning Stock Biomass of Sardine and Anchovy (SGSBSA) would take place in San Sebastian (Spain) between 11–13 November 2004 with the objective to:

a.) plan 2005 DEPM surveys for anchovy and sardine;

b.) 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;

c.) 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 along the northern border of the sardine stock;

d.) 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;

e.) refine models of vertical egg distribution and resolve selectivity problems with CUFES to assess its performance as a quantitative sampler.

1.2 Participants

The Study Group met in San Sebastian, Spain from 11–13 November 2004 with the following participants:

Alday, Ana (Observer)	Spain
Angelico, Maria Manuel	Portugal
Baldó, Francisco	Spain
Bernal, Miguel	Spain
Costas, Gersom	Spain
Ibaibarriaga, Leire	Spain
Franco, Concha	Spain
Jimenes, Paz	Spain
Lago de Lanzós, Ana	Spain
Millán, Milagros	Spain
Nunes, Cristina	Portugal
Pérez, José Ramón	Spain
Rueda, Ludi (Observer)	Spain
Silva, Alexandra	Portugal
Stratoudakis, Yorgos (Chair)	Portugal
Uriarte, Andrés	Spain

1.3 Progress with respect to recommendations of previous meeting

Within the past year and a half, satisfactory progress was registered in relation to most of the recommendations of the previous SGSBSA meeting (Malaga, June 2003). AZTI and IPIMAR advanced with the revision of POF stages for anchovy and sardine respectively based on clear morphological criteria, while information from auxiliary sources was explored to initiate the process of ageing staged POFs and to identify the potential impact of water temperature in POF degeneration rate. IEO (with the help of IPIMAR and AZTI) has made considerable ad-

vances in the preparation of software for performing traditional DEPM estimation and the elaboration of a manual to facilitate utilization. The first steps towards this development were taken during an ad-hoc meeting that took place in Lisbon during March 2004 with participants from IEO, AZTI and IPIMAR, as well as Simon Wood (the scientist responsible for the development of the software package egg that resulted from the EU Study Project on GAMs). IPIMAR has complemented previous analysis on the annual maturation cycle of sardine (macroscopic versus microscopic comparisons), while the EU project SARDYN and colleagues from Greece (Ganias *et al*.) have made considerable advances in describing the seasonality and dynamics of sardine spawning in the North-eastern Atlantic and the Mediterranean respectively. IEO has advanced with a pilot survey for anchovy in the Gulf of Cadiz during the summer of 2004, providing interesting preliminary results on egg distribution and adult biological properties during peak spawning in that area. Finally, AZTI has extended comparisons between CUFES and other samplers, while providing clear evidence and a possible solution for the problem of egg extrusion in CUFES mentioned in the last report.

1.4 Report structure

Chapter 2 is dedicated to the planning of DEPM surveys for sardine and anchovy in Atlantic European waters during 2005 (ToR a). It also includes the preliminary results of the pilot DEPM survey for anchovy in the Gulf of Cadiz and Algarve performed during the summer of 2004. In the light of these results, the design of a full-scale DEPM survey for anchovy in the southern coast of the Iberian Peninsula (provisionally set for the summer of 2005) is provided. Chapter 3 describes advances in the preparation of DEPM software that allows thorough comparisons between traditional and GAM-based estimation methods (ToR b). It also highlights several issues that seriously affect both traditional and GAM-based estimation, focusing on their clarification and resolution prior to taking final decisions on the comparison between traditional and GAM-based estimation for the existing sardine and anchovy surveys. This also resulted in a delay in the production of spatial maps for the two species (ToR c for this SG meeting), although average spatial maps of adult parameters from pooled survey data are shown in Chapter 4. Chapter 4 also provides updated morphological scales for sardine and anchovy POFs, discusses appropriate methods for POF ageing, describes the current microscopic maturation scales for the two species and reviews existing data and literature related to sardine spawning dynamics (ToR d). Chapter 5 updates results on the comparison of CUFES with other ichthyoplankton samplers (PAIROVET and LHPR), summarizes existing models to describe vertical egg distribution (necessary in order to consider CUFES as an appropriate sampler for the water column) and provides new evidence on the problem of egg extrusion in CUFES (ToR e). Chapter 6 summarizes the main findings and conclusions with respect to the terms of reference for this meeting, while chapter 7 lists the recommendations resulting from it. At the end of the report (after the list of references and the working document titles presented to this meeting), four Annexes illustrate the POF stages for anchovy (I) and sardine (II), provide a template for data registration during histological analysis (III) and describe the sardine microscopic maturation scale (IV).

2 DEPM Surveys in 2005

2.1 Introduction

This chapter is dedicated to the planning of DEPM surveys for sardine (Section 2.2) and anchovy (Section 2.3) in Atlantic European waters during 2005 (ToR a). It also includes the preliminary results of the pilot DEPM survey for anchovy in the Gulf of Cadiz and Algarve performed during the summer of 2004. In the light of these results, the design of a full-scale DEPM survey for anchovy in the southern coast of the Iberian Peninsula (provisionally set for the summer of 2005) is provided (Section 2.4). Section 2.5 summarizes the standardised DEPM data format (eggs and adults) devised during the EU project on GAMs, which is recommended for future use as it facilitates the use of the software that is being developed (see Section 3.2). Finally, Section 2.6 aims to initiate some discussion (beyond the remit of this SG) on ways to increase the international coordination of egg surveys in a cost-efficient manner. Sardine survey in ICES Subareas VIIIc and IXa

According to plan, a sardine DEPM survey will take place in 2005 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) will be surveyed by IPIMAR (Instituto de Investigação das Pescas e do Mar, Portugal), while IEO (Instituto Español de Oceanografía, Spain) will cover the north-western Iberian Peninsula and the Bay of Biscay (from ~ 42°N to 45°N). The Portuguese survey will take place in January/February 2005 onboard RV "Noruega" (see also WD by Stratoudakis *et al.*), while Spain will carry out two separate surveys in March/April, one for ichthyoplankton, using RV "Cornide de Saavedra", and another for adult surveying onboard RV "Thalassa".

Both national surveys will perform ichthyoplankton sampling on fixed stations with a PAIROVET (double CalVET - Smith *et al*. 1985) net and underway (3 m depth) CUFES (Checkely *et al*. 1997) sampling on fixed transects perpendicular to the coast and spaced 8 nm. The main ichthyoplankton sampler will be the PAIROVET, with the auxiliary use of CUFES for adaptive decisions. The inshore limit of transects will be determined by bottom depth (as close to shore as possible), while the offshore extension will be decided adaptively. The rules agreed by IEO and IPIMAR to apply during the ichthyoplankton survey are the following:

- CUFES samples (ongoing) will be taken every 3 nm throughout a transect.
- PAIROVET samples will be always taken every 3 nm in the inner shelf, up to 100 m depth or up to 200 m where the platform is narrow (*e*.g. Canhão da Nazaré, Cantabrian Sea).
- PAIROVET samples will be taken every 3 nm or 6 nm beyond the inner shelf, depending on the results of the most recent CUFES sample, collected every 2.8 nm (to allow time to look at the sample before reaching the grid position). When an ongoing CUFES is negative for sardine egg presence, the following PAIROVET, at 3 nm, is skipped.
- The outer limit of a transect is reached when two consecutive CUFES samples are negative beyond the 200 m depth.
- When finishing a transect offshore the vessel should proceed to the next line and start the CUFES on the way to check for egg presence. When eggs are present sampling continues in offshore direction, if no eggs are found sampling starts (always) with a PAIROVET at a point at the same latitude or longitude or equal distance from the isobath, depending on transect orientation, and then continue from there towards the shore with sampling using the criteria defined above.

The above rules are designed to guaranty intensive coverage of the inner shelf, adequate coverage of the outer shelf (intensified in the areas of egg presence) and minimal coverage be-

yond it, the use of CUFES aiming to improve the efficiency of sampling. With the above design is anticipated that the total number of PAIROVET hauls will range between 350 and 450 during the Portuguese survey and 300 and 400 in the Spanish survey.

The PAIROVET hauls will be performed using a net with 150 µm mesh size and fitted with flowmeters, operating vertically (1 m/s) to the surface from 5 m above the bottom to a maximum sampling depth of 100 m, or 150 m in the IPIMAR survey. Whenever a towing angle deviates from the vertical more than 30º the sample should be discarded and the haul repeated. After hauling, nets should be washed from the outside with seawater under pressure and stored in 2 jars with a fixative (see below). Samples from one codend will be preserved for sardine egg quantification, while samples from the other codend will be used for plankton biomass quantification, at the laboratory. Date, time, position (GPS), flowmeter readings, cable released and sampling and bottom depth data should be registered.

To determine temperature, salinity and maximum sampling depth IPIMAR's PAIROVET sampler has a modified design to include a CTD+flurometer profiler. IEO will carry out separate hauls for profiling using a CTD and plankton sampling and a Minilog sensor will be used with the PAIROVET to register maximum sampling depth. The CUFES sampler will be equipped with a 335 µm mesh size net and it will be used to delimit the spawning area and to modify adaptively the intensity of PAIROVET sampling. IPIMAR's CUFES system has a coupled CT (thermosalinometer) and fluorometer sensors that register temperature, salinity and chlorophyll (at 3 m depth) along the transects. IEO will collect environmental data from 3 m depth using a CT. In order to calibrate the conductivity and fluorescence sensors (from the CTD and CUFES) water samples will be collected by IPIMAR. For chlorophyll determination the water will be filtered after collection and the filters frozen for further processing. After collection, plankton samples will be preserved in formalin at 4% in distilled (or fresh, IEO) water, neutralized with sodium acetate. In laboratory, all sardine eggs will be sorted and staged. Eggs from the PAIROVET samples will be classified in 11 stages of development (adapted from Gamulin and Hure, 1955) while the individuals collected with CUFES will be staged in 11 phases by IPIMAR and in 3 categories (no embryo, early embryo, late embryo - ICES, 2003) by IEO.

Adult fish will be obtained from pelagic or demersal trawls, onboard RV "Noruega" by IPIMAR (concurrently with the plankton survey) and onboard RV "Thalassa" by IEO in parallel with the plankton survey using RV "Cornide de Saavedra". Additional samples will be obtained in collaboration with the purse seine fishing fleets operating in the vicinity of the research vessels. Fishing stations will be taken opportunistically during each day, aiming to collect 30–35 samples in the region to be covered by IPIMAR and around 30 in the area that will be surveyed by IEO. Extra fishery samples are expected to raise the total number of adult samples to at least 100 for the entire survey area.

Each fish sample onboard the research vessels will be constituted by 100 individuals randomly taken that will be biologically analysed. For the first 30 fish, the otoliths will be removed for posterior individual ageing. Moreover, for the first 30 females, the gonads will be immediately collected and preserved in formaldehyde solution (4% in seawater) for histological processing for identification of maturity stages and estimation of spawning fraction. Macroscopic stage I gonads will be included in the sampling for histology. To avoid sampling of fish clearly virgin, a minimum individual total length will be defined bellow which the gonads will not be preserved. The minimum length will be based on the L5 value (length at which 5% of the females are mature) estimated from an average maturity ogive from recent data. In addition, extra effort will be placed to obtain spawning females (macroscopic stage IV) for batch fecundity estimation. The objective will be to collect enough hydrated females for relative fecundity comparisons between geographical areas.

Laboratory processing of samples will be performed according to the procedures adopted in previous DEPM surveys. Histological analyses for spawning fraction and batch fecundity will be carried out at IEO using resin as embedding medium for all preserved samples. At IPIMAR, all samples will be processed in paraffin and those where POFs are observed will be reprocessed with resin for more precise POF staging.

2.2 Anchovy survey in ICES Subareas VIIIb and VIIIc

The anchovy DEPM survey for the Bay of Biscay will take place in spring 2005 on board RV "Vizconde De Eza" from 3–23 May and will cover the Spanish eastern Cantabrian Sea and the southern French coast covering the usual spawning grounds (ranging at least from 5ºW to 47ºN). Regular plankton samples will be taken throughout the whole potential spawning areas of anchovy for obtaining egg abundance estimates. In parallel, pelagic fish trawling will be made at places where relevant egg and/or acoustic detections indicate the presence of pelagic fishes. In recent years additional adult sampling has been obtained thanks to the help of the synchronous French acoustic survey, and the same help is expected to be achieved during 2005 in case that survey is scheduled for the same dates as the egg cruise. In any case, adult sampling during the survey will be complemented with samples taken opportunistically onboard the Spanish commercial fishing fleets of purse seines, as has been traditionally made in previous implementations of this DEPM survey.

The egg sampling will make use of the PAIROVET (double CalVET) net and will follow the historical systematic central sampling scheme. Samples will be taken 3 miles apart from each other, along radials perpendicular to shore spaced 15 miles (45 square miles sampling units). Flowmeters in both CalVET nets will be used; mesh size of 150 µm; a 45 kg depressor; and a CTD sensor will be deployed at every launch coupled to the PAIROVET net. CUFES will be continuously used every 1.5 nm (between PAIROVET hauls) making use of a 335 µm collector mesh size. It will be equipped with a flow meter to control thee pumped water per sampling time and with a CTD continuous monitoring of surface salinity and temperature. Offshore edge of the spawning area is considered to be reached by three consecutive samples with 0 anchovy eggs in PAIROVET or by 6 nm with the CUFES samples empty of eggs. CUFES samples will be checked on board, and can be used to relax (to space up to 6 nm) the Pairovet sampling in areas of low abundance.

Egg samples will be preserved in formaldehyde 4% buffered with Sodium Tetra borate. Most of the samples are analysed onboard after 6h of fixing for sorting, identification and counting of anchovy eggs. Afterwards in the laboratory the sorting made at sea is checked and anchovy eggs are staged according to Moser and Alshtrom, 1985. Ageing of eggs will be produced by the new Bayesian ageing procedure (ICES, 2004). And the subsequent estimates of Daily Egg Production will be produced either by the Traditional (Lo, 1985) or GLM procedures and will be tested by the GAM procedure.

Adult samples consist of about 2 kg selected at random for the catches during the cruises. Sampling finishes as soon as a minimum of 1 kg, or 60 anchovies are weighted and sexed, and the gonads of 30 non-hydrated females (NHF) are preserved in formalin. Sampling is in any case stopped when more than 120 anchovies have to be sexed to achieve the target 30 NHF. Otoliths are removed from the same sample in order to study the age composition of the anchovy population. Estimation of adult parameters will be made by following the standards established for this species (Motos 1994, Uriarte *et al*. 1998, Santos *et al*. 2003, Somarakis *et al*. 2004).

2.3 Anchovy survey in ICES Subarea IXa

2.3.1 Introduction

Traditionally, there has been a purse-seine fishery in the Gulf of Cadiz (ICES Subdivision IXa South), targeted to anchovy and sardine. The IEO began the studies on this fishery and the biology of its main target species at the end of the 1980s. These studies are subordinated to different IEO structural projects (PESQSUR and BIODAS). The state of the Gulf of Cadiz anchovy stock is currently unknown. Since 2001 several assessments trials (Algarve+Gulf of Cadiz) have been carried out in the ICES WGMHSA (Ramos *et al*., 2001; Anon., 2002, 2003, 2004). However, all are considered as exploratory exercises, as it was concluded that it is impossible to assess this stock reliably without the information provided by direct methods (DEPM and acoustics). For this reason, ICES strongly recommended to begin series of species biomass indices estimated by acoustic methods and DEPM in the study area. These facts moved the IEO to assess this resource by direct methods from the current year. The BOCADEVA 0604 survey is one of the research activities contemplated in 2004 within the new IEO research project PELCOSAT (see also WDs by Millan *et al*. and by Jimenez *et al*.). This project is mainly focused to the study of the fishery, biology and population dynamics of the Gulf of Cadiz anchovy. This survey tries to be the starting point of a historical series of (acoustic and DEPM) surveys that is expected that IEO will develop in the study area either yearly (acoustic surveys) or triennially (DEPM surveys).

2.3.2 The 2004 pilot survey

The scarce time available to carry out the survey (8 days only) led to consider the present survey as an exploratory one. Basically planned as an acoustic survey, the BOCADEVA 0604 also included the following DEPM-based objectives:

- the delimitation of the extension of the anchovy spawning area in the ICES Subdivision IXa South,
- a non-intensive collection of anchovy adult samples for a preliminary (exploratory) analysis of DEPM-adult parameters, and
- the evaluation of the CUFES performance as a quantitative sampler of the anchovy eggs abundance in the study area through a CUFES-PAIROVET validation exercise.

The survey was carried out between the 6–13 June 2004 with the RV "Cornide de Saavedra", covering a survey area comprising the waters of the Gulf of Cadiz, both Spanish and Portuguese (Algarve), between the 30 m and 200 m isobath. The shallowest depth limit of the surveyed area was established at 30 m as a security measure for vessel navigation, which entailed that a part of the coastal zone between the Guadalquivir and Guadiana River mouths was not sampled (Figure 2.4.2.1, see also Figure 2.4.3.1).

(i) Sampling methods

Spawning area delimitation: Continuous Underway Fish Egg Sampler (CUFES) was used. CUFES sampling design consisted in a systematic parallel grid with tracks equally spaced by 8 nm, normal to the shoreline. The volume of water filtered (600 l/min approximately) was integrated each three miles, and the collector was equipped with a 335 μm net. The sampling grid coincided with the one established for the acoustic sampling (21 radials in total), with both sampling carried out simultaneously and always in day light. A total of 99 CUFES stations were integrated along the study area, from the most eastern part of the Gulf of Cadiz to the Cape of São Vicente in Portugal. Sampling scheme was semi-adaptive, with the adaptive rule of enlarging the radials in case of anchovy egg presence at the end of each radial, until finding two consecutive negative stations (Figure 2.4.2.1).

Validation experience CUFES-PAIROVET: Validation was carried out using the two samplers simultaneously at night, after the end of the daily acoustic sampling. CUFES and PAIROVET sampling were carried out in selected radials in the following way: the samples of CUFES and PAIROVET were collected each 3 miles but with an interval of 1.5 mile among both. Therefore, for each PAIROVET station corresponds two (before and after) CUFES stations. To carry out the validation, CUFES (before and after) stations eggs densities (eggs/m^3) were calculated and then confronted with the egg densities (eggs/m^2) obtained in the corresponding PAIROVET station, and a linear model between them was fitted. A total of 26 PAIROVET stations were carried out, distributed on 7 radials (Figure 2.4.2.1). PAIROVET net, equipped with nets of 150 μm mesh size and flowmeters to estimate the volume of water filtered by the net. Vertical hauls to a maximum 100 m depth or to 5 m above the bottom in smaller depths were carried out. Sampling speed was approximately 1 m/s. The validation experience was carried out in radials spreading throughout the whole sampling area. This was also made in order to permit a crude preliminary estimate of egg production in the area using the obtained PAIROVET samples. In order to achieve this objective it is necessary to stage the obtained eggs. This task is not completed yet.

Adult anchovy samples were obtained from pelagic trawl hauls (20-m vertical opening). Fishing stations (13 valid in total) were opportunistic, but in the shallowest waters of the sampled area (between the 30-m and 50–60 m isobaths) fishing was not always possible because of the presence of artesanal fixed fishing gears. The sampled depth range in the valid hauls oscillated between 39–105 m in the Spanish waters, and between 63–121 m in the Portuguese ones (Figure 2.4.2.2). Length frequency distributions (LFD) by 0.5 cm class were obtained from these samples (either from the total catch or from a representative random sample of 100–200 fish). A two-stage individual biological sampling of anchovy by length, weight, sex and maturity stages was performed in each positive haul. In the first stage a random sample of 40 individuals was collected in order to biologically characterise the species' population. This initial sample was completed in a second stage with additional individuals following a size-stratified random sampling scheme (characterisation of the size classes in the population). A 6-stage maturity scale (Pinto and Andreu, 1957) was used for anchovy: virgin or resting (stage I); developing (stage II); pre-spawning (stage III); spawning (stage IV); partial post-spawning (stage V) and ultimate post-spawning (stage VI). Otoliths were extracted from all fish in the whole sample in each haul. An age-length key for the whole surveyed area was then constructed.

Sex-ratio (R), female mean weight (W) and spawning fraction (S): In each positive trawl, the first-stage random sample (40 fish) in the biological sampling was utilised for a further exploratory analysis of sex ratio and female mean weight. A goal of 20 non-hydrated females (NHF) per sample was collected in order to obtain a preliminary estimate of the spawning fraction. If such goal was not achieved from the initial random sample, the sampling continued up to achieve a maximum of 80 sexed anchovies. A total of 193 NHF gonads were finally collected, weighed and preserved with a 4% buffered formaldehyde solution for further processing in laboratory.

Batch fecundity (F): For a preliminary estimation of the batch fecundity, additional samples were collected in 3 hauls with occurrence of hydrated females (HF). A total of 87 hydrated females were selected and their gonads were weighed and then preserved with a 4% buffered formaldehyde solution for further processing in laboratory. For the histological processing of samples small ovary sections from the non-hydrated and hydrated mature females were embedded in resin. Three 3 μm-thick slides were cut and stained with haematoxylin-eosin.

ii) Results

Sampling and spawning area: All radials, except from the most easterly ones closest to the Strait of Gibraltar, registered positive stations for anchovy eggs. Delimitation of the survey

area and estimation of area represented by each sampling station was carried out using the R package *Geofun* (see WD by Bernal *et al*.). Positive area was continuous and spawning area was quantified by adding up the area represented by the stations included in the positive area (Figure 2.4.2.3). The obtained results were: a total sampling surface area of 9345 km^2 and a total spawning area surface of 4952 km^2 (positive area).

Eggs anchovy abundances in CUFES samples: A total of 14946 eggs with an average of 282 eggs/station were sampled in the positive area. The largest number of eggs (2513) was collected at a station off Huelva in a shallow region (35 m depth). Most of the eggs were obtained in the coastal strip between Huelva and Cadiz (Figure 2.4.2.3). Figure 2.4.2.4 shows the cumulative frequencies of eggs anchovy abundances in relation to depth: 90% of the eggs were fished below the 100 m depth line. Considering the relationship among egg abundance and physical parameters (temperature and salinity) the frequency bar chart (Figure 2.4.2.5) and the T-S graph (Figure 2.4.2.6), anchovy spawning shows a preference for a restricted range of temperature and salinity within the observed range of both variables in the survey. Most of the eggs have been sampled in a range of temperature of approximately 19.8–22.0 ºC and in areas with salinities between 35.9 and 36.4‰. In order to accept this hypothesis a large data series from futures surveys is necessary.

Anchovy occurrence, distribution and abundance: Anchovy occurred in 10 from 13 valid fishing stations, but only 9 anchovy samples were available for biological sampling (2 from Portuguese waters and 7 from the Spanish ones). Acoustic back-scattering values attributed to anchovy showed that the species was mainly distributed in the Spanish waters of the Gulf of Cadiz (86% of the total estimated biomass and 90% of the total abundance, Ramos *et al*., 2004), with higher densities occurring between 40 and 80 m depth. In the Portuguese waters the species was restricted to the easternmost area only (Figure 2.4.2.7). The total biomass estimated for anchovy was 13.2 thousand tonnes (894.4 x 106 individuals).

Population size/age structure and biological features: Data from fishing stations suggested an east-west size/age gradient, with the largest (and oldest) anchovies being more abundant in the westernmost limit of their distribution, and a recruitment area located in shallow waters close to the Guadalquivir River (Table 2.4.2.1, Figure 2.4.2.8). Results from the biological sampling of the population during the survey (Figure 2.4.2.9) indicate that 92.3% of females showed clear signs of reproductive activity (stages III–V). These mature females included both virgin and adult individuals with a size range between 97 and 186 cm. The occurrence of ultimate post-spawning females (stage VI) was very scarce in the surveyed area (3.4%, size range: 114–140 cm). Sex ratio (R) was estimated as the weight ratio of females in the mature population (Figure 2.4.2.9). In total, 476 mature fish (stages higher than II) from 9 independent samples were used for the estimation of the overall sex ratio in the surveyed area. This sex ratio for the Gulf of Cadiz was 0.566 (CV= 36%). A low number of both fishing stations and sampled fish per haul are the probable causes for the above high CV. A higher number of independent samples will be therefore needed for a more precise estimate in next surveys. It was noteworthy the scarce occurrence of females in the fishing station 7. However, the relatively high number of hydrated females occurring in this haul seems to suggest some reproductive aggregation behaviour (Alheit *et al*., 1984). Mean female weight (W) for the whole surveyed area was 17.64 gr (CV = 42%, n = 237), using data from 9 hauls. In the Spanish waters (7 hauls) the mean female weight was estimated at 14.57 gr (CV = 33%, n = 172) whereas in the Portuguese waters (2 hauls) this estimate was 25.88 gr (CV = 28%, n = 64). A total of 87 hydrated females (size range: 117–181 mm) were available from 3 samples from the whole surveyed area for preliminary batch fecundity estimation (F). Hydrated females were captured in a time range between 19:33 and 20:46 GMT. As stated above hydrated females were relatively more abundant in the fishing station 7, conducted in shallow waters (39–56 m depth) in front of Matalascañas. Histological processing is still in progress. The analysis of slides, including the classification of the ovaries according to the incidence of postovulatory follicles, has not started yet.

2.3.3 Survey plan for 2005

During June (peak spawning, see WD by Millan *et al*.) 2005 will take place the first anchovy DEPM survey in the Gulf of Cadiz (BOCADEVA-0605) onboard the RV "Cornide de Saavedra". The survey area will extend throughout the southern Iberian coast (Strait of Gibraltar-Cape of São Vicente).

The strategy for egg sampling will be identical to that used for anchovy in the Bay of Biscay. PAIROVET stations will be made every 3 nm, along transects spaced by 8 nm and perpendicular to the coast. Vertical hauls to a maximum 100 m depth or to 5 m above the bottom in smaller depths. PAIROVET will be equipped with nets of 150 µm of mesh size and flowmeters to estimate the volume of water filtered by the net. CUFES samples will be taken every 3 nm throughout the transects. The sampling scheme is semi-adaptive, with the adaptive rule of enlarging the radials in case of anchovy eggs presence at the end of each radial, until finding two consecutive negative stations. The collector will be equipped with a 335 µm net and the water intake will be 600 l/min approximately. In all stations temperature and salinity profiles will be obtained by CTD. An anchovy eggs incubation experiment will be carried out during the survey.

Adult sampling will be obtained from pelagic trawls hauls performed by the research vessel. Immediately after fishing, 60 fish will be randomly selected and biologically sampled onboard and 25 non hydrated-females (NHF) will be preserved. If such goal is not achieved from the initial random sample, sampling will continue until achieving a maximum of 120 sexed anchovies. In order to complement adult anchovy samples in shallower waters and hydrated females, additional samples will be collected onboard commercial purse seiners rented during the survey time. After fishing these samples will be boarding on the research vessel and processed following the above methodology.

During the pilot survey (BOCADEVA-0604) in 2004, shallower waters (< 30 m depth) could not be prospected due to the RV navigation security measures. As a result, a part of the coastal zone between the Guadalquivir and Guadiana River mouths was not covered (Figure 2.4.3.1). Previous studies have shown this area to be important for anchovy, which is corroborated by the distribution information for eggs and adults obtained in the pilot survey. In the 2005 DEPM survey this area should be sampled, and the inshore limit of transects should be as close to shore as possible (usually near the 20 m isobath), in order to avoid a sub-estimation of the spawning biomass.

2.4 Standardisation of DEPM data format

During the current SG meeting, attention was brought to the need to devise a standard format for the registration of DEPM data (eggs and adults). The benefits from using a standard format was demonstrated during the recent EU project on GAMs, where a first standardisation was agreed among partners permitting to built egg and adult databases for the two species that could readily be compiled into an R library and introduced for analysis with the R software. Tables 2.5.1 and 2.5.2 describe the basic format agreed during this project, which is recommended to be used routinely in future DEPM applications. The current format uses as basic information unit (row) the plankton station and fishing haul respectively, while the information registered (column) is the minimum required for traditional and GAM-based estimation. It is likely that in the future this format will be extended to include other variables, while in the case of adult sampling it will eventually be extended to include a database where the unit level is the individual fish (currently existing but not standardised among partners). To this end will also help the standardisation of the information collected during histological examination (see chapter 4 and Annex 3).

2.5 Improving the coordination and efficiency of international egg surveys

The DEPM surveys for sardine takes place every 3 years (1999, 2002, 2005 etc.) usually during January/February (IPIMAR) and March/April (IEO). In addition, the DEPM for anchovy takes place every year in May/June (AZTI). Beyond this, every three years (1998, 2001, 2004) egg surveys over the mackerel potential spawning areas take place during the first half of the year, including the areas and timing of sardine and anchovy spawning. The SG considered that there is scope to increase the coordination of these surveys to get further inputs about egg production of pelagic species and, eventually, further DEPM biomass estimates (at least for sardine).

For instance, a combination of the sardine and anchovy DEPM surveys could be devised to get estimates of egg production not only over the Cantabrian shelf areas but also covering a large part of the French waters in the Bay of Biscay (minimum up to the range of the anchovy northern spawning limits). Adult sardine sampling in the additional Bay of Biscay area could also be achieved in order to get daily fecundity estimates and hence SSB over this area. This expansion is possible thanks to the complementary coverage in time and space of both DEPM surveys. Depending on the European financial support through the national sampling programmes, it could also be considered that the anchovy DEPM survey could every three years include among its priorities the sardine and therefore guarantee a more thorough (northerly) coverage of sardine spawning areas in the Bay of Biscay.

For the triennial egg surveys, the extraction of the sardine and anchovy eggs for instance for survey coverage south of 52ºN would allow obtaining spatio-temporal images of sardine and anchovy spawning and egg abundances. But beyond this, if from the triennial egg cruises, the ones covering the southern region in March were selected for sardine then for those years egg abundances at peak spawning time for sardine would be obtained. Adult sampling is carried out in some of those surveys to obtain atresia correction values for mackerel spawners; if sardines were retained in formalin, then adult parameters could be obtained and the implementation of a complete DEPM in those years could even be devised for sardine with low additional effort. Similar approach could be devised for anchovy if selecting the triennial May-June cruises but that is of lesser importance since DEPM is carried out on an annual basis.

With the two collaborations outlined above, 2 out of every 3 years sardine egg production could be generated not only for the Iberian region, but also for the areas south of 52 ºN. And if adults were available from the March triennial egg surveys then sardine DEPM SSB estimates could also become available. One of the major reasons for not making nowadays the sardine DEPM surveys simultaneous with the triennial egg surveys is linked to the amount of laboratory work involved (particularly for the histological analysis of adults). However, sorting out the eggs of sardines and anchovy in addition to those of mackerel and horse mackerel is not a major job. Another caveat is that the sampling grid applied to mackerel surveys in the southern areas (based on Bongo nets) is considered to be too wide for the sardine egg production estimates (where about half of the positive stations are found within the inner shelf). However, given the interest of Portugal and Spain on sardine it could be considered that it is worth the effort of increasing sampling intensity with some PAIROVET hauls in the mid-points between Bongos, or even increasing the number of radials to allow for simultaneous DEPM estimation.

If adult samples or PAIROVET net samples could not be processed during the years of AEPM surveys, they both could be delayed till the next years with official DEPM surveys and the effort required to accomplish either a complete daily egg production or SSB estimate for the triennial egg surveys of March could be devoted in parallel to the official DEPM applications of next year. For quick images of sardine spawning egg abundances the Bongo processing for the triennial egg surveys would suffice, but in addition the ICES MHSAWG would get two egg production estimates and either 1 or 2 new SSB estimates from the DEPM methods every

three years and knowledge about the population structure and levels beyond the currently managed areas. The implications in terms of additional funding from the National programmes should be evaluated, but it is anticipated that the cost would be minor in relation to benefit from obtaining additional sardine abundance indices. The question about the utility of expanding the sardine DEPM estimates to the Bay of Biscay and south of 52ºN when the current boundaries of the unique managed sardine population is south of 44º30'N is open and depends on the connections that the SARDYN project and other studies could establish between sardines in different areas; but also on the general EU policy and interest for assuring general monitoring of the non currently managed exploited resources. Given the fact that the current proposal benefits from existing routine surveys and that parallel multi-species surveys (for instance with acoustics) in the Bay of Biscay are already being funded by the EU, it seems plausible that the extra cost involved in the additional exploitation of these egg surveys explored in this section could be as well funded by the EC.

Table 2.4.2.1: BOCADEVA 0604 acoustic survey. Estimated anchovy abundance (thousands of individuals) and biomass (tonnes) by age groups (Ramos *et al.*, 2004).

AGE CLASS	ALGARVE	CADIZ	TOTAL
	Number	Number	Number
0	0	0	0
I	82348	798175	880523
II	8423	5423	13846
III	0	0	0
TOTAL	90771	803598	894369
Age class	**ALGARVE**	**CADIZ**	**TOTAL**
	Weight	Weight	Weight
0	0	0	0
I	1546	11224	12771
II	246	151	398
III	0	0	0
TOTAL	1793	11376	13168

Table 2.5.1: Summary description of DEPM egg database format agreed in the EU project on GAMs and recommended for standard use in DEPM applications (minimal requirements).

NAME	DESCRIPTION	UNITS	TYPE
Station	Unique identifier of station	Integer	Factor
Lat	Latitude of station	4 decimals	Number
Long	Longitude of station	4 decimals	Number
Depth	Bottom depth at station (in m)	Integer	Number
Time	Sampling time (Greenwich)	2 decimals	Number
Efarea	Effective area of the sampler (in m^2)	3 decimals	Number
I, II, XI	Number of eggs at stage (1 per column)	Integer	Number
Dest	Destroyed eggs (unstaged)	Integer	Number
Toteggs	Total number of eggs in the sampler	Integer	Number
Temp	Sub-surface measure of temperature (CUFES)	2 decimals	Number
Sal	Sub-surface measure of salinity (CUFES)	2 decimals	Number
Chlor	Sub-surface measure of fluorescence (CUFES)	3 decimals	Number

Table 2.5.2: Summary description of DEPM adult database format agreed in the EU project on GAMs and recommended for standard use in DEPM applications (minimal requirements).

NAME	DESCRIPTION	UNITS	TYPE
Survey	Survey name	Text	Factor
Vessel	Vessel performing fishing station. First appears the type of vessel (RV=Research Vessel and CV=Commercial Vessel) and then its name.	Text	Factor
Gear	Sampling gear, coded as MT=Midwater Trawl, PS=Purse Seine, PT=Paired Trawl and BT=Bottom Trawl	Text	Factor
Haul	Number identifying haul order in a survey	Integer	Number
Day	Day (GMT) of sampling	Integer	Number
Month	Month (GMT) of sampling	Integer	Number
Year	Year (GMT) of sampling	Integer	Number
Time	Time (GMT) of sampling	Hours, 2 decimal points	Number
Long	Longitude of sampling station	Decimal degrees 4decimal points, positive north	Number
Lat	Latitude of sampling station	Decimal degrees 4decimal points, positive east	Number
Ntot	Number of fish in the sample. These individuals are used for the estimation of sex-ratio	Integer	Number
Nfem	Number of females in the sample	Integer	Number
Nhist	Number of mature females used in histological preparations in order to estimate spawning fraction.	Integer	Number
Nmatfem	Number of mature females. These individuals are used for the estimation of the expected mean batch fecundity and the mean female weight	Integer	Number
Fexp	Expected mean batch fecundity - number of eggs spawned by a female per batch - in the fishing station. Relationship between the number of eggs per batch observed (Fobs) and the ovary-free weight observed in the hydrated females is used to estimate the expected batch fecundity (Fexp) for non-hydrated females from the gonad-free weight	Decimal	Number
R	Sex ratio - proportion of biomass of females in the fishing station. It is estimated as the female proportion of biomass in each haul using all fish (mature and immature).	Decimal	Number
S	Spawning fraction - proportion of mature females spawning per day in the fishing station.	Decimal	Number
W	Mean mature female weight in the fishing station. Weight of hydrated females must be corrected downward to account for temporary weight gain	Grams	Number

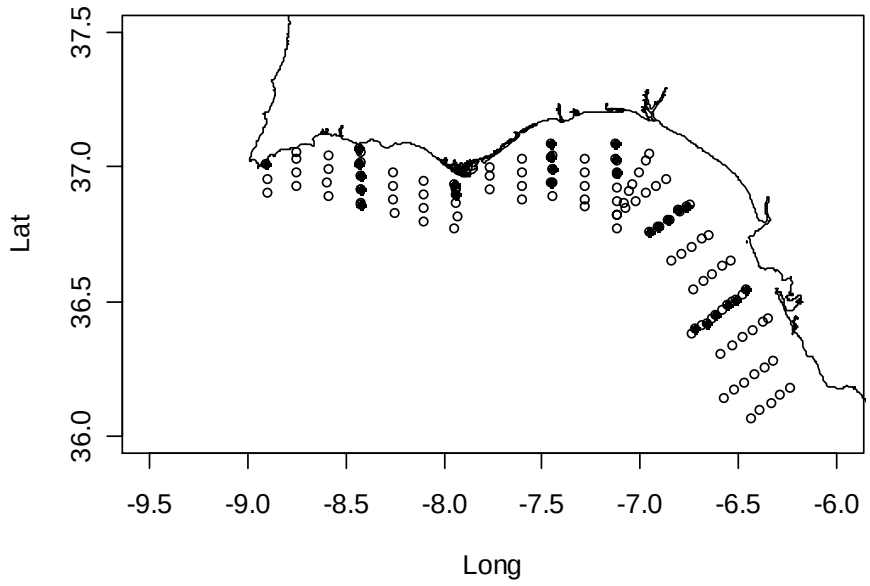


Figure 2.4.2.1: BOCADEVA-0604 plankton survey. Survey area, (o) CUFES and (●) PAIROVET stations.

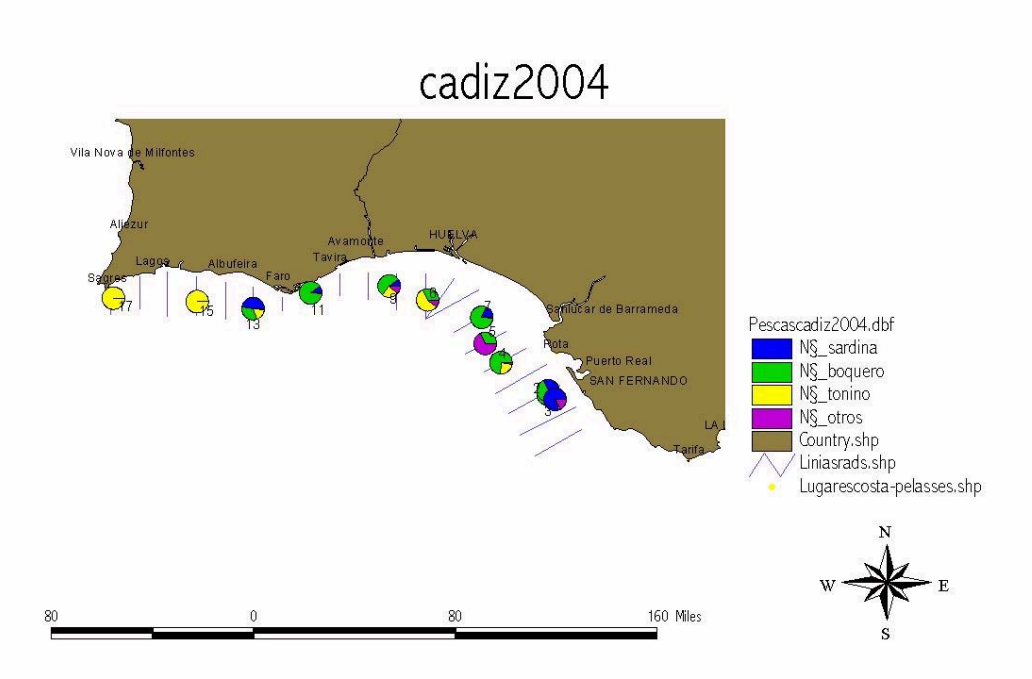


Figure 2.4.2.2: BOCADEVA 0604 survey. Location of valid fishing stations and species composition (percentages in number), (Ramos *et al.*, 2004).

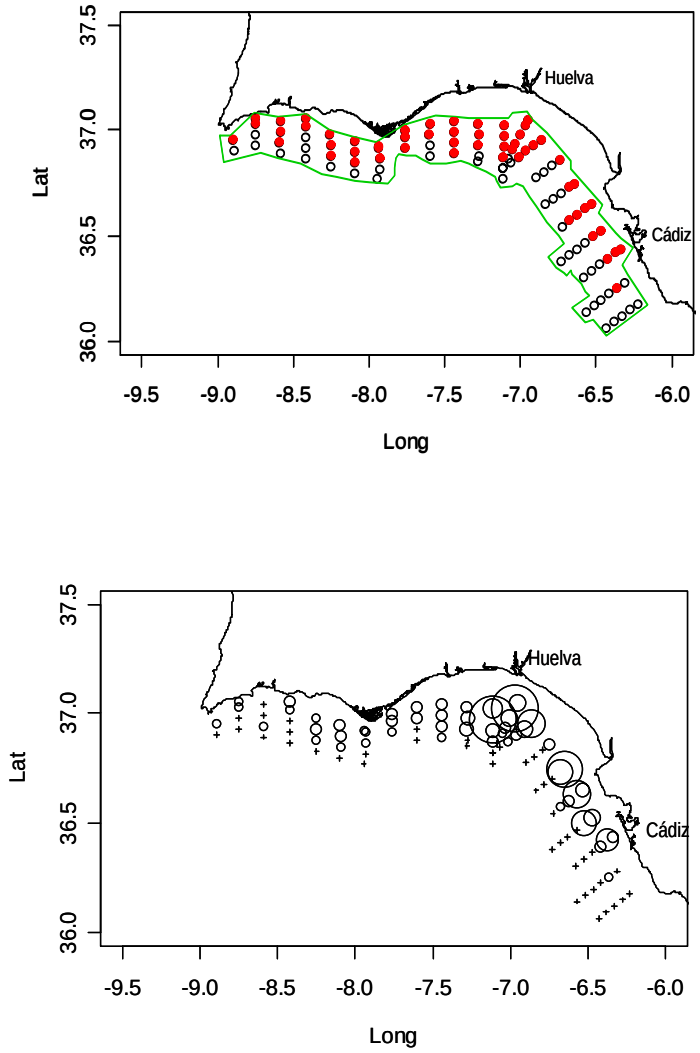


Figure 2.4.2.3. BOCADEVA-0604. CUFES stations (● stations with presence of anchovy eggs) and sampling area delimitation (upper panel). Abundance (nº/m^3) of anchovy eggs (lower panel).

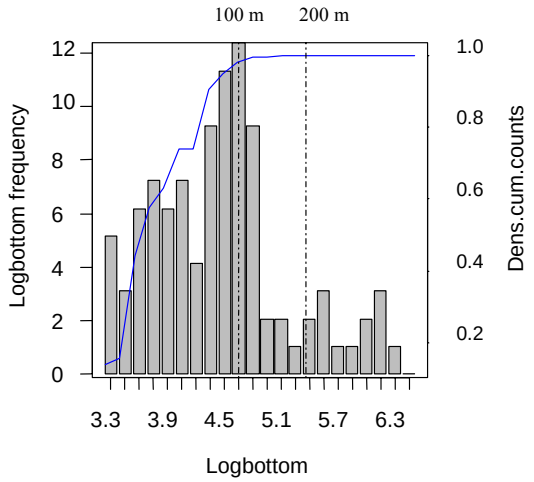


Figure 2.4.2.4: BOCADEVA-0604. Anchovy eggs abundance (density in n°/m3) in the Gulf of Cadiz with regard to the sampling depth. Graph of accumulated frequencies.

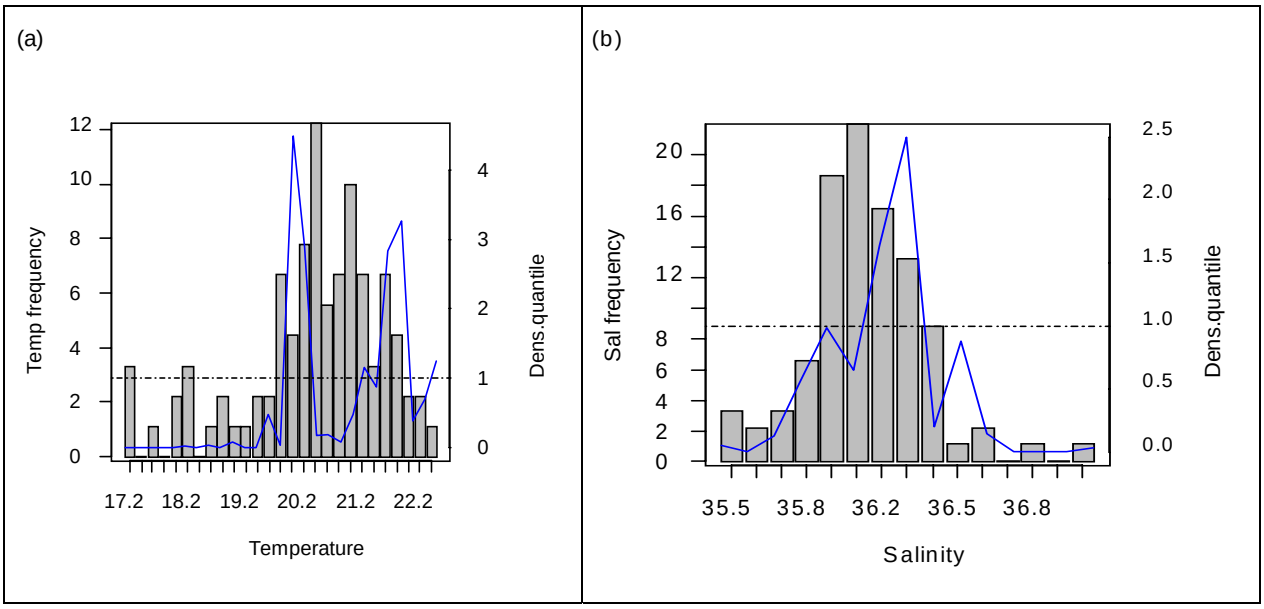


Figure 2.4.2.5: BOCADEVA-0604. Anchovy eggs abundance in the Gulf of Cadiz with (a) temperature and (b) salinity. Graph of frequencies.

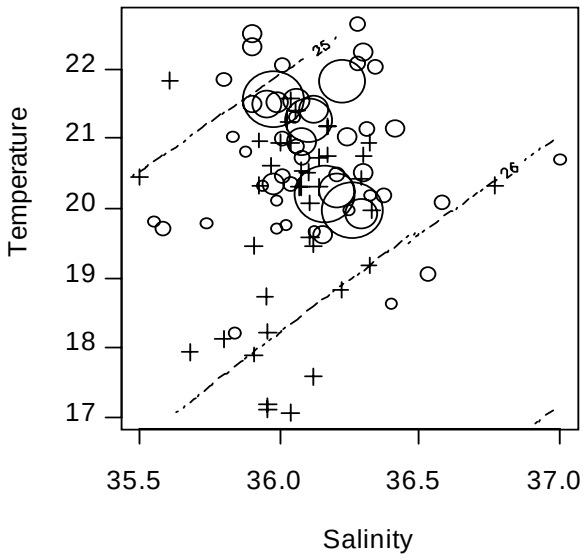


Figure 2.4.2.6: BOCADEVA-0604. Anchovy eggs abundance (density in n°/m^3) in the Gulf of Cadiz considering temperature and salinity. Graphic T-S.

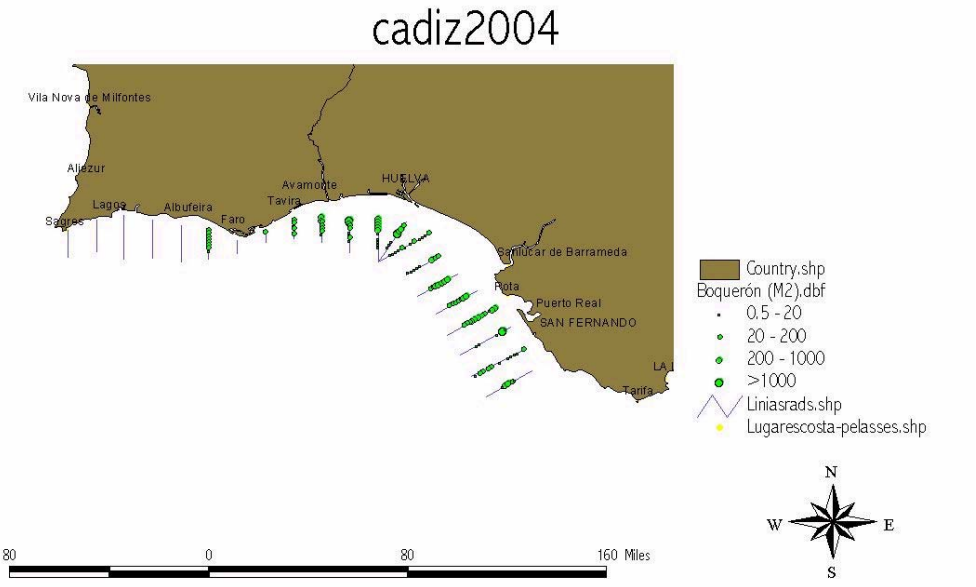


Figure 2.4.2.7: BOCADEVA 0604 survey. Anchovy distribution derived from the back-scattering energy attributed to the species (Ramos *et al.*, 2004).

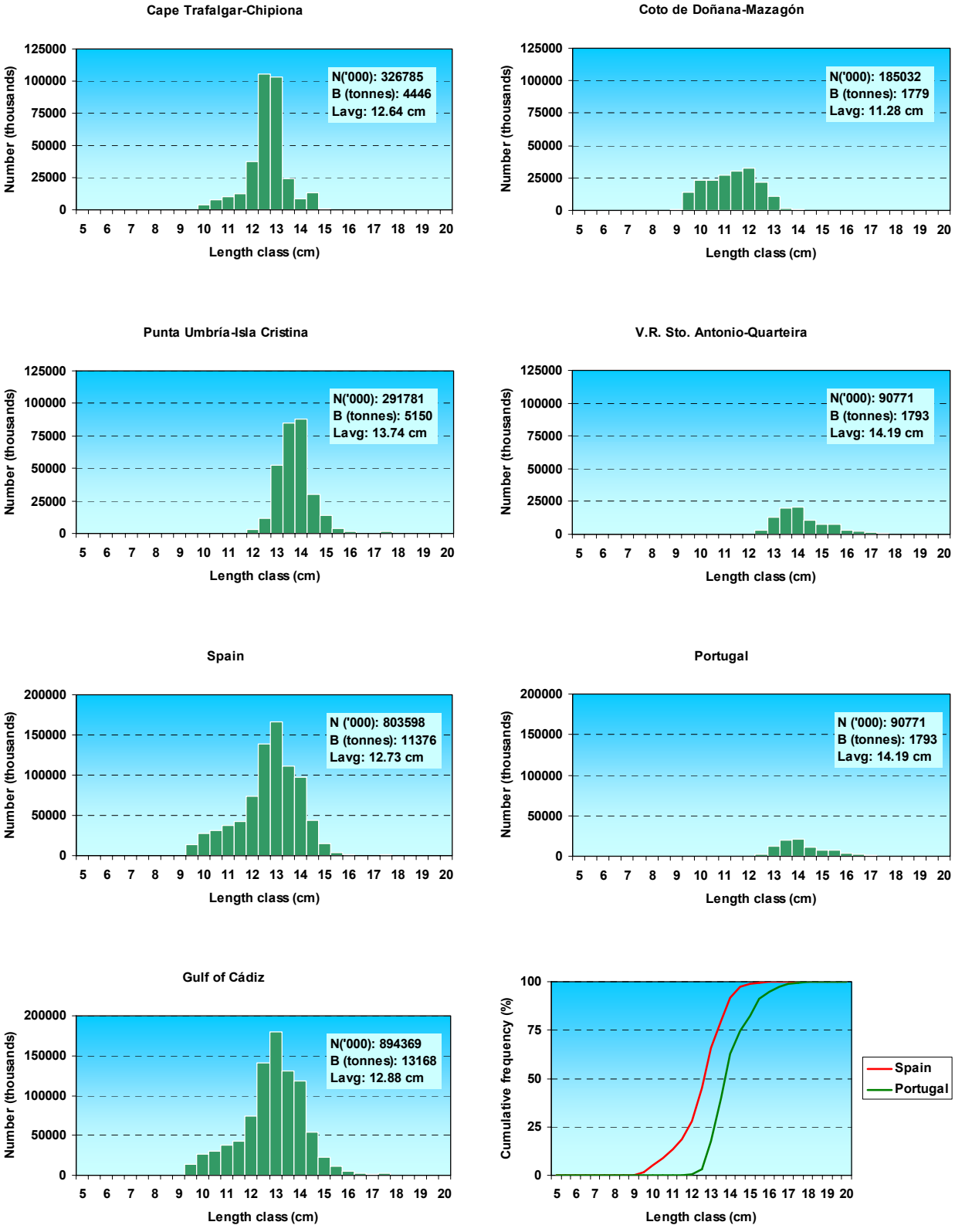


Figure 2.4.2.8: BOCADEVA 0604 survey. Estimated anchovy abundances by length class by sector, region and total area. Bottom right: cumulative frequency (%) by length class and region (Ramos *et al.*, 2004).

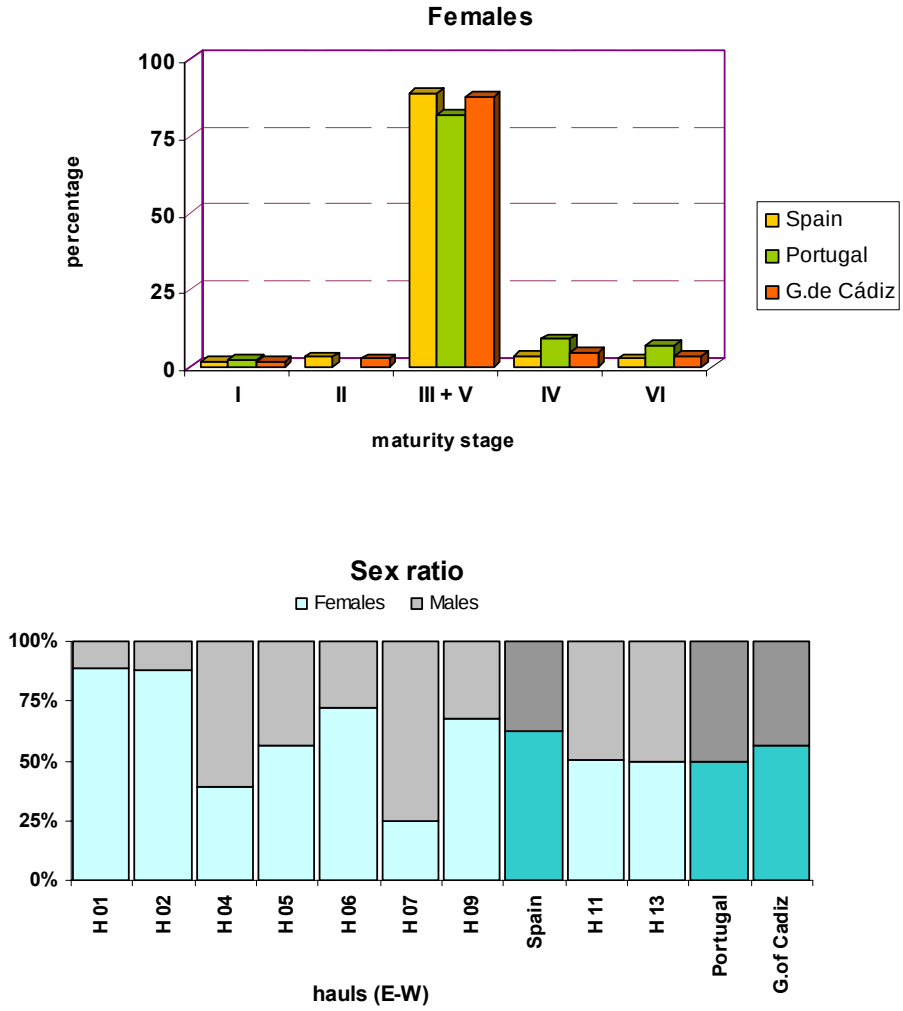
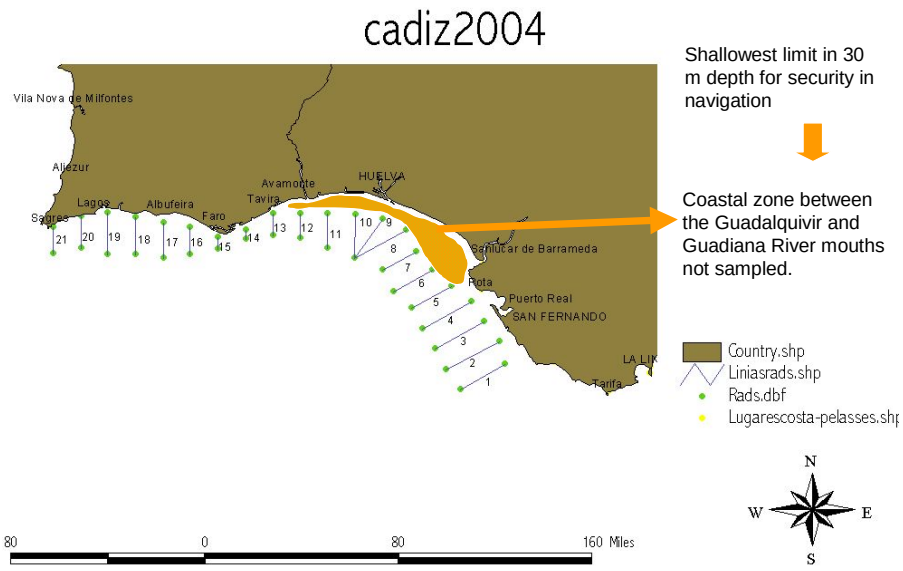


Figure 2.4.2.9: BOCADEVA 0604 survey. Anchovy sex ratio (in weight) of the mature population.

Figure 2.4.3.1: BOCADEVA-0604. Surveyed area. Yellow zone shows the area not prospected in 2004.



3 Traditional *vs.* GAM Estimation

3.1 Introduction

In the previous SG reports and the recent ICES Cooperative Research Report resulting from them (ICES 2004a), emphasis was placed on the potential use of GAMs (Generalized Additive Models) in DEPM estimation. ToR b for this meeting was set to compare traditional with GAM-based estimates of SSB to decide whether GAMs can be recommended as the standard methodology for routine DEPM estimation. However, in the last year consensus has been built up among SG members that this question is, in fact, misleading and cannot be answered universally. Instead, the emphasis should be placed in obtaining software that can allow the use of a range of options of increasing complexity for DEPM estimation, and in identifying the situations where each estimation procedure is more adequate in the light of the data available and the spatial complexity of the population under study.

Following this rationale, Section 3.2 describes the advances in the preparation of DEPM software (freely available on request to SG members), providing concrete examples of its utility. This utility is further demonstrated in Section 3.3, where a series of important decisions/assumptions affecting egg production estimation are highlighted, using simulations and exploration of existing survey data. Given that these issues can seriously affect both traditional and GAM-based estimation, it was considered important to invest in their clarification and resolution prior to taking final decisions on the comparison between traditional and GAM-based estimation for the existing sardine and anchovy surveys. This also resulted in a delay in the production of spatial maps for the two species (ToR c for this SG meeting), although average spatial maps of adult parameters from pooled survey data are shown in Section 4 (Section 4.4).

3.2 Available software for DEPM estimation

ICES (2004) provided a description of the software (R libraries) available at the time for DEPM estimation. Since then, the following modifications/additions have taken place:

- *Depmodel* (currently called *Egg*): GAM-based egg production estimation functions have been extended to permit estimation (rather than setting) of the parameters describing the daily spawning probability density function. Although not yet introduced in the library, code permitting bootstrap estimation of the variance associated to each adult parameter and spawning biomass for GAM-based estimation has been produced.
- *Geofun*: A new and more robust version has been produced for the transformation of spatial variables, interactive delimitation of survey limits and positive stratum, estimation of sea area associated to each survey point and the plotting of results at the appropriate spatial scale.
- *Eggsplore*: A new library to perform traditional egg aging (based on the original FORTAN code produced by Lo in 1985) and egg production estimation using non-linear least squares or generalized linear models. In conjunction with the library *egg*, this library facilitates comparisons of traditional with GAM-based estimation
- *Shachar*: A new library to perform exploratory data analysis for GAM-based estimation and for spawning habitat characterization.

In addition, the process of producing a manual describing the use of these libraries with worked examples has been initiated (WDs by Bernal *et al*. and Stratoudakis and Bernal presented to this SG meeting). The following sections briefly describe (including worked examples) some aspects of these libraries, aiming to demonstrate their utility for comparing alternative options in DEPM estimation.

3.2.1 Survey area delimitation and estimation

Within the library *Geofun*, survey area delimitation and estimation can be performed interactively for each survey (or survey segment) based on a robust estimation method (Dirichlet tessellation performed by the library *Deldir*). Outer area delimitation is defined by the user (by mouse clicks on an interactive plot), while the inner limits are defined by a smooth coastline (although can also be delimited by the user). For positive area delimitation (positive stratum in DEPM terminology) it is important that the exercise is performed on a spatial scale with adequate resolution, since at low resolution the line can intersect with survey points leading to computation problems. Area estimation (in km^2) is based on Dirichlet tessellation, which calculates the total area within the delimitation and distributes it among the data points considered. As an example, Table 3.2.1.1 shows the estimates of total and positive area for each Iberian DEPM survey for sardine based on *Geofun*, while Table 3.2.1.2 compares with existing estimates for the two national surveys. Overall, estimates of total area are very similar between methods, although positive area is in general larger with *Geofun*. This can be partly due to user subjectivity but also to the fact that with *Geofun* delimiting small irregular patches can be more cumbersome and, occasionally, computationally unstable.

3.2.2 Egg ageing

The data to be used for the parameter estimation of an egg development model come from incubation experiments that should span the temperature range observed in surveys. For example, during the period 1988 to 2002, sardine eggs have been found off the Iberian Peninsula over the temperature range of 10.9–19.3°C. Until 1999, the egg development model used for DEPM purposes was based on the incubation experiment performed by Miranda *et al*. (1990). The advantage of this experiment is that encompasses the above range of temperatures (incubation temperature range 11–20°C), but the raw data are not available. Given that the data were used to fit a rather restrictive non-linear model, the scope for future use of this experiment is limited. In 2002, IEO performed another incubation experiment for sardine, this time in the Gulf of Cadiz. The disadvantage of this experiment is that it did not consider the upper range of observed temperatures in the field (incubation temperatures 11–17°C), but the raw data are available and can be used to fit any model of egg development.

There are currently three different ways for obtaining egg numbers and mean age per daily cohort per sample (the basic information needed to estimate egg production). With increasing order of complexity these are:

- the method of Lo (1985);
- the Bayesian method (ICES 2004);
- the extended Bayesian method to include daily spawning PDF estimation (not yet reported).

All methods can currently be performed within the R library *eggsplore*, provided the raw data from an incubation experiment are available. Figure 3.2.2.1 shows the cohort numbers at age for each sardine survey and ageing method to explore for discrepancies between them. In 1988 (and 1990, not shown), there are no points with high leverage and both methods produce very similar results, despite the Bayesian method producing a visibly longer right tail of low densities at old ages. In 1997, despite the apparent similarity in the density plots, the Bayesian method provides lower estimates of production and mortality (see Section 3.2.3 below). These differences are not related either to differences in the right tail nor at the highest density points. In 1999, there is a highly influential point with a different relative contribution to the two methods: it belongs to a station where 779 eggs were found, with 449 being at stage VI and the rest being destroyed. The main difference in the two methods is that in the Bayesian one, destroyed eggs are distributed proportionately to all estimated ages, while under the current implementation of the Lo method in *eggsplore* destroyed eggs are excluded. Removing this station leads to a 16% reduction in the Bayesian mean production density and a 7.5% reduction in the Lo one. In 2002, both methods estimate a negative mortality rate (i.e. egg density increasing with age) due to three stations with high densities at old ages. The three stations were all

sampled during the same day in an offshore area north of Lisbon (depth 110–140 m), where stages VI to X eggs dominated. Their coincidence in space suggests that this is not likely to be a sampling artifact, implying that either older eggs were advected offshore or that daily spawning activity was interrupted for some reason. In either case, removing these three stations leads to slightly higher production estimates and inverts the sign of the slope for both ageing methods, although mortality estimates remain non-significant.

Overall, the above observations and the shape of quantile-quantile plots of mean cohort ages and densities obtained by the two ageing methods (Figure 3.2.2.2) indicate that the Lo method:

- Has an abrupt-ending right tail (older ages) which is relatively insensitive to temperature variations
- Attributes considerably fewer cohorts at the daily cohort age tails
- Is more prone lead to large changes in mean cohort age when small modifications in the spawning PDF assumptions are made
- Attributes slightly higher ages than the Bayesian method and the difference increases in older daily cohorts.

3.2.3 Egg production estimation

Egg production estimation from aged egg observations is based on the assumption that egg abundance/density follows an exponential decay model from fertilization to hatching:

$$E[D_t] = D_o e^{(-z\,t)}$$

Where Do is the original egg production (in number or density), Dt is the abundance/density at time t within the period of development and z is mortality. Traditionally, densities at age in a cohort (over unit area of sampling) and mean cohort age are used in a non-linear least squares estimation (NLS), where observations are weighed by the relative area associated to each sampled point to estimate egg Do and z. A statistically more elegant estimation procedure is to use a GLM with an appropriate error distribution for parameter estimation (usually negative binomial or over dispersed Poisson). In this case observed abundance by cohort is used and standardized by unit area using an offset variable, while the relative area of each station is again used to weight observations. A generalization of this procedure is to use a GAM where Po (and eventually mortality) can be locally estimated as a function of covariates (subject to global constrains).

To evaluate the impact of ageing and estimation method, the staged egg data from all sardine DEPM surveys were aged according to the Lo and Bayesian methods 8see Section 3.2.2 above) and the three estimation methods (NLS, GLM and GAM) were applied to the combined Iberian data in each year (Figure 3.2.3.1). For NLS and GLM only the stations within the positive stratum were considered, while for the GAM all stations were considered. The negative binomial error distribution was used in the GLM and an over dispersed Poisson for the GAM. GAMs were fitted as a bivariate surface of Lat and Long and in several cases did not converge. Initialization parameters had to be refined for NLS estimation to converge. Confidence limits in Fig. 3.2.3.1 are based on 2 standard errors of the Po and Z estimates (not available for GAM Po because bootstrapping was not performed).

The results show that Po estimates vary considerably among years (from <2 to >7 10^{12}, note that 1990 refers only to the northern Spanish coast), but in most cases Po estimates are not significantly different among ageing and estimation methods (CVs for Po ranging within 15–30%). On the contrary, Z estimates are generally less variable among years (generally between 0.01–0.02 per hour, with the exception of 2002 where negative mortality was observed) and significantly different from 0, but systematic patterns between ageing methods can be observed: the Bayesian method generally leads to lower and less precise estimates of Z, irrespective of the estimation method. These results where confirmed by an ANOVA, where (apart from the significant year effect both on Po and Z),

there was a marginally significant ageing method effect for Z ($p = 0.02$). It is worth noting that GAM estimates are generally similar to those of the other methods despite the use of a very simple bivariate smooth and the occasional lack of convergence.

3.3 The impact of spawning daily synchronicity, young egg availability and temperature-dependent hatching time on egg production estimation

An essential component of the DEPM is the estimation of mean egg density (number of eggs per unit of survey area) produced daily by the population. This is usually obtained fitting a log-linear model to aged daily cohort densities obtained from the ageing of staged egg counts in an ichthyoplankton survey. However, in linear and generalised linear models, parameter estimation is highly influenced by the observations at the extremes of the distribution range of the explanatory variable (here mean cohort age). In the case of the DEPM, this implies that samples taken at times of the day where very young (i.e. recently spawned) and very old (i.e. very close to hatching) eggs should be observed, will determine to a large extend the estimated egg production (exponent of the intercept) and mortality (slope).

Egg densities at extreme ages are naturally affected by the processes of birth and hatching respectively, which in most DEPM applications are accounted for based on the best available biological information. Small pelagic fish spawning is known to show daily synchronicity, usually towards the end of the day e.g. ICES 2004; Ganias *et al*. 2004). However, the degree of synchronicity in the population can affect the earliest time at which the daily cohort is entirely available to the sampler. When daily spawning activity cannot be assumed to be knife-edge, estimation usually excludes cohorts whose estimated age is less than half of the assumed total duration of daily spawning (e.g. Lo, 1985). Similarly, cohorts whose age is larger than the age at the onset of hatching for a given temperature are truncated for estimation purposes. However, these decisions require some knowledge/assumptions on the shape of the daily distribution of spawning activity and the temperature-dependent duration of egg development up to hatching and their importance for estimation are usually not sufficiently highlighted in DEPM applications

During this SG, two other processes, rarely discussed in the literature, but can considerably affect observed egg densities at the tail of the cohort age distribution and thus influence estimation were identified. The first is reduced availability of young eggs to the sampler, which, if present, would invariably lead to negative bias in egg production estimation. The second is temperature-dependent production and/or mortality, which, if present, can either lead to positive or negative bias in estimation. Although at first sight both processes may seem far-fetched and unlikely to occur at any significant rate under natural conditions, experience in the SG indicates that their potential impact may not be negligible. In the rest of this section, simulations are used to explore separately the impact of daily spawning synchronicity patterns, young egg availability to the sampler and temperature dependent production and mortality rates on egg production parameter estimation.

Ichthyoplankton survey "observations" (egg densities and mean ages for daily cohorts) were generated from a known population. To test the processes affecting young eggs, observations were generated from a negative binomial distribution with mean equal to a production of 100 eggs m^{-2}, adjusted for an hourly mortality rate of 0.02. The parameter theta of the distribution was fixed to 0.3. To avoid the influence of processes affecting observations at old ages, it was assumed that sampling occurred at a temperature where hatching starts beyond the third day from spawning, so observations were truncated to a maximum cohort age of 72 hours for estimation purposes. Fifteen realizations for each age in the [1, 72] hourly intervals were generated for each simulated set of observations, which can be seen as the result of a survey where one plankton haul is performed hourly for 15 days (360 hauls). Daily production density (exponent of the intercept) and hourly mortality rate (slope) were estimated in each case using a GLM with a negative binomial error distribution. Generation of survey observations and estimation was repeated 500 times for each simulation.

Figure 3.3.1 shows the impact of the shape of the daily spawning probability density function (PDF) on egg production and mortality estimation. When the PDF is reasonably tight (i.e. most of the daily spawning occurring within a couple of hours) the impact due to the incomplete recruitment of the young daily cohort is minor for estimation purposes under the assumed levels of natural variation in the observations. However, as the PDF becomes progressively flatter, negative bias in the estimated egg production and mortality becomes progressively more noticeable. At the most extreme case considered here (a PDF from a normal distribution centered about the peak spawning time and with a standard deviation of 4 hours), egg production and mortality are underestimated by 10% approximately.

Figure 3.3.2 shows the impact of reduced young egg availability to the sampler on egg production estimation. For these simulations the spawning PDF was substituted by an "availability" PDF coming from a normal distribution with mean equal to the number of hours after peak spawning where half of the young eggs become available to the sampler (H50) and a standard deviation of 1 (left) or 2 hours (right)). These results show that the impact of reduced availability has a considerably larger effect on egg production estimation with a negative bias in the order of 25% when H50 is 5 hours later than peak spawning time. As expected, the shape of this PDF is less important in this case, with the flatter PDF showing some evidence of higher negative mortality for low H50s (1 or2 hours from peak spawning).

The above results clearly show that, if present and not accounted for, reduced young egg availability can greatly influence egg production estimation under both traditional and GAM-based estimation. In the case of sardine, the extremely rare observation of stage I eggs (always in low densities) had alerted scientists on the potential problem of reduced young egg availability since the first application of the DEPM in the Iberian Peninsula in 1988 (Cunha *et al*. 1992). At the time though it was considered more likely that the problem was due to gear selectivity (stage I eggs of sardine are considerably smaller because the chorion is still closely attached to the embryo). However, in more recent surveys the problem persisted despite the use of finer meshes. From the 1322 hauls with sardine eggs in all Iberian DEPM surveys until now, only 20 contained stage I eggs. In addition, only in 8 of these hauls more than 1 stage I eggs were found and all cases less than 20 per sample.

To explore further the problem of young sardine egg availability, the data from all DEPM surveys were pooled and aged with the Bayesian method assuming a very tight spawning PDF (mean= 20 hours, sd = 0.5 hours). All aged observations with egg presence from the first daily cohort (i.e. mean age < 24 hours) where binned in hourly intervals and divided by the number of ichthyoplankton stations sampled within the respective hourly bin. The same calculation was performed for observations from the second daily cohort (i.e. with mean cohort ages between 24 and 48 hours). Figure 3.3.3a shows that the hourly distribution of sampled stations around the 24 hours cycle is approximately uniform (around 140 by hour), despite a small increase during night hours (when fishing for adults does not take place). Figure 3.3.3b shows the proportion of sampled stations with egg presence in each hourly interval over the first 48 hours of sardine egg life-time. The results show that from the 8th hour onwards the mean proportion remains constant at approximately 0.22. However, from 20:00 to 04:00 in the following morning (i.e. first 8 hours of life), there is a sharp linear increase in the proportion of sampled stations where the youngest daily cohort is observed. The shape of this curve corresponds closely to an availability PDF with H50=4 hours and sd=2 hours, thus indicating that if this reduced early availability is not accounted for, negative bias in the order of 20% can occur in sardine egg production estimation.

To test the process affecting old eggs, observations were separately generated from distinct populations in two equal-sized geographical strata. To avoid the influence of processes affecting observations at young ages, it was assumed that daily spawning was knife-edge and there were no young egg availability problems. To simulate temperature-differences in the two strata, it was assumed that in the "colder" stratum hatching started occurring at the end of the fourth day (96 hours) and at the "warmer" stratum after 3.5 days (82 hours). Egg observations in each stratum were once more generated from a negative binomial distribution with theta=0.3, up to the maximum age prior to the onset

of hatching in each stratum. Eight realizations for each age in the respective age range of each stratum were created under distinct scenarios for egg production and mortality in the two strata. Once more, generation of survey observations and estimation was repeated 500 times for each simulation.

Figure 3.3.4 shows the resulting estimates for egg production (top panels) and mortality (lower panels) for 5 population (x-axis) and two estimation scenarios (left *vs.* right panels). When production and mortality are equal in the two temperature strata, estimation is unbiased irrespective whether older ages are truncated according to the stratum temperature (left panel) or the high temperature stratum (right panel). When production in the two strata differs, truncating according to stratum temperature leads to bias. As expected, negative bias is observed when production is lower at the high temperature stratum and positive mortality is observed when production is higher at the high temperature stratum. When only production is different between strata, estimation remains unbiased if older ages at both strata are truncated at the age that hatching starts in the high temperature stratum. However, when mortality varies between the two temperature strata, resulting estimation is always biased and the only solution is to post-stratify and estimate separately in the two strata.

Table 3.2.1.1: Total and positive area (in 10^3 km^2) in sardine DEPM ichthyoplankton surveys by year and stratum.

SURVEY AREA	YEAR	SOUTH	W PORT	GALICIA	W CANT	E CANT	TOTAL
Total	1988	8.7	41.3	23.5	19.4	9.0	101.9
	1990	-	-	26.0	27.3	9.5	62.8
	1997	20.0	38.7	29.0	17.8	9.3	114.7
	1999	22.1	34.2	14.9	8.8	4.5	84.6
	2002	16.1	32.4	14.0	11.5	6.1	80.1
Positive	1988	3.5	24.2	10.3	14.4	6.1	58.5
	1990	-	-	11.4	20.2	5.6	37.2
	1997	13.2	13.4	0.0	5.0	3.2	34.8
	1999	15.0	19.6	0.0	4.7	3.1	42.3
	2002	11.2	26.7	2.1	8.4	4.9	53.2

Table 3.2.1.2: Comparison of total and positive survey area (in 10^3 km^2) by country, year and method (Iberian DEPM surveys for sardine).

YEAR	COUNTRY	TOTAL AREA		POSITIVE AREA	
		Geofun	**Literature**	***Geofun***	**Literature**
1988	Portugal	50.0	48.9	27.7	22.3
	Spain	51.9	51.7	30.8	26.4
1990	Spain	62.8	60.6	37.2	30.6
1997	Portugal	60.4	66.6	26.7	17.8
	Spain	54.4	NA	8.3	7.4
1999	Portugal	57.4	38.6	34.5	16.2
	Spain	26.6	16.9	7.8	8.4
2002	Portugal	48.5	45.7	37.9	16.6
	Spain	31.7	NA	15.3	10.0

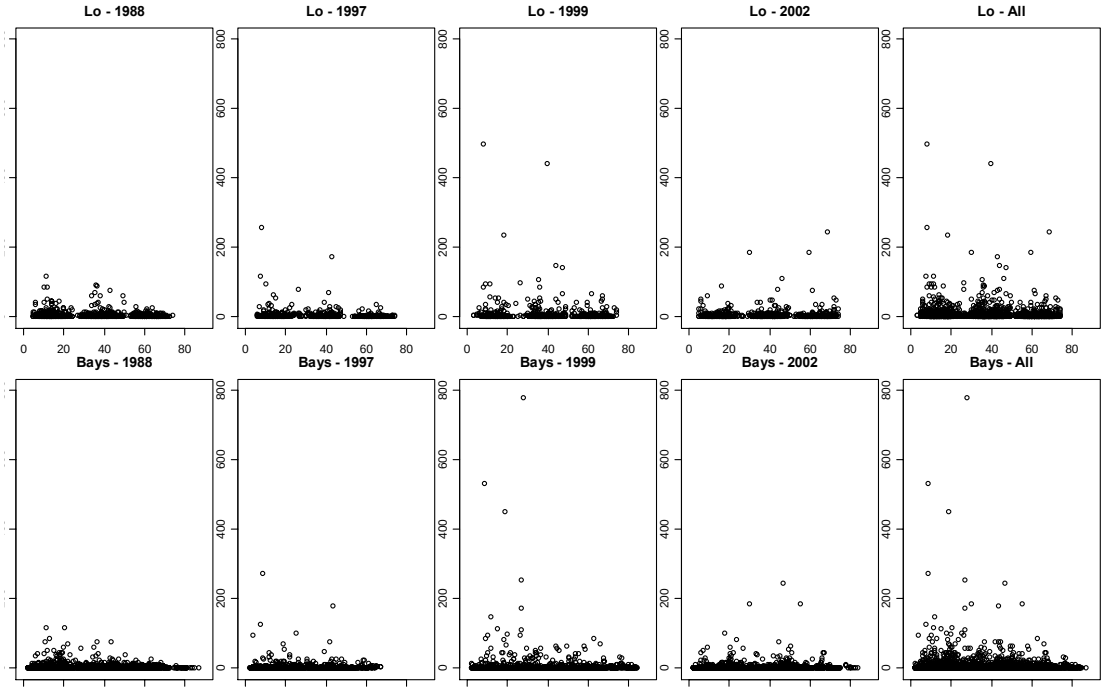


Figure 3.2.2.1: Sardine daily cohort density against mean cohort age (hours) from all Iberian DEPM surveys based on the Lo ageing method (upper panel) and the Bayesian ageing method (lower panel).

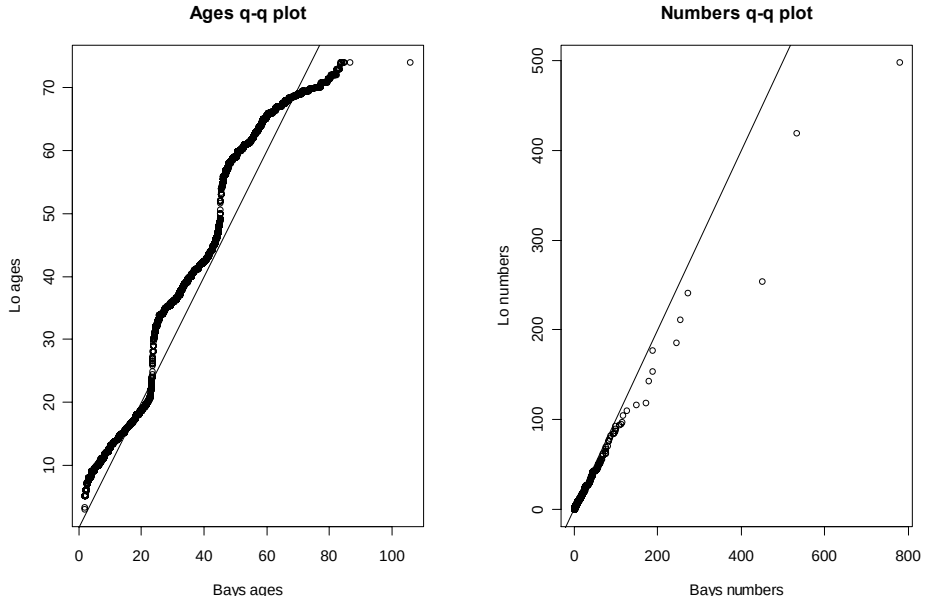


Figure 3.2.2.2: Quantile-quantile plots of mean cohort age (left) and cohort numbers (right) based on the Lo and Bayesian ageing methods respectively (data pooled from all Iberian DEPM surveys for sardine). Lines indicate 1:1 relationship.

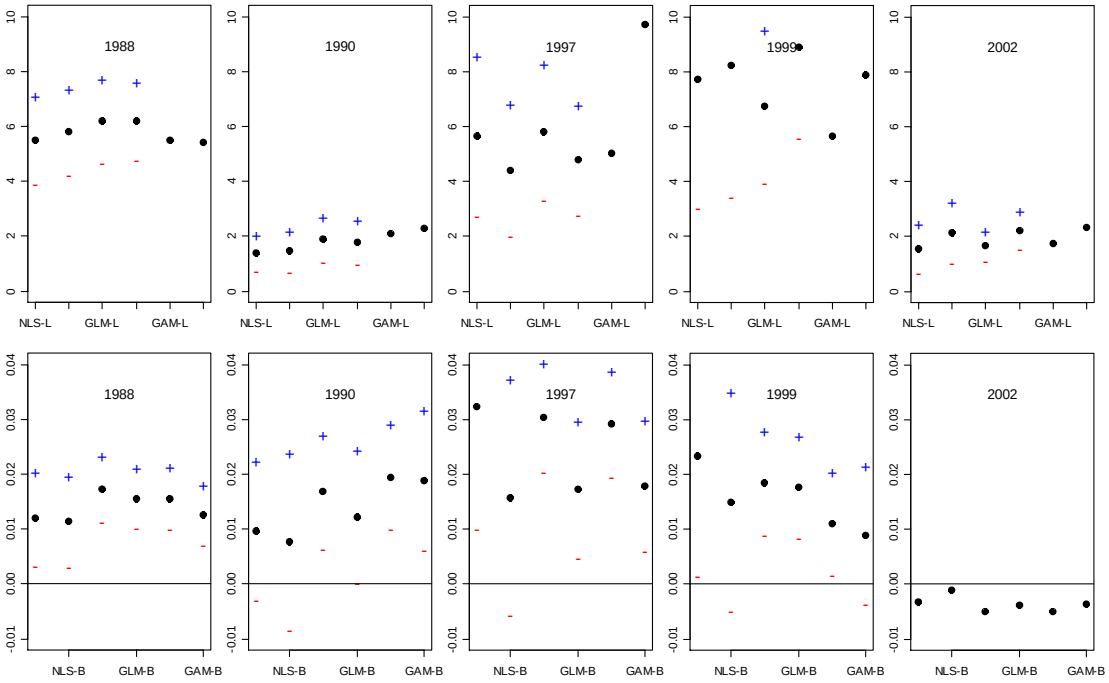


Figure 3.2.3.1: Po (top) and Z (bottom) estimates for each sardine DEPM survey in relation to ageing method (Lo or Bayesian) and estimation method (NLS, GLM or GAM). Confidence limits are based on 2 standard errors of the Po and Z estimates (not available for GAM Po because bootstrapping was not performed).

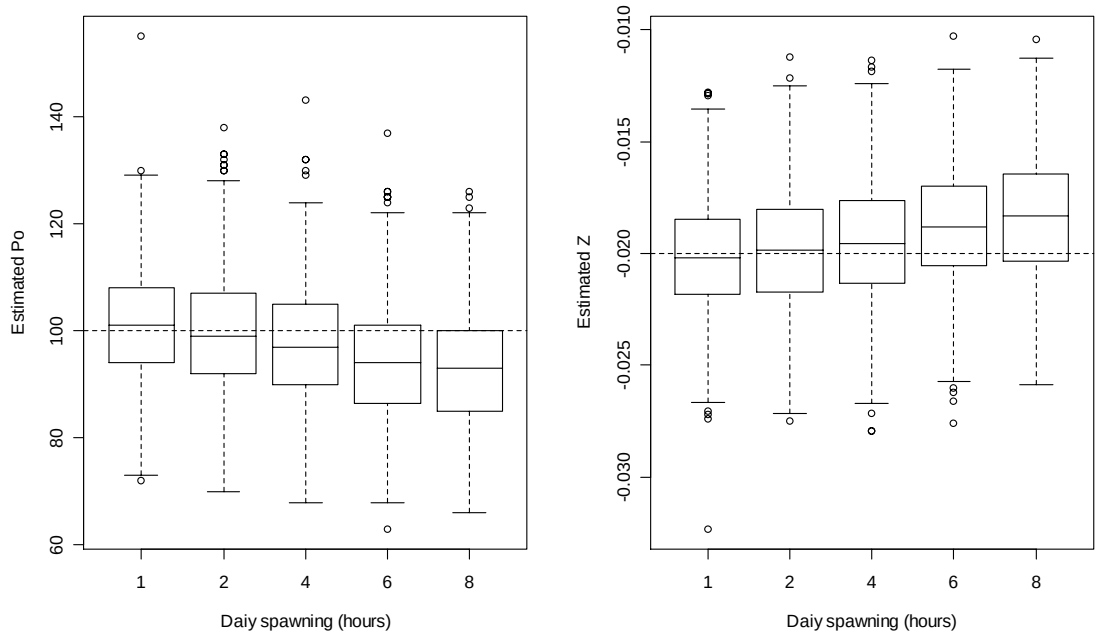


Figure 3.3.1: Simulation results on the impact of spawning PDF shape (x-axis) on estimated egg production (left) and mortality (right)). Each boxplot represents the distribution of the 500 estimates of egg production/mortality under each scenario. Broken lines indicates true population production/mortality.

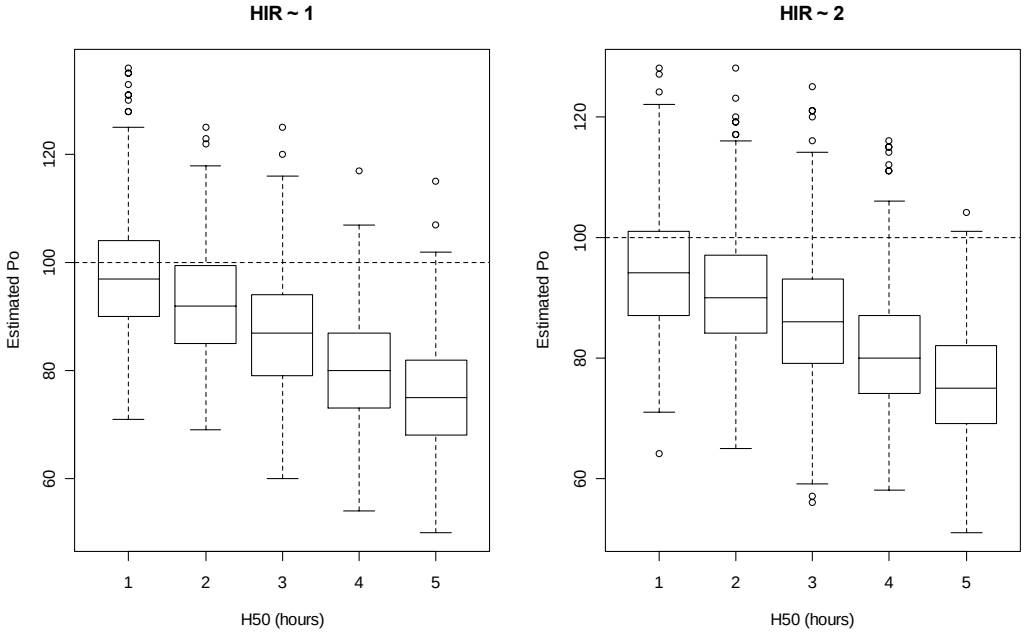


Figure 3.3.2.2: Simulation results on the impact of reduced young egg availability to the sampler on estimated egg production as a function of the shape (left *vs.* right panel) and location of the PDF (x-axis). Each boxplot represents the distribution of the 500 estimates of egg production under each scenario. Broken line indicates true population production.

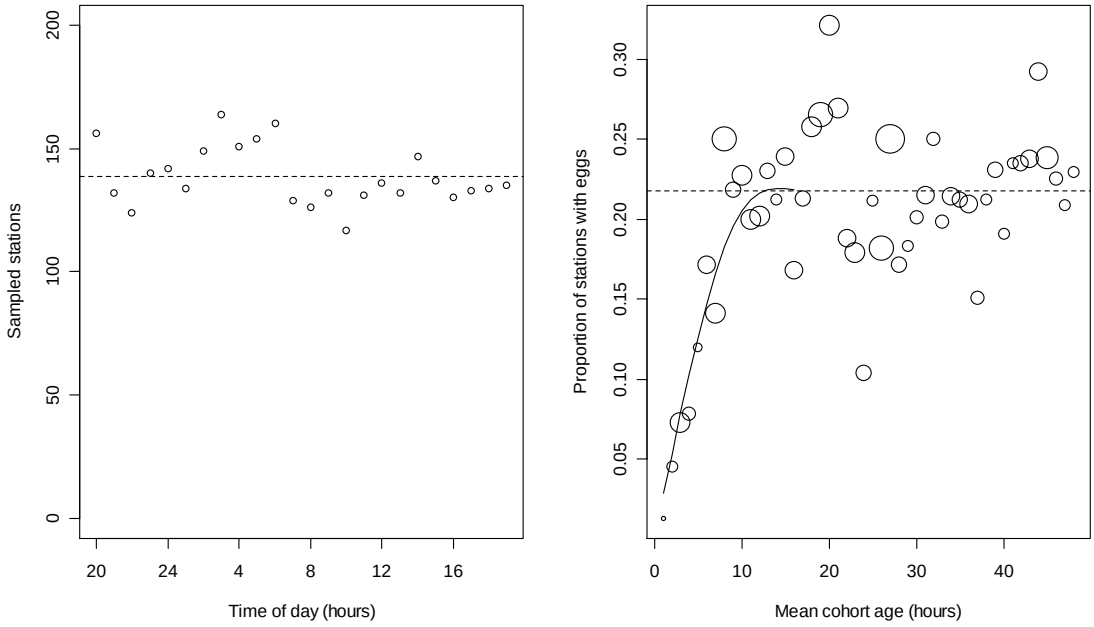


Figure 3.3.2.3: Number of ichthyoplankton stations sampled hourly within the daily cycle (left) and proportion of stations with eggs from the first (1–24 hours) and the second (24–48 hours) daily cohorts (right). Estimates are based on pooled data from the 1988, 1997, 1999 and 2002 Iberian DEPM surveys for sardine. In the left panel, broken line indicates mean number of stations sampled hourly. In the right panel, broken line indicates mean proportion of sampled stations with egg presence after the first 8 hours of life, solid line indicates smooth spline fitted to the data from the first 16 hours of life and circle size is proportional to the logarithm of the mean hourly cohort abundance.

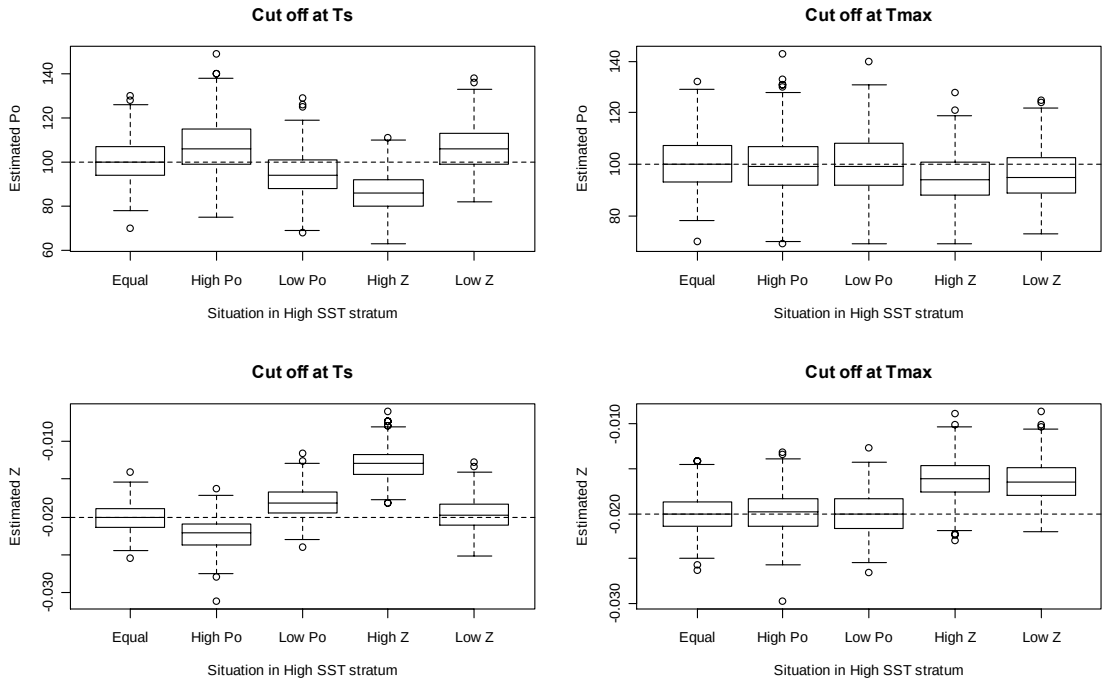


Figure 3.3.2.4: Simulation results on the impact of variable production/mortality in the two temperature strata (x-axis indicating situation in high temperature stratum) and of estimation method (left panel: truncating at stratum temperature; right panel: truncating at high temperature) on estimated production (top) and mortality (bottom). Each boxplot represents the distribution of the 500 estimates of egg production/mortality under each scenario. Broken line indicates true population production/mortality.

4 Reproductive Parameters and Spawning Dynamics

This chapter focuses on aspects of sardine and anchovy reproduction that influence adult DEPM sampling, laboratory analysis and estimation (ToR d). Section 4.1 presents new developments on post-ovulatory follicle (POF) staging and ageing for the two species, an issue with important implications for the estimation of spawning fraction. Section 4.2 reviews (anchovy) and revises (sardine) information on microscopic female gonad maturation, while section 4.3 reviews information on sardine spawning dynamics based on data and literature from both the Iberian waters and the Mediterranean Sea. Finally, Section 4.4 provides average spatial maps of adult DEPM parameters for sardine and anchovy, using GAMs on the pooled data from various surveys.

4.1 Post-Ovulatory Follicles: staging and ageing

A postovulatory follicle is mainly composed of two cell layers - the granulosa (epithelial tissue) and the theca (connective tissue). These two layers together form the structure that surrounds a developing oocyte, which remains in the ovary after the ovulation (and spawning) of the hydrated egg. After ovulation, this structure collapses, degenerates and is reabsorbed within a few days (West 1990). The similarity between the processes of identifying egg daily cohorts from the morphological characteristics of developing eggs and identifying cohorts of daily spawners from the morphological characteristics of degrading POFs was acknowledged in the last SG meeting, where it was suggested that the methodology available for egg staging and aging could be adapted for the identification of POF daily cohorts for spawning fraction estimation.

The attribution of POFs to daily cohorts would use information on daily spawning activity and on the rate of temperature-dependent follicle degradation. As with egg ageing, the first step in this process should be the classification of POFs into distinct morphological stages, which could then be converted to ages using independent information on the time of spawning, temperature and sampling time. Based on work developed by Ganias *et al*. (2003) on the Mediterranean sardine, the compilation of data on POF morphological characteristics from discrete daily periods was considered adequate to develop such a classification scale. This approach has been used to develop a scale of POF morphological stages for sardine (Section 4.1.1) and to obtain preliminary information on the conversion of POF stages to daily cohorts (Section 4.1.2). In the case of anchovy, the detailed analysis of the morphological characteristics of a large set of histological sections of ovaries was the basis to establish a description of POF stages. Preliminary data on anchovy POF ageing was collected during an experiment in which the degradation of POFs was followed in adult anchovies caught close to spawning time and maintained in captivity during several days.

4.1.1 POF stages

For the Bay of Biscay anchovy, POF stages were initially established by Motos (1996). The degeneration process was classified into three stages: POFs 0 (0–6 h); POFs 1 (7–30 h) and POFs 2+ (31–54 h). In practice however, some problems were identified when following this classification and subjective decisions were often necessary. In addition, in this procedure staging and ageing were unified. Hence, after the histological examination of the gonads (and taking into account the collection time), only the final cohort allocation was retained but not the stage of degeneration of the follicle. In this SG meeting (first WD by Alday *et al*.), a new classification key of seven stages was presented, based exclusively on the state of degeneration of the postovulatory follicles. These POF stages were defined by selected objective degeneration criteria among those previously described in literature (Hunter and Macewicz 1985, Hunter and Goldberg 1980, Goldberg *et al*. 1984). A detailed description of each POF stage from this new POF classification key for anchovy is presented in Annex 1 and summarised in Table 4.1.1.1.

For Atlanta Iberian sardine, the pattern and rate of POF degeneration was first described by Pérez *et al*. (1992) from laboratory-spawned individuals. This classification has been used since for the

spawning biomass estimation of sardine in the Iberian Peninsula (Cunha *et al*. 1992, Cunha *et al*. 1997, Lago de Lanzós *et al*. 1998, Quintanilla and Pérez 2000). Further, an inter-calibration exercise took place in 2001 and a reference collection of follicle images was built for future use (ICES 2002). It was then agreed to continue to classify POFs in four stages or daily cohorts, Day-0, Day-1, Day-2 and Day-3, based on Pérez *et al*. (1992). In 2003 a revision of the POFs dating was performed for the 1997 and 1999 Portuguese survey samples by a different reader and important discrepancies between these two readings were obtained for the spawning fraction of 1997 (ICES 2003), suggesting that problems existed either in the sampling procedures and/or quality of histological slides or in the identification of the POF daily cohorts.

During the past year, histological samples from the 1999 and 2002 DEPM surveys were used to delimit distinct morphological stages, by only considering samples from discrete periods of the day [in the evening – around the peak spawning time (19–20h), and in the morning (7–10h)]. Digital images of the POFs observed in the histological slides of the selected samples were obtained and "blindly" classified (i.e. irrespectively of sampling time), based uniquely on the POF morphology. The criteria used were mainly related to: the POF overall aspect and size, the aspect and cellular integrity of the granulosa cells and of their nuclei, the presence of oval structures (or vacuoles), the relation between the granulosa and theca layers, and the presence of a lumen. On the whole, images from 155 samples processed histologically on resin or paraffin, were analysed. However, the samples embedded in paraffin appeared to be of very difficult classification as the cytological details (especially of the granulosa) – very important in the assessment of the intensity of the follicular degeneration - were not as clearly visible. Therefore the classification of morphological stages was elaborated only with the resin embedded samples. The different samples processed in resin could be classified morphologically into seven distinct stages (Annex 2).

4.1.2 POF ageing

For anchovy, in order to validate the duration of the stages proposed above, several captivity experiments were carried out at warm temperatures (17–21ºC), as described in the second WD by Alday *et al*. The analysis of the results obtained in these experiments seems to suggest that the POF degeneration process is faster than what has been traditionally believed using the conventional staging method. In addition, the analysis of the field samples corresponding to years 2001 and 2003 (mean T= 17ºC, similar to the experiments) seem to correspond largely with the results obtained from the captivity samples. However, the results obtained from the field samples corresponding to the colder years 1995 and 2004 (mean T=14ºC) suggest 4 or more hours of delay in the degeneration process, which would need to be checked by performing more captivity experiments at cold temperatures. Therefore the impact of these results on the estimation of the spawning frequency for anchovy is still uncertain and more research is required for different temperatures.

For sardine, the samples of the 1999 DEPM survey collected during two periods of the day – evening (19–20h) and morning (7–10h), were blindly classified into seven distinct morphological stages and the pattern of POD degeneration was described. For each sample, the follicular morphological stage was related to the spawning time, and the results have shown a complete correspondence between the two parameters, i.e. each morphological stage was observed only during a discrete period of the day (evening or morning), with the exception of stage 6 which could be found in samples from both periods considered (see Table 4.1.2.1).

Although the present results do not allow assigning a precise follicular age (in relation to the assumed spawning time) to each morphological stage, an indication of the probable elapsed time from the spawning event can be inferred from the sampling time information. For instance, stage 2 occurred only in the evening in our samples and corresponds to a very recent POF, with no signs of degeneration, suggesting that this stage may appear only a few hours after the spawning event. Stage 3 was observed only in samples from the morning and the POFs were just initiating degeneration, suggesting that these postovulatory structures were originated from the preceding day in the evening

(presumptive elapsed time from spawning: half a day). The same reasoning was conducted for all the other stages (Table 4.1.2.1).

In conclusion, the identification of POF daily cohorts in Iberian sardine can still not be based on precise stage/age classifications as it has been achieved for eggs. The postovulatory follicles can be classified into morphological stages, and according to the sampling time, a possible elapsed time from the spawning can be deduced, giving indications on the POFs daily cohorts. Furthermore, this classification should also be considered with some care in years with very different mean sea temperatures in relation to 1999 (year from which samples were taken to build this classification); indeed the duration and rate of POF degeneration may differ with environmental conditions, which would imply a different relation between a given morphological stage and its presumed age.

4.2 Anchovy and sardine maturation scales

Knowledge of the overall developmental stage of the ovary is useful to complement information on POF degradation and to understand the process of multiple spawns and ovarian regeneration. Microscopic staging of gonads can also be used to improve the precision of macroscopic maturity keys. Criteria for the classification of oocyte stages and general development stage of the ovary were presented in last years meeting and have been improved based on new data available to the SG (Section 4.1).

4.2.1 Anchovy

The traditional classification of oocytes used for anchovy ovaries has been based on the five oocyte stages of Motos (1994) described as follows:

Unyolked oocytes (1): All oocytes without yolk that are between about 0.04 and 0.35 mm. The smaller oocytes within this class are spherical and become oval as his development advances. At the beginning they have a large nucleus and a narrow cytoplasm homogenous, very densely stained with haematoxylin. The nucleus contains several nucleoli.

Partially yolked oocytes (2): Oocytes in this class are in the early stages of yolk deposition. Yolk deposition starts at the periphery of the oocyte and spreads internally until it nearly reaches the finely granular perinuclear zone. Usually by this time the granules have become small spherules.

Yolked oocytes (3 and 4): Oocytes in this class range from 0.45 to 0.70 (major axis) and all contain yolk spherules throughout the region between the periphery of the oocyte and the perinuclear zone. As vitellogenesis continues, the yolk varies from spherules in the smaller oocytes to large globules in the larger ones.

Migration of the nucleus: Prior to hydration, the nucleus starts his migration to the animal pole (initial migration, **stage 5**) what occurs approximately 24 hours before the spawning. Once the nucleus has arrived at the animal pole the nuclear membrane disintegrates (**stage 6**), dispersing its contents into the cytoplasm. In the same way, the yolk globules fuse into plates (12 hours before spawning).

Ovaries from the samples collected in previous DEPM surveys (during the anchovy peak spawning period, i.e. May-June) were examined in detail in order to establish the series of oocyte maturation stages and the degeneration process of postovulatory follicles. When a stage was considered to be morphologically identified, microphotographs were obtained and a brief description of the features was made. As a result, a new classification scale was proposed (first WD by Alday *et al.*), which agrees almost completely with the previous one, except for two modifications. First, the hydrated gonads were assigned an independent oocyte class (hydrated oocyte) and the hydrated oocytes have been classified into two different classes depending on the hydration process:

Partially hydrated oocytes (stage 7): Oocytes that have started the hydration process. Large yolk globules fuse forming yolk plates. The oocyte expands greatly stretching the granulosa and thecal cell layers but his shape remain oval.

Completely hydrated oocytes (stage 8): Oocytes that have completed the hydration process. Now the yolk plates have fused forming one single plate. The oocyte continues expanding acquiring a star shape.

Secondly, this new classification takes into account not only the stage of development of the oocytes present in the ovary but also the morphological characteristics of the POF degeneration as well as the incidence of oocytes atresia (α and β atresia) (see Table 4.2.1.1). Based on this classification, a list of morphological characteristics of oocytes, POFs and atresia types that should be observed when analyzing histological slides of both of sardine and anchovy ovaries for DEPM studies has been established. The SG agreed that this list should serve as a guide for future work within DEPM and a template of the information to register during laboratory work is presented in Annex 3.

4.2.2 Sardine

The agreement between macroscopic and microscopic classification of maturity stages was one of the topics discussed in the last SG meeting. This topic is relevant to adult sampling within DEPM since only macroscopically mature (stage 2 and above) females are analyzed histologically. A large misclassification rate of those females could introduce bias in the estimation of adult parameters. A comparison between microscopic and macroscopic maturity stages based on survey samples was reported to the last SG meeting. In this exercise, 24.5% of macroscopically stage 1 (virgin/resting) gonads were classified microscopically in stage 2 and 3, i.e. gametogenesis was advancing and should therefore be included in the spawning component of the population. Furthermore, in the last DEPM survey (2002), ca. 12% of the individuals sampled had macroscopic stage 1 gonads, which is a non-negligible fraction of the sampled population. These samples were not processed histologically and therefore we do not know if they have been misclassified.

In DEPM surveys, a microscopic maturity scale of four stages has been used to confirm macroscopic classifications. This scale was based uniquely on the presence of the most advanced type of oocytes, considering the four stages of oocyte development of West (1990): chromatin nucleolar and perinucleolar, cortical alveoli, vitellogenic and mature or hydrated. This scale had the advantage of a high objectivity in its application but on the other hand complicated the comparisons with the existing macroscopic scale for sardine (composed of 6 stages). A microscopic scale of six stages was also presented last year, considering not only on the characteristics of the most advanced type of oocytes, but also on other aspects as the organization of ovigerous lamellae, the presence of post-ovulatory follicles, the incidence of atretic oocytes and debris in re-absorption . Since this scale was based on data from a single month (November) it was decided to explore a limited set of histological slides of gonads from monthly market samples between January and November 2002, to complement the results. This analysis was expected to improve the description of microscopic maturity stages and to complement to the macro-micro calibration exercise with data from outside the spawning season.

A revised microscopic scale of maturity based on these additional samples is presented in Annex 4. The scale of 6 stages allowed characterizing microscopically a final post-spawning stage (which corresponds to the macroscopic stage 5). Ovaries in that stage have shown a variety of microscopic aspects, which at first sight may be difficult to identify, but in all cases they indicate that the female is probably ending its spawning activity for that spawning season, whether because they already contain only undeveloped oocytes (chromatin nucleolar and perinucleolar) or because most of the developing vitellogenic oocytes are atretic. A difference from the scale presented last year is the distinction between virgin and resting individuals: although the two stages share the same type, size and organization of oocytes, the presence of blood vessels identifies fish which have already spawned. However, this characteristic is not detectable externally and since the overall size and morphology of the oocytes is similar, the two stages are not distinguished macroscopically.

The microscopic separation between pre-spawning and partial post-spawning is maintained, however their similarity at the microscopic level is also acknowledged. The differences between the two stages are related to the spawning activity: in a partial post-spawning female that has already spawned several times, signs of this spawning activity become progressively visible in the ovary (resorbtion structures, atresia, and smaller density of vitellogenetic oocytes). But in a female that has spawned few times, these signs may still not be as conspicuous and the microscopic aspect of the gonad may still have the typical aspect of a pre-spawning (stage 3) female. Indeed, typical microscopic stage 3 females can be observed during the spawning season, being impossible to determine if these females have already spawned a few times (while keeping a microscopically stage 3 aspect) or are about to spawn for the first time. This is in accordance with the macroscopic maturity scale used in routine in AZTI, which joins within stage 3 pre-spawning females and females that have recently entered the spawning cycle. Therefore, the precision of the macroscopic scale can be increased either by considering both pre-spawning and early partial spawning females in stage 3 or by merging pre-spawning and partial post-spawning stages.

Overall, the microscopic scale is expected to "overestimate" maturity in the early spawning season and to "underestimate" maturity in the late spawning season, comparatively to the macroscopic scale. This discrepancy arises from the fact that microscopic stages are defined by the presence of the most advanced type of oocytes and other characteristics that are not reflected immediately on the external appearance of the ovaries. When only a few oocytes of a given stage are present, it is possible to detect them microscopically; however they do not affect the overall ovary volume or aspect. Therefore, an ovary in initial development may have a few microscopically detectable stage 2 oocytes (cortical alveoli) but still shows the macroscopic characteristics of a stage 1 ovary. On the contrary, in the late spawning season, a macroscopic partial post-spawning ovary may have a high incidence of oocyte degeneration suggesting no further spawning activity (characteristic of a post-spawning ovary). Table 4.2.2.1 shows the correspondence between macroscopic and microscopic classification of sardine ovaries in 2002 samples (monthly data pooled due to the limited sample size). These data essentially confirm the low precision of initial maturity stages (1 and 2) based on macroscopic criteria and the large misclassification between pre-spawning and partial post-spawning ovaries.

4.3 Sardine spawning dynamics

This section presents information on several topics of sardine spawning dynamics that have a direct link to the estimation of spawning biomass within DEPM or provide auxiliary information to the interpretation of DEPM results. Most of this information results from analyses of existing data from DEPM or acoustic surveys, from samples of the fishery or from both data analyses and literature review. Section 4.3.1 describes the seasonal cycle of spawning off the Portuguese coast and the discusses the appropriate definition of maturity ogives for the estimation of spawning stock biomass. Factors affecting the seasonality of sardine maturation and spawning are discussed based on research on the Mediterranean sardine (Section 4.3.2).

4.3.1 Annual maturation cycle and maturity ogives

DEPM estimates are based on a snapshot of the reproductive activity in the peak of the spawning season. However, a continuous monitoring of the reproductive cycle is essential to track changes in reproductive activity which helps both to interpret past results and to plan future DEPM surveys. With this aim, the monthly evolution of sardine macroscopic maturity was explored based on data from market samples off the Portuguese coast for 1998–2003 and a detailed description of female gonad development is provided. Data from acoustic and DEPM surveys carried out in the same period were used as complementary information on the spawning activity of the population. Finally, maturity ogives (by length) were compared from the start, middle and end of the spawning season (November, January and March 1998/1999 and 2001/2001) to illustrate how small and large individuals are recruited to the spawning season.

Figure 4.3.1.1 shows the monthly percentage of female sardines by maturity stage and the mean GSI in catch samples from January 1998 to December 2003. Stage 1 and 2 females are rare in catch samples during the spawning season; however, their percentage in the population estimated from survey samples can be high depending on recruitment strength (Table 4.3.1.1). Fractions of stage 1 individuals above 60% were observed in the 2000/2001 spawning season, following the strong 2000 recruitment. Stage 3 individuals are generally more abundant early in the spawning season (November) while stage 5 (final post-spawning) fish peak around March; the cycles of these two stages overlap considerably with that of stage 6 (partial post-spawning) what may be partly a consequence of misclassification among these stages (see Section 4.2.2). The spawning peak defined by the gonadosomatic index (which does not include females in spawning condition) occurs between December and January and is consistent with the peak in the percentage of spawning females (maturity stage 4 in Figure 4.3.1.1).

There is some indication of a secondary spawning peak in spring in some of the years (1999/2000 and 2000/2001). The correlation between the successive series of maturity stages suggests that the processes of early development and post-spawning regression of gonads are slower than progression from pre-spawning (stage 3) to spawning (GSI series) (Figure 4.3.1.2). While there is only one month lag between the peaks of stage 3 and GSI series, the progression from stage 2 to stage 3 and from spawning to post-spawning takes about three months. In summary, these results mainly confirm previous observations that sardine shows an extended winter spawning in the Portuguese waters with a peak in December-January and possibly a secondary spring peak in some years. Similar results are reported for the Mediterranean and other sardine populations (see Section 4.3.2).

Maturity ogives based on data from the beginning (November), mid (January) and end (March) spawning season off the Portuguese west coast in 1999 and 2002 are presented in Figure 4.3.1.3. The percentage of adult females by length class was higher in January than in November in both years suggesting that smaller individuals entered the spawning population during the first part of the spawning season. Ogives from March surveys do not show a consistent relation with those from the beginning or mid-spawning season. A different relation between November and March ogives from year to year was also evident from an earlier analysis of maturity ogives including a large number of acoustic surveys (November 1997–2001 and March 1996–2003) However, a higher maturity-at-length (difference of 0.6 cm in L50) was observed in November when pooling data from the different years. The decrease in maturity-at-length in the late spawning season suggests that smaller individuals not only recruit later but also exit earlier from the spawning population. This type of spawning dynamics is expected since similar differences in spawning period have been reported for other populations of small pelagics (see Section 4.3.2).

As pointed out in last SG report, the spawning fraction estimated in the 2002 DEPM for sardine was particularly low and several other indicators of spawning activity had unusual values in comparison with previous years. Monthly data on macroscopic maturity from market samples also suggests that the 2001/2002 spawning cycle was unusual with a higher and more extended occurrence of stage 1 (virgin/resting) females preceding the spawning season and an earlier appearance of post-spawning (stage 5) females (Figure 4.3.1.1). Data on maturity stage fractions in surveys also show a disproportionate abundance of post-spawning individuals during the 2001/2002 spawning season (Table 4.3.1.1). Another indication of an unusual maturation pattern in that season comes from the maturity ogives: maturity-at-length estimated along that spawning season show an increase in the proportion of resting individuals at intermediate lengths suggesting either a delay or a cessation of the spawning activity (Figure 4.3.1.1). In January 2002, the maturity ogive appears to branch; an inspection of the raw data showed that the left branch (early maturation) is based on samples north of 39ºN while the right branch (late maturation) comes from samples south of that latitude (Lisbon area). Data from the previous November survey (2001) confirm this pattern with most of the large resting females distributed in the northern part of the area (around 38º30'). All available evidence points to atypical spawning dynamics in the 2001/2002 spawning season and the SG recommends that more effort is dedicated to the clarification of this issue.

In addition to the above contributions to sardine reproductive biology, some results were also presented on methodological issues, such as the boundary between virgin/adult individuals and the consistency between maturity ogives used within DEPM and the assessment model. Increasing the boundary between virgin/adult individuals from stage 2 (current practice) to stage 3 did not show a consistent effect on the maturity ogives in the two years analysed (there was a decrease in maturity-at-length in 1998/1999 but no effect in 2001/2002). Since no new information was available on the early development or incidence of atresia in the early spawning season, stage 2+ females should be considered as part of the spawning stock biomass. The concept of spawning stock biomass (SSB) was further discussed and it was agreed that within DEPM the SSB refers to the potential spawning biomass of the stock in a certain year. As seen in the comparison of maturity ogives based on November (used in the stock assessment model) and January (used in DEPM for the Portuguese waters) surveys, the SSB from the DEPM is possibly below the assessment SSB in the two years analysed. However, these results should be considered cautiously since the effects on the actual SSB estimates were not evaluated and may be negligible.

4.3.2 Biological and environmental factors affecting maturation and spawning

A WD discussing the factors which affect the reproductive activity of sardine was presented to the SG (WD, Ganias *et al*.). This discussion is based on research on sardine populations from central Greece waters, however most of the conclusions may be applied to sardine populations in general.

In the first sections of the WD the appropriateness of several indices that are commonly used to assess the seasonal evolution of reproductive and somatic condition (gonosomatic and hepatosomatic index, condition factor) in sardine populations is discussed. The most serious problem is that for many fish species, these indices are correlated with fish size and consequently their use in between sample comparisons produce biased estimates when samples are comprised of different sized individuals. Gonosomatic index in the Mediterranean sardine is independent of body size for all females except from those with hydrated ovaries (Somarakis *et al*. *in press*). This is attributable to ovarian growth being isometric in all stages of ovarian development except from hydration when it is altered to positively allometric (Somarakis *et al*. *in press*). Despite, its validity in monitoring the ovarian condition of the individual, the use of *GSI* in population studies has been argued due to continuous *de novo* vitellogenesis, which is a normal event in the ovaries of multiply spawning species. For instance, fish with hydrated ovaries (just prior to spawning) have much higher *GSI* values compared to those that have recently spawned (Somarakis *et al*., *in press*). However, these two groups of females actually belong to the same spawning state. In that case, instead of using the arithmetic mean of *GSI*, the average spawning state of the population should be expressed via the fraction of active spawners. Ideally, the latter are identified histologically, or alternately by the use of a critical *GSI* value (GSI_{50}=1.2 for the Mediterranean sardine), derived from an histologically based maturity ogive, that separates active spawners from inactive/immature females.

Similarly to ovarian growth, hepatic growth in the Mediterranean sardine is isometric, both prior and after vitellogenin secretion. Therefore, the resulting hepatosomatic index (*HSI*) is independent of body size, and may be used without restrictions in population statistics. On the other hand, the pattern of somatic growth in sardine is positively allometric, and thus the Fulton condition factor (*K*), which presupposes isometric growth, depends significantly on fish-size. In that case, it is advisable to use the relative condition factor (*Kn*), which incorporates the allometric coefficient of the overall length-weight data equation, and which is consequently supposed to overcome size-dependency and be more appropriate in population studies.

The seasonal evolution of the fraction of actively spawning females (September 1999– May 2001) showed that the population of sardine in coastal Greece (c. Aegean and Ionian Seas) has an extended reproductive period during the colder months of the year (Figure 4.3.2.1). This pattern seems to coincide with most sardine populations around the world's oceans (Table 4.3.2.1). An exception to this general pattern is observed for sardine populations inhabiting colder seas (e.g. Black Sea, N. Sea, the

Channel), where reproduction occurs during the warmer months of the year. Another exceptional pattern displayed by some sardine populations (e.g. the Japanese sardine) comprises the partition of spawning in two discrete seasons within a single yearly period.

The process of ovarian development and the ultimate release of mature oocytes is synchronized by an interrelated series of internal (endogenous) and external (exogenous) stimuli. Exogenous factors such as temperature, photoperiod, food availability, water quality and a variety of social factors (e.g. visual, chemical or tactile contact with conspecifics) are perceived by the brain and translated into neural impulses that stimulate the endocrine pathways of the hypothalamo-pituitary-gonadal axis to respond in appropriate fashion (Nicolas, 1999; Coward and Bromage, 2000). Concerning the populations of sardine, water temperature is speculated to be the most important external cue regulating gonadal development (Matsuyama *et al*. 1992).

Furthermore, several studies report additional factors affecting spawning activity in sardine populations, such as upwelling (Lluch *et al*., 1991; Ward and Staunton-Smith, 2002) and plankton biomass (Lynn 2003, Somarakis *et al*. *submitted*). The synergetic effect of different of environmental parameters in the gonadal development of sardine is somehow demonstrated in Matsuyama *et al*. (1992) who report that estradiol and MIH (maturation inducing hormone) in Japanese sardine do not undergo the same seasonal cycles, implying that vitellogenesis and final oocyte maturation (FOM) respectively might be triggered by different environmental cues (e.g. synergetic effect of temperature and photoperiod). Sardine spawning occurs over a large temperature range, 13.5–25^{0}C, with an optimal range at 13.5–17^{0}C (Lluch *et al*. 1991, Lynn 2003). Therefore, the aforementioned exceptional pattern exhibited by high latitude *Sardina* stocks, which spawn at late-spring and summer is probably attributed to the fact that it is only in that season that the temperature range for reproduction is achieved. Differences in seasonality of spawning as a response to different temperature regimes are clearly exhibited by sardine stocks inhabiting the Australian coasts. The latter belong either in the subtropical or in the temperate zone, and as a result the respective sardine stocks exhibit remarkable differences in the timing of spawning, e.g. comparison of sardine populations in temperate South Australia and sub-tropical southern Queensland by Ward and Staunton-Smith (2002). Some might hypothesize that such differences displayed by stocks inhabiting different climatic zones are induced by respective latitude-related differences in the photoperiod. However, differences in the spawning period of sardine stocks with similar latitudinal but different longitudinal distributions (e.g. the Mediterranean and the Japanese sardine stocks; Table 4.3.2.1), indicate that similar photoperiod regimes are not enough to induce similar spawning seasonality. Apart from its function as an environmental stimulus determining the onset of gametogenesis, temperature may also affect reproduction in terms of metabolic rates.

Figure 4.3.2.2 illustrates variability in SST within the geographic range of *Sardina pilchardus* and summarizes the seasonal extent of spawning season for several *Sardina* stocks along with respective values of maximum length and maximum age. It seems that populations inhabiting colder habitats (e.g. the Channel, or the Black Sea) display increased lifespan, and limited spawning seasons. Moreover, sardines in the S.E. Mediterranean seem to attain first maturity in smaller lengths (11.8cm; Ganias *et al*. 2003) compared stocks inhabiting colder environment, such as the Bay of Biscay (15cm, *Fishbase* online). This plasticity may be interpreted as phenotypic response to differential temperature regimes. Stocks inhabiting warmer habitats grow faster, and have shorter reproductive life spans (Jennings *et al*. 2001). We assume that in order to mediate the effect of life-shortening on life time reproductive output, populations of sardine inhabiting warmer environments (like the Mediterranean sardine) decrease length at maturity and increase annual reproductive output by elongating their reproductive season. Similar pattern is also described for several Atlantic herring, *Clupea harengus*, stocks across the species' latitudinal range, by Jennings and Beverton (1991). The net effect of this plasticity is that lifetime reproductive output remains relatively constant across the distributional range (Jennings *et al*. 2001).

Recruitment or exit from the spawning population was not simultaneous for all females of the Mediterranean sardine population, as individuals belonging to different size classes (11.8 - 12.9cm, 13-

15.9 cm, and >16cm) exhibited differences both in timing and duration of their spawning period (Figure 4.3.2.3). More intense were differences between the non-fished (<13cm) and the fished (>13cm) population, as the former exhibited reduced spawning season with a delayed seasonal peak, which shifted towards spring. Similar differences in the spawning period of different size-classes are also reported for other populations of clupeids. Herrera *et al*. (1994) and Le Clus (1989) report for the Chilean sardine and sardine of the ecosystem of Benguela respectively, differences in the seasonal evolution of ovarian mass among different length classes. Millan (1999) reports for the anchovy, *Engraulis encrasicolus,* delayed maturation and smaller reproductive period for females <1 year. The same author suggests that inter-annual differences in the extent of the spawning season of the population might be induced by differences in its demographic composition. Similarly, Parrish *et al*. (1986) report for the Californian anchovy, *Engraulis. mordax*, that smaller females have reduced spawning period which occurs towards the end of the spawning period of the whole population. In his review Solemdal (1997) reports for several teleost species (mostly for gadoids and flatfishes), that young recruits tend to have smaller and delayed reproductive period compared to the older. Hence, we may contemplate that the two successive seasonal peaks that are often observed within the spawning season of several sardine populations (e.g. Japanese sardine: Shoji *et al*., 1999; Californian sardine: Quiñonez-Velásquez *et al*., 2000; N. Aegean sardine: Voulgaridou and Stergiou, 2004), might be due to different size/age classes having different spawning periods. Furthermore, we may assume that intense fishing of larger/older females is expected to affect, and more specifically to reduce the reproductive period of the population.

The previously mentioned differences in the spawning period of younger and older females might be related to the common in teleosts dependence of the balance of energy-allocation on growth or reproduction on the size/age of the individuals. For instance, it is widely known that the rate of somatic growth is higher before the beginning of first sexual maturation (Roff, 1983). By delaying sexual maturation and investing energy primarily on growth, individuals achieve sooner the advantages of having larger body size, i.e. increase in fecundity, decrease of the possibility of being preyed etc. (Jennings *et al*. 2001). Hence, the delayed and smaller spawning season of the "young" might be attributed to the fact that when older females begin to invest energy on gamete production, these "prefer" to keep investing on growth. Probably this consists the reason why young sardines synchronize their spawning period with months of increased productivity (see below for: *spring-bloom*); having already spent almost all energetic reserves on growth, their reproduction may only be supported by direct food-intake. The fact that female sardines from the same spawning stock display differences in their spawning periods was also supported histologically, by the occurrence of early post-breeding females (i.e. females with 100% a-atresia) throughout the spawning period of the population (Ganias *et al*., 2003). In their analysis, Ganias *et al*. (2003) showed that even if the beginning of spawning in the Mediterranean sardine depends primarily on size, i.e. the acquisition of size at first maturity by recruit spawners, cessation of spawning is independent of body-size. This coincides with results of the present study, that the end the spawning period is fairly synchronized for all size classes. On the other hand, in Ganias *et al*. (2003), cessation of spawning was shown to be related to the somatic condition of the individual and more specifically to hepatic mass. The present study, demonstrates that actively spawning females display increased hepatic mass not only compared to females that recently abandoned the spawning stock but also to adult females that have not even joined it, i.e. immature females, larger than the size at maturity, without incidence of atresia.

The liver displays a crucial role in teleosts reproduction, by consisting the basis of vitellogenin secretion (Nicolas, 1999; Coward and Bromage, 2000). Furthermore, it consists the conduit for the transfer of lipids from the visceral and muscle deposits to the ovary (Henderson *et al*., 1996). However, a question that emerges is whether increased hepatic weight in active spawners is solely attributed to vitellogenin metabolism, or it also reflects the better nutritional condition of the individuals. Mean hepatosomatic index of sardines in the study area shows a seasonal maximum during late winter-early spring. In the eastern Mediterranean, mixing of the surface waters is generally completed by the end of January and the period of late winter-early spring (February-March) exhibits maximum chlorophyll concentrations, high primary productivity values and a diatom dominated bloom over the

continental shelf (Drakopoulos *et al*., 2000; Psarra *et al*., 2000). This seasonal coincidence of high *HSI* values with the so-called spring-bloom implies that energy from the increased diatom production might be directly stored in the liver. Hence, we may assume that increased hepatic mass of active spawners also indicates better nutritional condition compared to inactive females. The positive effect of *HSI* on reproduction has also been demonstrated by its significant relationship with sardine's ovarian mass (Somarakis *et al*., *in press*) and fecundity (Ganias, 2003). Apart from *HSI*, actively spawning females were also in better somatic condition, but only during the months of maximum reproductive activity. Breeding performance in the Mediterranean sardine is not only modified by the current rate of consumption but also depends on previous consumption history. This is indicated by the reverse seasonal evolution of spawning activity with the relative condition factor. The latter peaks during the summer, i.e. in months of increased zooplankton productivity for temperate habitats (Mann, 1993, Christou, 1998). Thus, we may assume that energy from the nutritionally rich zooplankton is primarily stored as fat in the summer, and is consecutively transferred to gonadal proliferation in order to support energetically the following breeding season. In months of energetic exhaustion, which coincide with population's reproductive maxima (Aegean: December-January; Ionian: February-March), non-spawning adults are in poorer somatic condition compared to actively spawning fish. This implies that reduction of somatic condition below a certain threshold leads to cessation of spawning .

4.4 Average spatial distribution of adult DEPM parameters

An important aspect of GAM-based estimation is the ability of the method to provide spatial information on all DEPM parameters. Such patterns can be easily explored for all years with sufficient data and either lead to a full-scale GAM estimation of spawning biomass or facilitate decisions on post-stratification for the traditional method. In addition, when broad spatial patterns are time invariant, GAMs can provide average spatial maps (from pooled survey data) that can be useful to describe the spatial dynamics of the population under study.

4.4.1 Anchovy

In the last SG report, a first attempt for applying GAMs to estimate DEPM adult parameters and for obtaining a fully GAM-based spawning stock biomass estimate for the Bay of Biscay anchovy was presented using the 2002 survey data. Then GAMs emerged as a powerful tool not only for modelling egg production but also for spatial mapping of the adult parameters.

In this section we briefly illustrate the use of GAMs to explore general spatial patterns of each of the adult parameters for the Bay of Biscay anchovy using pooled data of 10 years surveys (1989-1992,1994-1995, 1997-1998 and 2000-2002). As the aim was to get preliminary ideas on spatial mapping for the adult parameters, only a simple bivariate smooth of Longitude and Latitude was used and environmental covariates were not considered.

Mean female weight (W): This is one of the key adult parameters for the Bay of Biscay anchovy. A GAM with gaussian error distribution and an identity link function using a simple bivariate smooth of Lat and Long coordinates was fitted. Summary statistics are shown in Table 4.4.1.1. The model is able to pick up a general trend of increasing mean female weight as moving offshore with largest weight females located close to the 200m depth contour line (Figure 4.4.1.1). However, the model is not able to explain the inter-annual variability. Figure 4.4.1.2 shows that the yearly pattern observed in the mean female weight is detected in the residuals of the fitted GAM.

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. Alternatively a GAM similar to the one fitted to W could be fitted to F. Figure 4.4.1.3 shows the batch fecundity surface predicted from a GAM with Gaussian error distribution, identity link and bivariate smooth of Long and Lat. The differences in the spatial distribution between the fitted surfaces of mean female weight and batch fecundity are due to inter-annual changes in the regression between both parameters.

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. However, this model explained less than the 4% of the variability and the fitting was not significant (Table 4.4.1.1). No other significant pattern was found for the spawning fraction. All the analysis carried out up to now have not showed any spatial pattern of this parameter.

Sex ratio (R): Sex ratio in numbers was fitted using a GAM with a simple bivariate smooth of Long and Lat, assuming a binomial error distribution with logit link and weights equal to the total number of fish sampled. Even if the model was significant, it only explained 8% of the variability (see Table 4.4.1.1 and figure 4.4.1.4). In addition, the sex ratio in numbers is almost invariant in time (figure 4.4.1.5), supporting the hypothesis validated for the Bay of Biscay anchovy in 1997 and 1998 (Uriarte *et al*. 1999) of 1:1 sex ratio in number. Under this assumption the expected sex ratio in weight could be inferred from the ratio of mean weight of females to the total weight (sum of female and male weights).

4.4.2 Sardine

Adult data from all DEPM surveys for the Atlanto-Iberian stock of sardine were pooled to explore average spatial patterns for adult parameters within the Iberian Peninsula. In total, data from 298 hauls were available, although sampling intensity and spatial coverage varied considerably among years (limited coverage in the southern Iberia in 1988, no data from Portugal in 1990, limited coverage from northern Iberia in 1997 and 1999). GAMs were fitted to the pooled data for each DEPM adult parameter according to the description provided in Section 4.4.1 for anchovy, with two modifications:

- A log link was used for female weight and batch fecundity;
- Daily fecundity was estimated from the predicted values of the 4 models on each grid point and plotted in space.

The resulting models explained 71.4% of the deviance for female weight, 69.6% for batch fecundity, 22.5% for spawning fraction and 4.7% for sex ratio. Figure 4.4.2.1 shows the average spatial map for sardine female weight, spawning fraction and daily fecundity, while Figures 4.4.2.2 and 4.4.2.3 show residual inspection plots for the models fitted to female weight and spawning fraction respectively.

The GAM fitted to female weight (and batch fecundity) explained a large proportion of the variation in the data and led to a spatial map that is in large agreement with existing knowledge from acoustic surveys and the fishery. Despite some problems with the distribution of residuals (longer tail of negative residuals) the overall quality of the fit can be considered good and confirms that most variation in sardine female weight within the Iberian Peninsula is spatial rather than temporal. Indeed, the general spatial pattern remains unchanged when data from each DEPM survey are considered separately (results not shown). The spatial map of mean weight indicates that considerably larger/heavier fish are found in the Cantabrian Sea (almost double the mean weight of sardine in the western and southern coast of the Iberian Peninsula) and that the smallest/lightest fish are found off northern Portugal and in the Gulf of Cadiz. Interestingly, these two sites correspond to the areas where sardine recruitment is known to be most pronounced within the Atlanto-Iberian stock area.

The GAM fitted to spawning fraction also revealed the presence of some spatial structure in this variable, albeit in this case the proportion of deviance explained being considerably lower. This can be explained by the fact that pronounced inter-annual variations in spawning fraction have already been detected, particularly in 2002 off Portugal (see Section 4.3.1). However, the general North-South gradient present on the average spatial map is consistent with that provided when analysing the data from each survey separately. Overall, spawning fraction seems to be highest in the Cantabrian Sea and lowest in southern Iberia, a pattern that is likely to be related to overall duration of the spawning season in these areas. Stratoudakis *et al*. (2004) showed that, in the period 1990-2000, the sardine spawning season n the Cantabrian Sea was narrower than in the western Iberian coast, which

again was narrower than in the southern Iberian coast. Combining the above results demonstrates that a North-South gradient is also detected in daily fecundity, with DF values 4 times higher in the Cantabrian Sea than in the southern Iberia. Apart from the biological interest, this observation also indicates that larger sampling effort is required in the Portuguese survey during adult DEPM sampling in order to obtain similar levels of precision in the estimated spawning biomass resulting from the two surveys.

Table 4.1.1.1: Characteristics of the anchovy POF morphological stages.

		1	2	3	4	5	6	7
SIZE		Large	Large	Smaller size	Smaller size	Small	Very small	POF remains
LOOK		Form loose folds or loops	More tightly folded	Slightly reduced	Notably reduced	Few folds and a more regular form	Very deteriorated	Long or polygonal remains between oocytes
GRANULOSA	**Cells**	Arranged, columnar slightly hypertrophied	Marked alignment characteristics	Alignment characteristics still visible	Noticeable disorder	Complete disorder	Absence of cell walls	Absence of cells
	Nuclei	Very large	Prominent few of them pycnotics	Mostly pycnotics	Pycnotics	Pycnotics	Scarce Pycnotics	Very scarce Pycnotics
	Vacuoles	Absence	Few	Affecting to <50% of the cells	Affecting to >50% of the cells	Massive	Few	Absence
THECA		Noticeable Separated from the granulosa	With capillaries Separated from the granulosa	Noticeable Adheres to the granulosa	Becomes thinner and more closely adhered to granulosa	Still visible Pycnotic nuclei	Less distinct incorporating to stroma	Not visible
LUMEN		Large irregular with granular material	Large with granular material More regular	Easily visible Granular material still possible	Reduced	Very reduced - Absence	Absence	Absence

Table 4.1.2.1: Sampling times at which each sardine POF morphological stage was observed on the histological slides (P.E.T.S.: presumptive elapsed time from spawning, in days).

Sampling time	POF Stage						
	1	2	3	4	5	6	7
7:00			X		X		
7:59					X	X	
8:15			X		X	X	
9:00			X		X	X	
9:30					X	X	
10:00					X	X	
//							
19:15	X			X		X	
19:30				X		X	
20:00	X	X				X	X
P.E.T.S. (d)	0	0	0,5	1	1,5	2/2,5	3

Table 4.2.1.1: New codes and criteria or the identification of the oocyte classes and postspawning stages for the anchovy ovaries and references that should be taken into account for the ovaries examination within the DEPM.

		Code	Comments
OOCYTE TYPE	**NV**	**1**	Only unyolked oocytes
	PV	**2**	The most advanced are partially yolked oocytes
	V	**3**	Less than 50% are yolked oocytes
	V	**4**	More than 50% are yolked oocytes
	MIn	**5**	Beginning of the nuclei migration
	MAv	**6**	Arrival to the animal pole and nuclei disintegr. Partially hydrated oocytes. Yolk plates visible
	Hyd(Pl)	**7**	Completely hydrated star-shaped oocytes
	Hyd	**8**	
POST-SPAWNING STAGE	**No POFs**	**0**	Ovaries with no postspawning signs
	POFs(1)	**1**	New POF's
	POFs(2)	**2**	First signs of POF degeneration
	POFs(3)	**3**	POF with pronounced degeneration
	POFs (4)	**4**	Regressing follicle
	POFs (5)	**5**	Regressing follicle. Absence of lumen
	FPOs(6)	**6**	Very reduced POF
	FPOs(7)	**7**	Tissue remains between the oocytes
ATRESIA	**ATNV**	**%**	Percentage of occurrence of α atresia in the different oocyte classes
	ATPV		
	ATV		
	ATHID		
	ATß		Percentage of occurrence of ß atresia
	TOTAL	**%**	Sum of the atresia percentages

Table 4.2.2.1: Correspondence (number of individuals) between macroscopic and microscopic classification of female sardine ovaries in the 2002 monthly samples (data were pooled due to the limited sample size).

Sample	Macrosc. Matur. Sc.	Microscopic Maturity Scale						
		I	II	III	IV	V	VI	n
2002	I	3	0	0	0	2	0	5
	II	5	4	0	0	0	0	9
	III	0	0	4	0	0	2	6
	IV	0	0	0	0	0	0	0
	V	3	1	0	0	30	3	37
	VI	0	0	9	0	6	17	32

Table 4.3.1.1: Percentages of sardine macroscopic maturity stages (both sexes) off Portugal and Cadiz in the spawning seasons 1997-2002, based on data from acoustic and DEPM surveys.

Spawning season	Month	Survey	Maturity stages					
			1	2	3	4	5	6
1997/1998	November	Acoustic	3	6	29	7	3	52
	March	Acoustic	14	26	10	4	3	43
1998/1999	November	Acoustic	33	12	5	1	1	48
	January	DEPM	6	6	47	3	0	36
	March	Acoustic	6	15	0	2	20	57
1999/2000	November	Acoustic	25	15	10	2	1	46
	March	Acoustic	28	21	0	2	19	29
2000/2001	November	Acoustic	67	17	2	1	2	11
	March	Acoustic	66	0	0	1	21	12
2001/2002	November	Acoustic	21	12	10	3	10	44
	January	DEPM	12	3	2	3	12	68
	March	Acoustic	33	0	0	1	42	24

Table 4.3.2.1: Reproductive periods for several *Sardina* and *Sardinops* stocks.

Genus	Species	Subspecies	Region	Month											
				S	O	N	D	J	F	M	A	M	J	J	A
Sardina	*pilchardus*	*pilchardus*	North Sea[1]										*	*	*
			Channel[1]								*				
			Portugal[2]	*	*	*	*	*	*	*	*	*			
		sardina	Greece (Thermaikos gulf)[3]		*	*	*	*	*	*	*	*			
			Greece (central)[4]	*	*	*	*	*	*	*	*	*			
			Black Sea[1]										*	*	*
			Morocco[1]		*	*	*	*	*	*	*	*	*		
Sardinops	*melanostictus*		Japan[1]							*	*	*			
			Japan[1]		*	*	*	*			*	*	*		
	neopilchardus		Australia (Victoria)[1]	*	*	*	*	*	*						
			Australia (Western)[1]				*							*	
			Australia (S. Queensland)[5]	*	*	*							*	*	*	*
			Australia (South)[6]					*	*	*	*				
			Australia (S.Western)[1]	*						*	*	*	*	*	*
	sagax	*caerulea*	California[1]					*	*	*	*	*	*		
			Peru[1]	*	*	*	*	*	*	*					
			Chile[1]	*					*	*				*	*
		ocellata	South Africa[1]	*	*	*	*	*	*						

[1]FishBase online (www.fishbase.org), [2]Zarrilinki et al. (2004), [3]Voulgaridou and Stergiou (2004), [4]Present study, [5]Schoji et al. (1998), [6]Ward & Staunton-Smith (2002).

Table 4.4.1.1: Summary statistics of the fitted GAM for each DEPM adult parameter for the Bay of Biscay anchovy using the pooled series of data.

VARIABLE	MODEL	N	FITTED DF	GCV	% DEVIANCE
W	s(Long, Lat)	508	20.8	41.6	38.7
F	s(Long, Lat)	432	21.9	-	42.8
S	s(Long, Lat)	337	3.25	-0.22	3.8
R	s(Long, Lat)	437	25.8	6.18	7.9

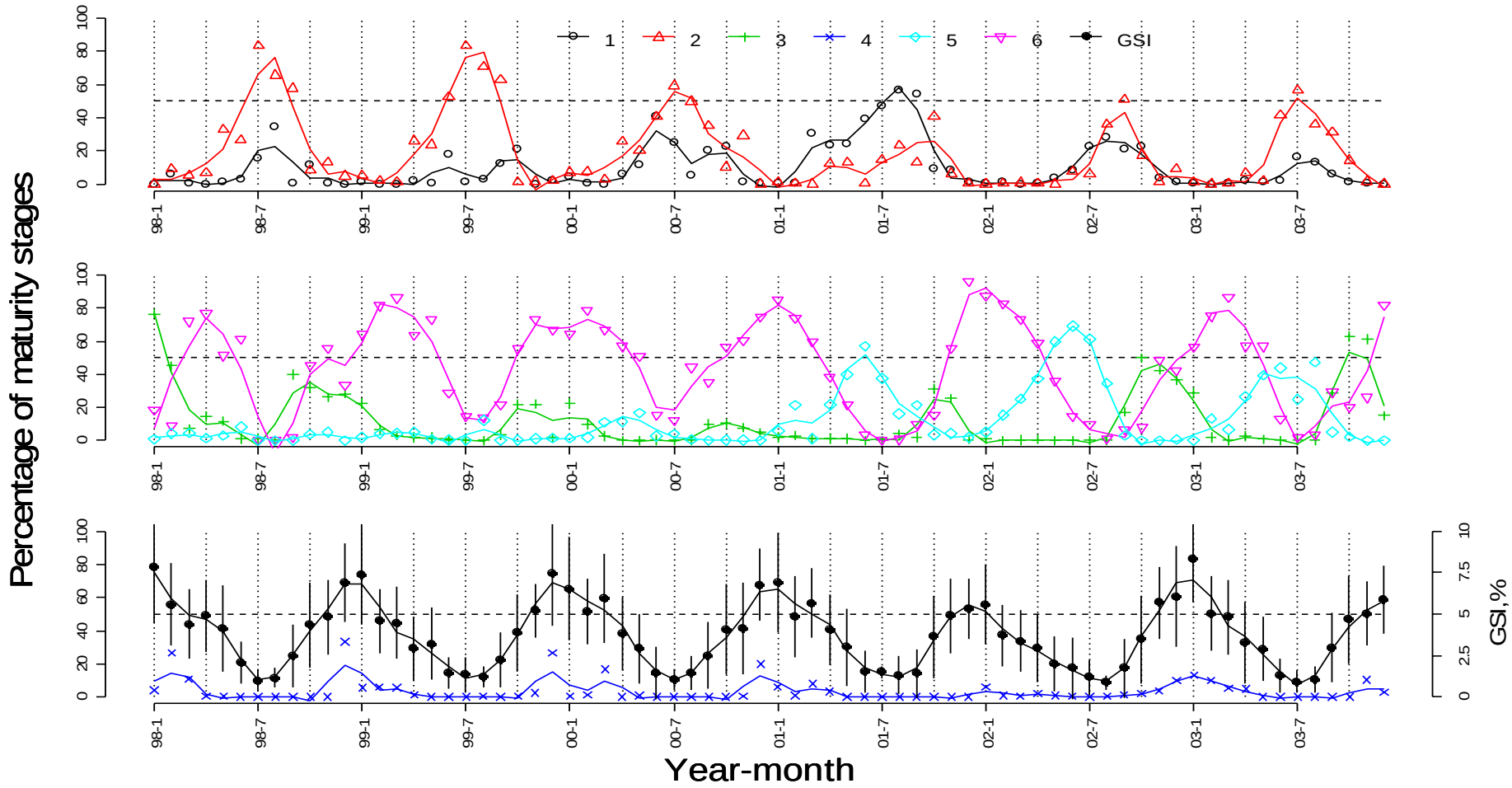


Figure 4.3.1.1: Percentage of sardine macroscopic maturity stages and mean GSI (±std) by month off the Portuguese coast, from catch samples in 1998-2003. Top panel: stages 1 and 2, middle panel: stages 3, 5 and 6 , bottom panel: stage 4 and GSI. A loess (span=0.1) is superimposed on each series. Stage1: virgin/resting; stage 2: initial development; stage 3: pre-spawning; stage 4: spawning; stage 5: final post-spawning; stage 6: partial post-spawning.

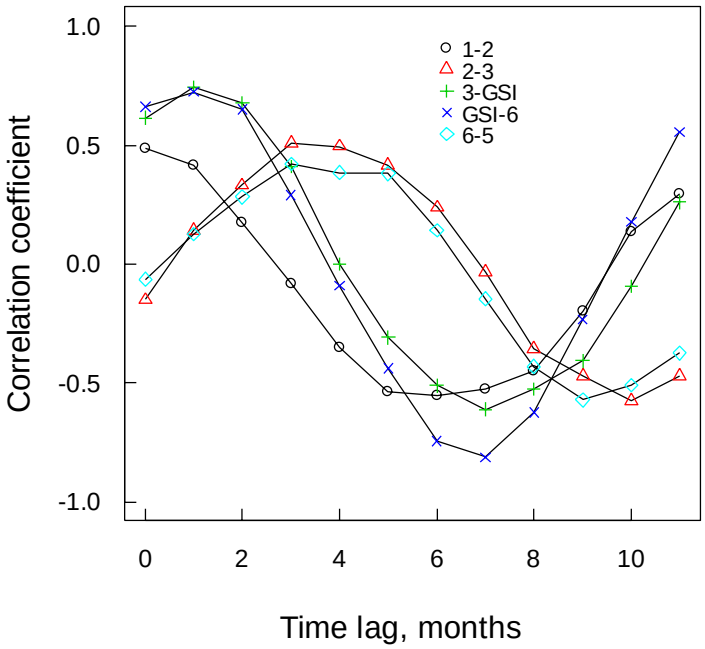


Figure 4.3.1.2: Spearman rank correlation between series of monthly percentages of sardine macroscopic maturity stages, with lags of 0-11 months. . Stage1: virgin/resting; stage 2: initial development; stage 3: pre-spawning; stage 4: spawning; stage 5: final post-spawning; stage 6: partial post-spawning.

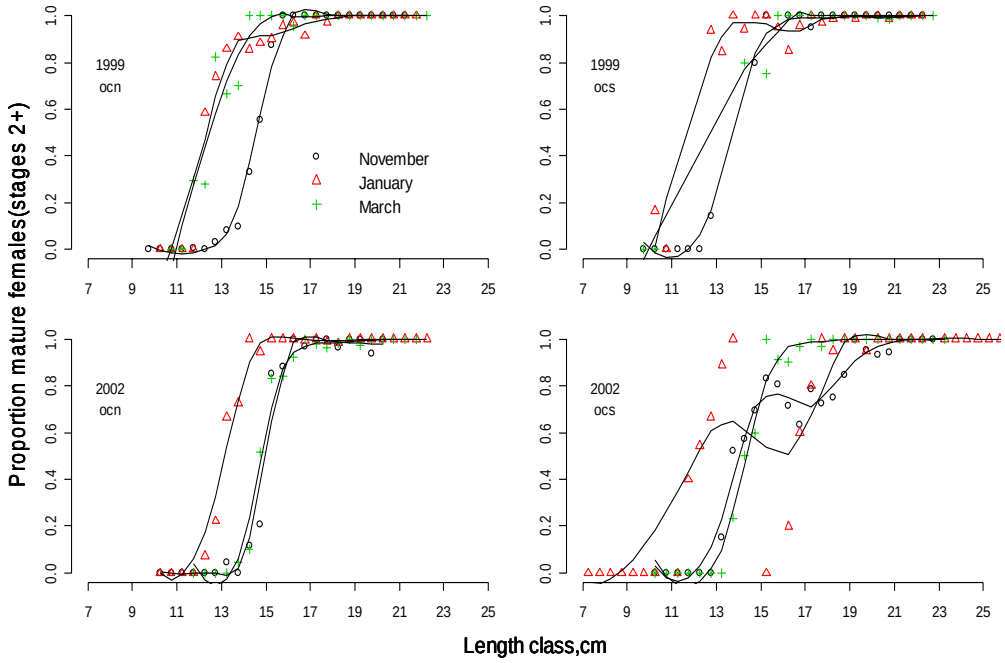


Figure 4.3.1.3: Proportion of mature female sardine (stages 2+) by 0.5 cm length class in November (acoustic), January (DEPM) and March (acoustic) surveys 1999 and 2002 off the north (ocn) and southwest (ocs) Portuguese coast.

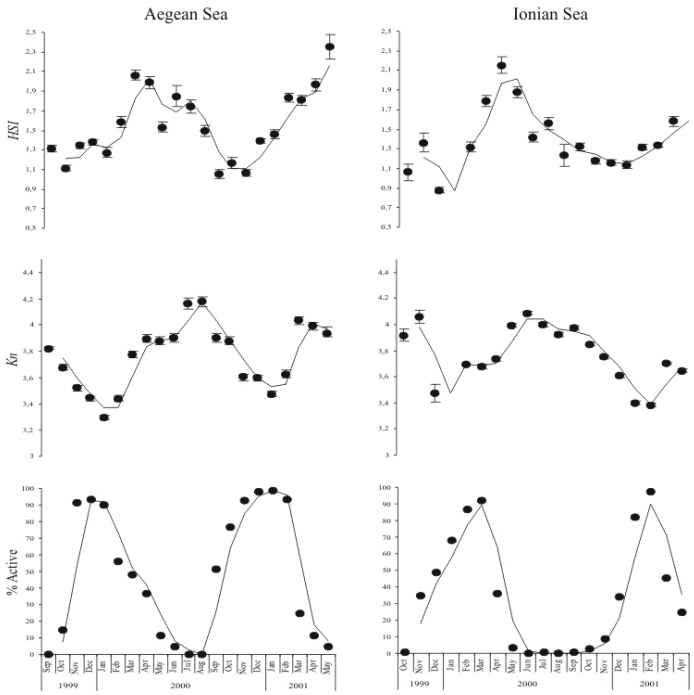


Figure 4.3.2.1: Monthly evolution of reproductive activity (fraction of reproductively active females) and somatic condition of the sardine populations of the central Aegean and Ionian Seas. HSI: hepatosomatic index; Kn: relative condition factor. Trend lines represent the moving average (set at two months interval) and vertical bars correspond to standard errors.

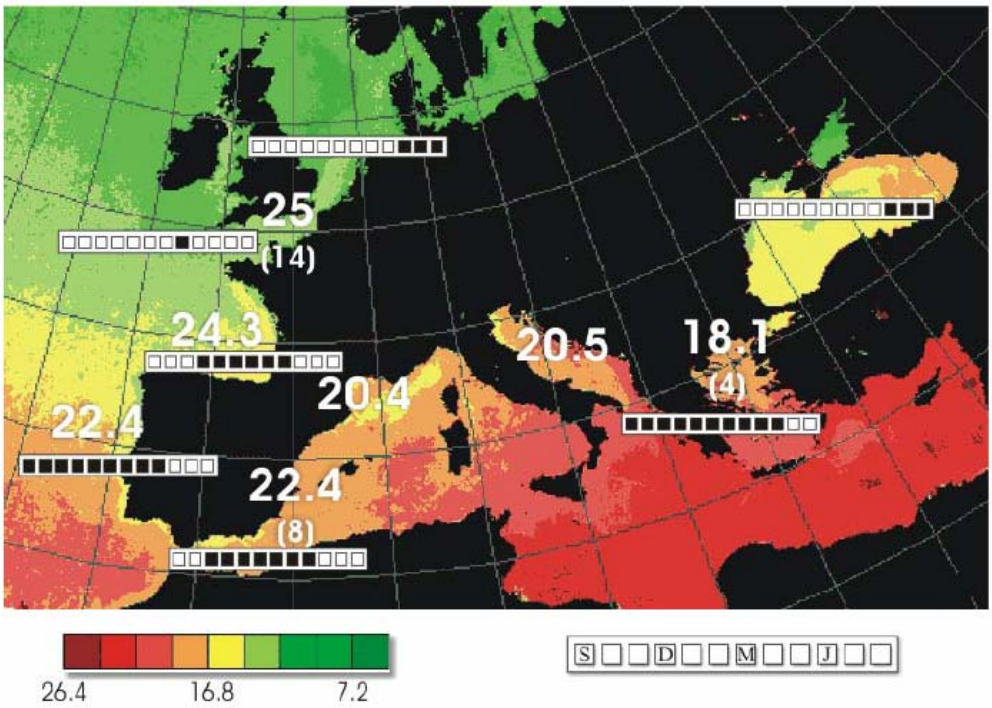


Figure 4.3.2.2: Horizontal distribution of average yearly SST through the geographic range of *Sardina pilchardus* along with spawning periods and estimated values of maximum length and maximum age (in years; given in parentheses) for several Sardina stocks. Biological data were extracted from Fishbase (www.fishbase.org). Left index: temperature scale (in 0C); right index: extent of reproductive period indicating the sequence of months.

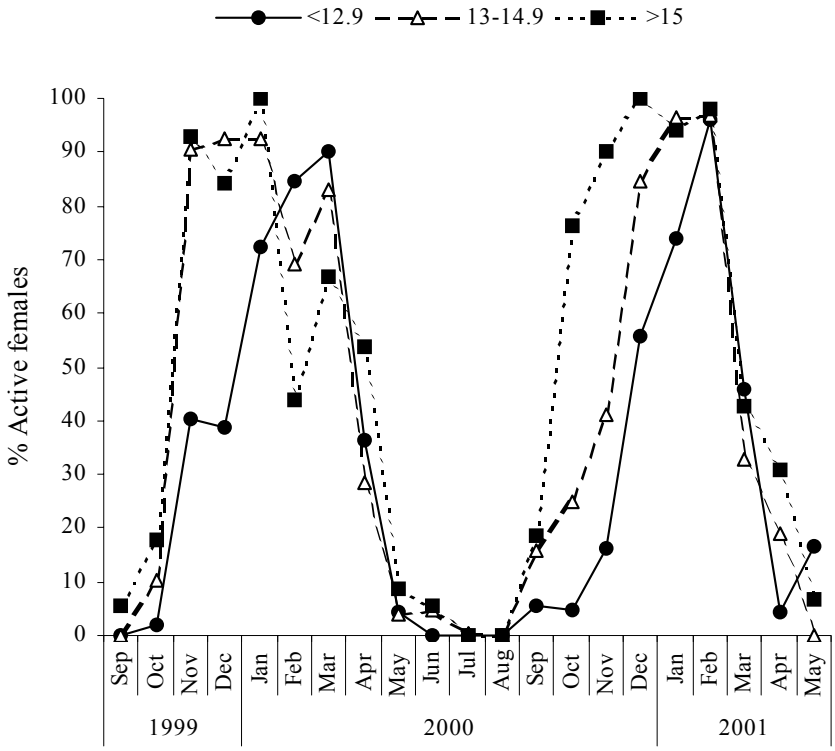


Figure 4.3.2.3: *Sardina pilchardus* sardina; coastal central Greece. Monthly evolution of the fraction of reproductively active females in different length (cm) classes.

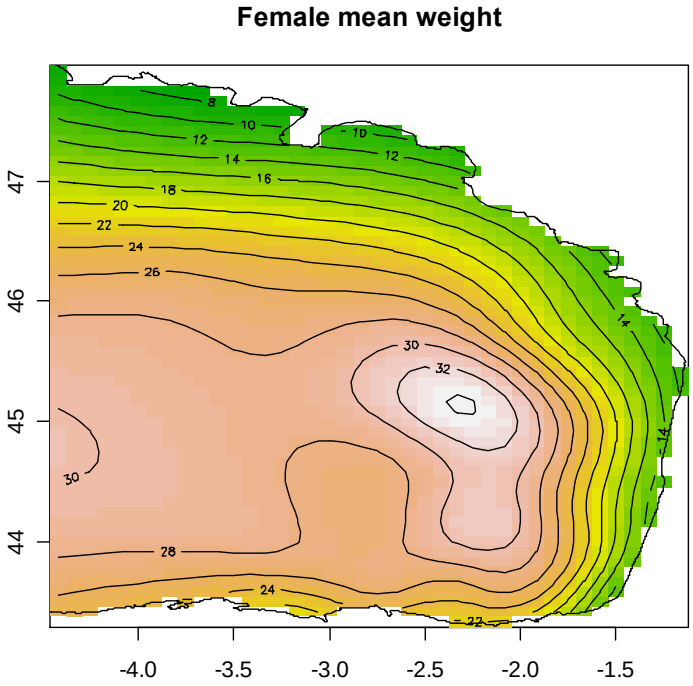


Figure 4.4.1.1: Predicted surface for female mean weight of the Bay of Biscay anchovy from the pooled data series.

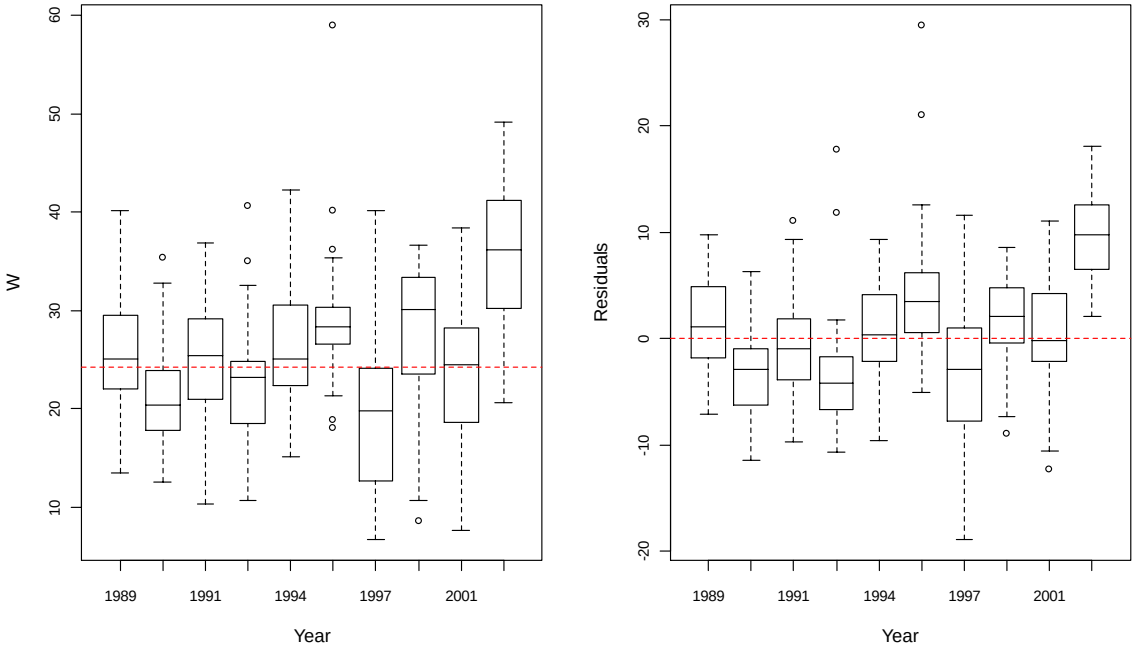


Figure 4.4.1.2: Box-plot of the female mean weight (on the left) and the residuals of the GAM fitted to female mean weight (on the right) of the pooled data series. Dotted red line represents the median of the female mean weight on the left and 0 on the right.

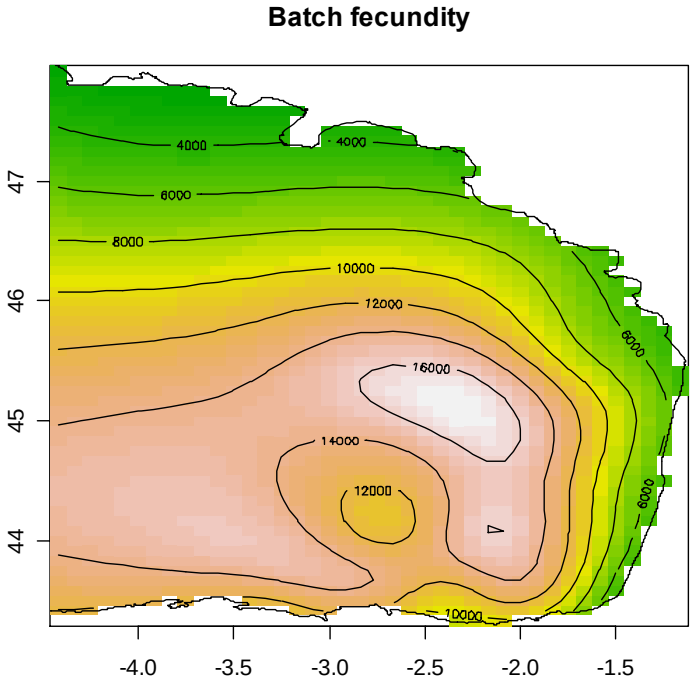


Figure 4.4.1.3: Predicted surface for female mean weight of the Bay of Biscay anchovy from the pooled data series.

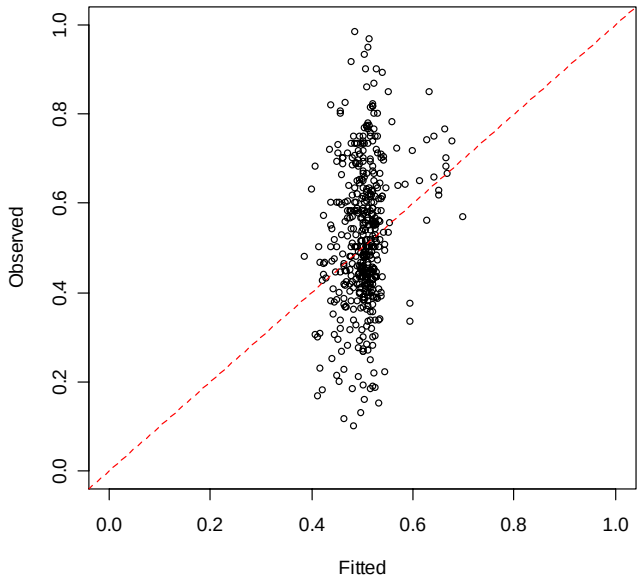


Figure 4.4.1.4: Observed vs. fitted values of sex ratio in numbers.

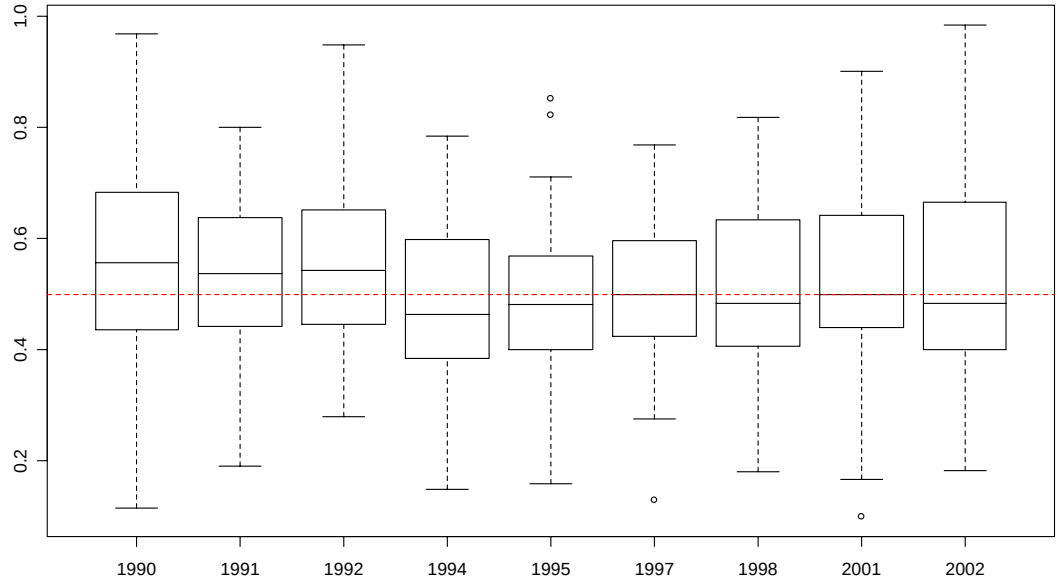


Figure 4.4.1.5: Box-plot of sex ratio in numbers for the Bay of Biscay anchovy. Red dotted line represents the hypothesis of 1:1 sex ratio.

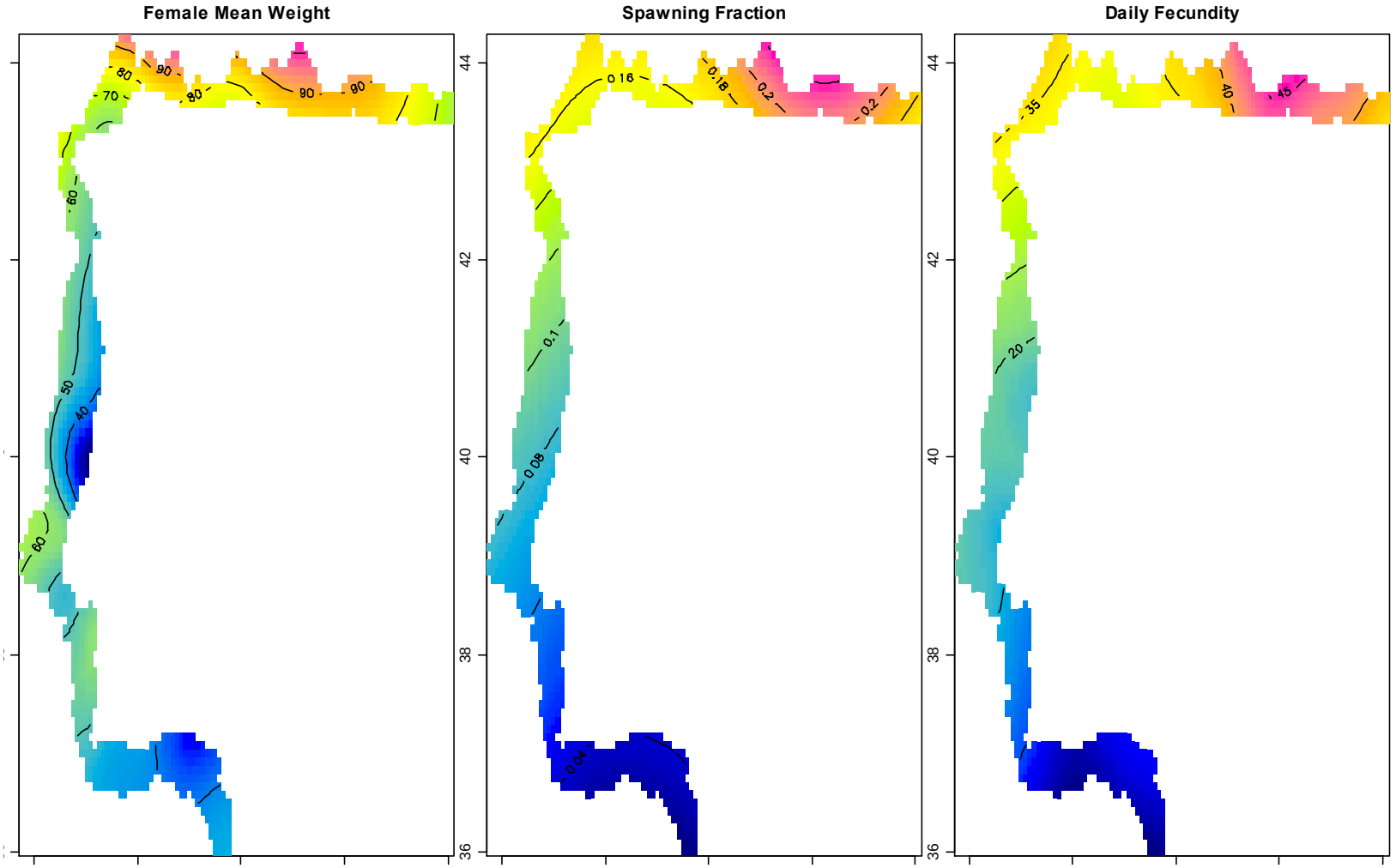


Figure 4.4.2.1: Spatial distribution of sardine female weight (left) spawning fraction (centre) and daily fecundity (right) from GAMs fitted to pooled DEPM data from all Iberian surveys.

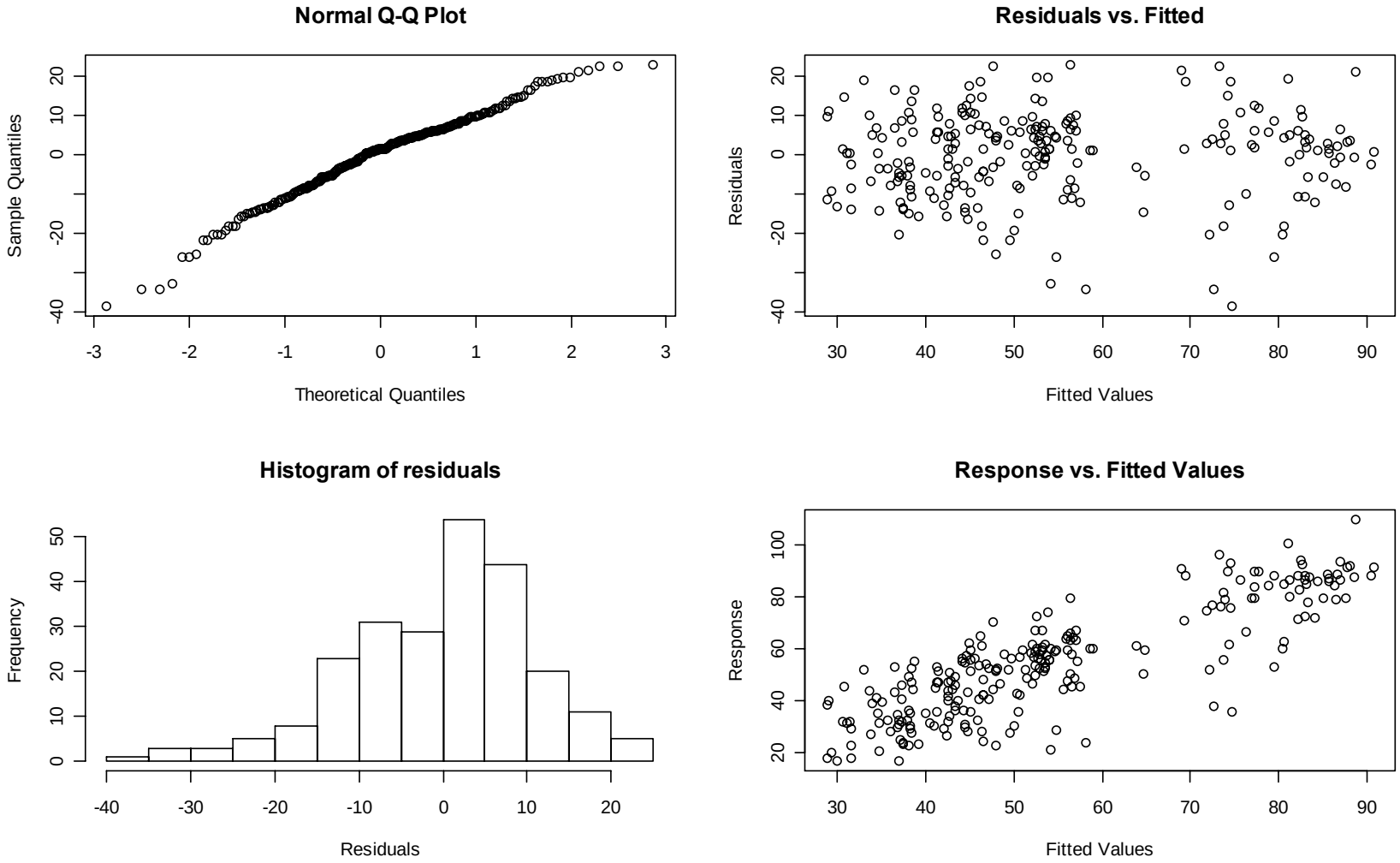


Figure 4.4.2.2: Residual inspection plots for GAM fitted to sardine female weight (pooled data from all surveys).

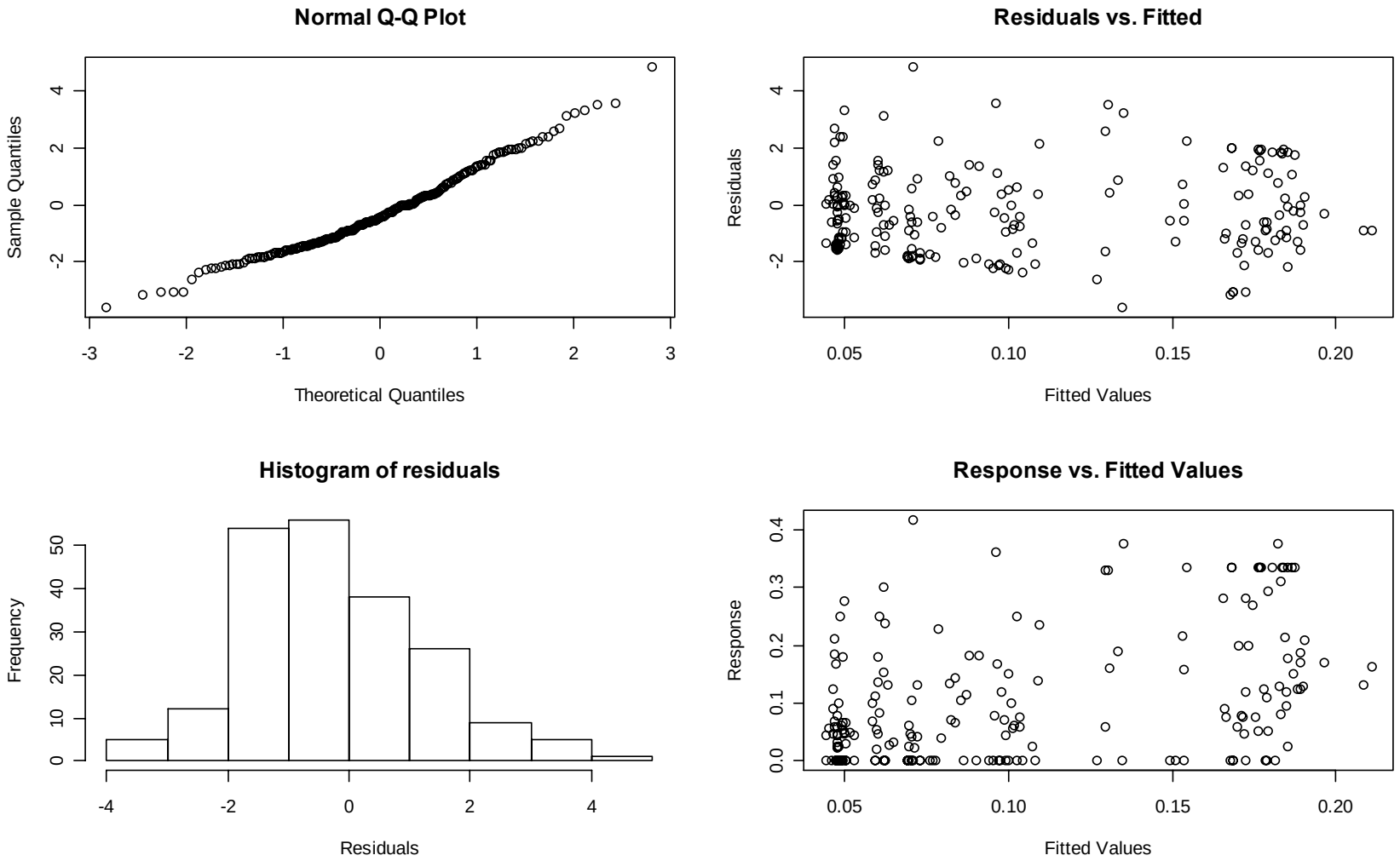


Figure 4.4.2.3: Residual inspection plots for GAM fitted to sardine spawning fraction (pooled data from all DEPM surveys).

5 CUFES Updates

5.1 Introduction

CUFES (Continuous Underway Fish Eggs Sampler, see Checkley *et al*, 1997) is a new system that provides a continuous, real-time spatial mapping of eggs at a fix depth (3m). The use of continuous samplers, such as CUFES, has the potential to reduce sampling time, provide better horizontal coverage, operate continuously and under nearly all sea conditions, be used simultaneously with other towed instruments and facilitate adaptive sampling. The samples obtained by CUFES are smaller and easy to sort at sea, reducing the laboratory work. In this section, advances on the application of this sampler are presented. Section 5.2 describes the egg extrusion problem with CUFES that was briefly mentioned in the previous SG report. Section 5.3 presents comparisons between CUFES and other samplers in order to check the reliability of CUFES to estimate egg abundance. Finally, Section 5.4 summarizes existing vertical egg distribution models, whose appropriate development is an important requirement in order to be able to use CUFES for egg abundance estimation in the water column.

5.2 Egg Extrusion

Extrusion of eggs through the 500 micron net of the CUFES collector is a generally admitted problem, particularly for anchovy eggs, which implies the loss of a presumed constant percentage of eggs in CUFES samples. Experiments were carried out by AZTI in 2003 (during BIOMAN 03 and SAVOR 03 surveys) and 2004 (during BIOMAN 04 survey) with the aim of estimating the percentage of anchovy and sardine eggs lost by extrusion (se also WD by Rueda *et al.*). The experiments compared the results of the use of two different mesh nets: the traditionally used 500 μm mesh net and a 335 μm mesh net. The number of lost eggs was analyzed, taking into account the flux of the pump in each case.

For anchovy, when the 500 μm mesh was used, the results show a mean percentages of lost eggs (weighted to the number of eggs) of 92.3% in BIOMAN 03, and 29.3% in BIOMAN 04. With the 335 μm mesh the mean percentages were reduced to 28.3%, 6.7% and 9.5% for the experiments during BIOMAN 03, SAVOR 03 and BIOMAN 04 respectively. For sardine, the obtained mean percentage of lost eggs during BIOMAN 04 with 500 μm was 32.3% and using 335 μm this percentage dropped to 1.4%.

The results show remarkable differences, not only between mesh size but also between surveys. This suggests that there are other factors also affecting egg retention rates other than mesh size. The current experimental results suggest that the pump's flux can influence the percentage of lost eggs through the mesh. In Figure 5.2.1, simple regression plots between the percentage of lost eggs and the flux are presented for each species and mesh size. For anchovy, an increasing trend of the percentage of lost eggs with the flux can be observed. This tendency is observed in both cases, using 500 and 335 μm mesh net, with an appreciable reduction in the percentage of lost eggs when the 335 μm mesh net is used. For sardine, no clear tendency in response to increasing flux is observed for the 500 μm mesh net, but the number of observations is low. Probably the amount of sardine eggs lost can be considered almost constant over the range of fluxes of the experiments for the 500 μm mesh net. The reduction of the mesh net to 335 μm leads sardine eggs to be almost entirely retained. Overall, using a 335 μm mesh net, notably reduces the percentage of egg lost by extrusion for both species, usually to values at or below 10%. Despite the differences between the three surveys, the tendency in each survey is the same, so, the use of the 335 μm is recommended for future CUFES applications.

5.3 Egg abundance comparisons with other samplers

Previous calibrations between CUFES and more traditional ichthyoplankton samplers have demonstrated that additional work is needed before being able to consider CUFES to obtain estimates of egg abundance for the entire water column. In this section the new comparisons between CUFES and other samplers are presented for the Bay of Biscay (Section 5.3.1) and Gulf of Cadiz (Section 5.3.2), the former presenting for the first time comparisons with LHPR and the latter demonstrating CUFES/PAIROVET comparisons under different environmental conditions.

5.3.1 Comparisons in the Bay of Biscay

In the Bay of Biscay, samples were taken within the PELASSES project, in the surveys developed by AZTI in 2000 and 2001, using CUFES, PAIROVET and LHPR samplers. In this section, the abundances given by these three samplers are compared (see also WD by Rueda *et al*.).

The main differences between PAIROVET and CUFES samplers are that vertically hauled nets, produce a depth integrated sample at discrete points, whereas CUFES provides horizontal continuous sampling but at a discrete depth (3m). The use of vertical distribution models should allow to obtain vertically integrated abundances from egg abundance at 3 meters given by CUFES. So when correcting CUFES with vertical distribution models, similar results to those obtained by PAIROVET net should be obtained. With this aim, the model developed by Boyra *et al*. (2003), was tested to correct CUFES data, and the results were compared with vertically towed plankton samples (PAIROVET). The model developed by Boyra *et al*. (2003) calculates the percentage of eggs by depth in the first 50 meters of the water column, so, knowing the percentage of eggs at CUFES sampling depth, the total amount of eggs in the water column can be calculated.

In this analysis, comparison with the LHPR (Longhourst Hardy Planckton Recorder) sampler was performed for the first time. This sampler is a towed plankton net system in a mounting frame about 2 m in length. A single filtering net (200 m mesh aperture) terminates in a mechanical cod end unit in which a series of samples are taken at pre-set time intervals (30 seconds, 1 or 2 minutes in this study) as the sampler is towed on an oblique haul at around 2.5 knots. A self-contained data-recording unit provides data on the depth range and volume of water filtered for each sample.

Integrated egg abundance estimates of LHPR and CUFES at surface (3m) *vs.* PAIROVET were compared in order to check the relative performance of these samplers for sardine and anchovy eggs, after correction of the former samplers by the vertical egg model distribution of Boyra *et al*. (2003). For the comparison, the samples were restricted to the set of CUFES and PAIROVET hauls associated to LHPR hauls. Several simple regressions were performed in order to see the differences between the three samplers. The analysis was done for all stations, but also distinguishing high and low surface salinity stations, with the aim of see differences related to environmental conditions. The results obtained from this analysis are presented in Table 5.3.1.1, where the R-square values obtained from each comparison, for both anchovy and sardine eggs can be seen.

For both species, LHPR integrated vertical egg abundances were in close agreement with PAIROVET estimates, giving confidence to their use as an unbiased estimator of egg abundance over the water column. However, CUFES consistently produced lower egg abundances (in densities) than those integral samplers. And these lower estimates were not corrected by the application of the Boyra's model to infer the total egg density of eggs. This could be either due to insufficient egg retention in CUFES (see Section 5.2 above) or to the worse performance of Boyra's *et al* model (2003) in low salinity areas. The comparison of the LHPR densities of eggs at 3 m depth with those obtained in CUFES reveals a rather close agreement be-

tween the catching efficiency of the two samplers (just a bit less efficient CUFES than LHPR at 3m) (Figure 5.3.1.1). This implies that the major disagreement between CUFES modelled vertical egg abundance and LHPR or PAIROVET estimates should arise from the Low salinity areas. However in the case of anchovy the same comparison (Figure 5.3.1.1.) reveals that CUFES was consistently catching less anchovy eggs than the LPHR at 3m. This implies that the major differences already appeared at the retention capacity of CUFES sampling and hence at the extrusion problem for anchovy eggs described in Section 5.2.

5.3.2 Comparisons in the Gulf of Cadiz

Previous results from the Bay of Biscay have shown that the performance of CUFES as a quantitative egg sampler in the water column is highly dependent on the environmental characteristics of the sampled area. As this was the first time CUFES was used in the Gulf of Cadiz during summer peak spawning for anchovy, a calibration exercise with PAIROVET was considered necessary (see also WD by Jimenez *et al*.). Methodology validation CUFES-PAIROVET experience was described in Section 2.4.2.1. In PAIROVET sampling, anchovy eggs were found in 11 of the 26 stations. Within the positive stations, a total of 151 eggs were counted with an average of 13.7 eggs / station. In stations 7 and 8 (radial 7) the largest number of eggs was found. Both stations are very shallow (32 and 47 m depth) and they are located off the National Park of Doñana (between the towns of Huelva and Sanlúcar of Barrameda) (Figure 5.3.2.1).

As was the case for the CUFES, the largest densities of eggs obtained with the PAIROVET net were found in the coastal strip between Huelva and Cadiz cities. Nevertheless, one of the radials (Stations 7 and 8) located within this area showed low density of eggs in the daylight CUFES sampling (Figure 2.4.2.4), while shows the largest densities in the night PAIROVET sampling (Figure 5.3.2.1). These stations are the outliers of a clear relationship represented in Figure 5.3.2.2.

Possible causes of these differences could be:

- Water column mixes
- Depth
- Recently spawning eggs

The observation of temperature and salinity profiles (obtained by CTD) and the fact that stations 7 and 8 are shallow discard the first two possible causes of the differences. To analyze the third possible cause samplings hour were determined. Only five of the CUFES validation stations (from a total of 99), were carried out between 20:00 and 22:00 hours (Figure 5.3.2.3). Both stations 7 and 8 were among the ones sampled between 20:00 and 22:00. As anchovy show a night spawning peak (assumed to be around 00:00 for the case of the Bay of Biscay anchovy), possible hypotheses is that spawning in stations 7 and 8 was intense but occur immediately before the sampling, and CUFES may have under sampled those eggs. These can be due to spawning happening at depths larger than 3 m depth, and sampling just after spawning did not allow the eggs to reach the surface. In order to corroborate this hypothesis, eggs found in station 7 and 8 should be in early stages (I or II; eggs not staged yet). Excluding these considerations a clear relationship between CUFES and PAIROVET observed densities was found (Figure 5.3.2.4).

5.4 Modelling vertical egg distribution

Understanding and modelling the vertical distribution of fish eggs in the water column is a major challenge for future use of the underway – continuous egg samplers as estimators of the total egg abundance (Boyra, *et al*., 2003). The factors affecting the vertical distribution of eggs

are related to the vertical velocities of the eggs, egg buoyancy, dimension and form, but also environmental factors, such as turbulent processes and vertical movements in the mixed layer.

5.4.1 Brief review of existing models

Several authors have developed models to describe the vertical distribution of fish eggs:

- Sundby (1983) developed a one dimensional model for the vertical distribution of pelagic fish eggs. This model described the physical properties of pelagic fish eggs and a method for computing the corresponding ascending velocity. Eddy diffusivity was calculated using the wind stress, and the eggs were considered as spheres of constant density.
- Westgard (1989) developed a model that includes a full set of equations for the physical properties of the water column, including the concentration of eggs. The eddy diffusivity was modelled using a k-epsilon closure scheme.
- Page *et al*. (1989) modelled the vertical distribution of haddock (*Melanogrammus aeglefinus*) eggs, depending on their stage of development. The theory developed by these authors is an extension of Sundby's model, but modified to include a depth-dependent water density and a constant eddy diffusivity coefficient for simplicity.
- Boyra *et al*. (2003), based also on the model of Sundby, modeled the vertical distribution of anchovy and sardine eggs. This model includes a modification in the calculation of the eddy diffusivity coefficient profile, which follows the profile inverse to that of the density gradient. They also allow for a Gaussian variability of egg density and adaptability of the egg density to the surrounding water by the permeability of the egg chorion. This model could not obtain the observed sub-surface maximum.
- Petitgas *et al*. (2004), based on the model of Westgard, developed other one-dimensional model for the vertical distribution of fish eggs. This model has a turbulent closure achieved by a k-l scheme, including the tidal current effect and river plume effect in the vertical distribution. Combining this with the variation in the egg density and diameter, the observed subsurface maximum could be obtained.

To conclude, more work is needed in order to improve the results obtained by the vertical distribution models, related to the turbulence closure scheme, the permeability of the eggs and the affection in the vertical distribution, and the problems to obtain the subsurface maximum.

5.4.2 Factors affecting vertical stability in the water column

The vertical distribution models described in Section 5.4.1, calculate the vertical distribution of eggs being in equilibrium in the water column. But, reaching the equilibrium in the water column depends on the spawning depth and also on the egg ascending velocity. For anchovy, which spawns near the surface, the eggs are close to their density, so they can reach the equilibrium in a sort time. On the other hand, sardine eggs, which are spawned near the bottom, need more time to reach the equilibrium. This process is affected by the ascent velocity of eggs, which depends on the difference between the egg density and the surrounding water density, and also on the egg morphology. Eggs with larger periviteline space are more adaptable to the surrounding water density (Coombs, *et al*, 2004, Rueda *et al*. in preparation). More work is needed in order to improve the vertical distribution models, taking into account the depth at which the eggs are spawned and the egg morphology.

Table 5.3.1.1: R-square values adjusted for d. f. obtained in the simple regressions comparing the observed values and the model obtained values for anchovy and sardine eggs. First is calculated using all the stations and also distinguishing High and Low surface salinity stations. The number of observations is also indicated in each analysis.

ANCHOVY	Total	High Salinity	Low Salinity
CUFES observed vs Pairovet observed	0.1760	0.2838	0.3207
CUFES model vs Pairovet observed	0.0596	0.1974	0.2849
CUFES model vs LHPR total	0.1219	0.3215	0.0437
CUFES observed vs LHPR 3m	0.4418	0.5764	0.3737
LHPR total vs Pairovet	0.5290	0.5770	0.6179
n observations	34	21	13
SARDINE			
CUFES observed vs Pairovet observed	0.5542	0.7505	-0.0976
CUFES model vs Pairovet observed	0.4279	0.5841	-0.0979
CUFES model vs LHPR total	0.5521	0.5265	0.8147
CUFES observed vs LHPR 3m	0.8463	0.8394	0.8841
LHPR total vs Pairovet	0.4901	0.4918	-0.0853
n observations	26	14	12

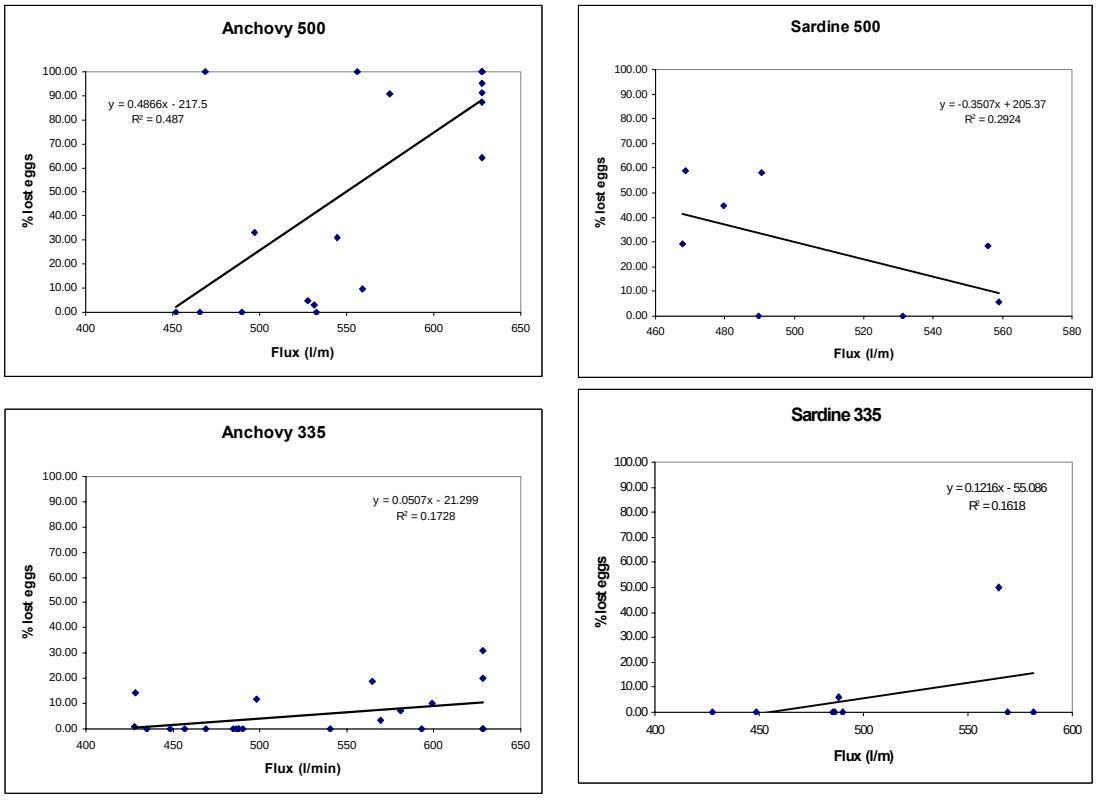


Figure 5.2.1: Plots of the simple regression between the percentage of lost eggs and the flux of the pump, using all the data from all surveys, for anchovy and sardine. In X axis the Flux (l/m) is presented and in Y the percentage of lost eggs. In the plots also the R- square obtained value and the proposed equation is indicated.

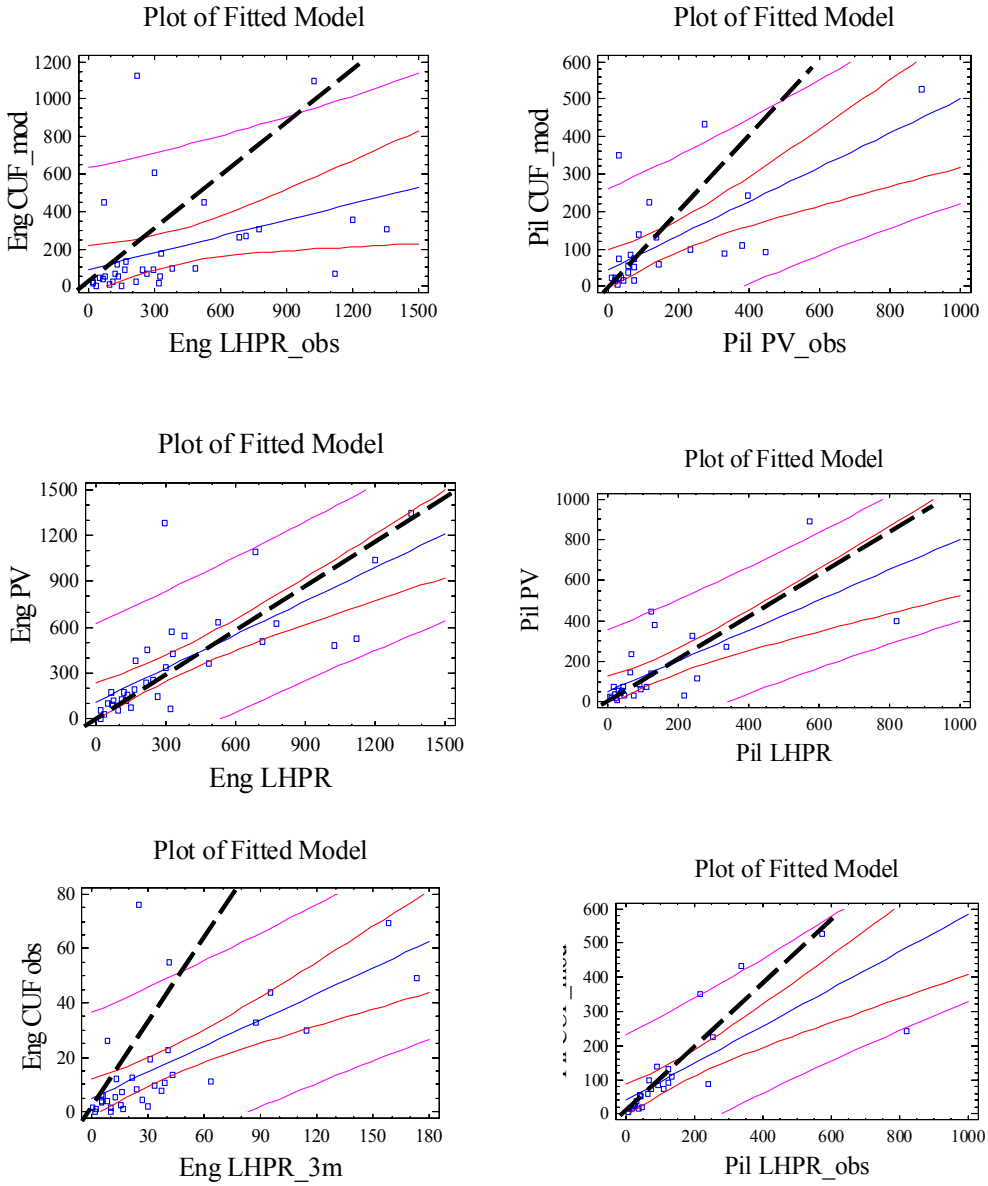


Figure 5.3.1.1: Plots some of the simple regressions for anchovy (left) and sardine (right) eggs, using all the stations from 2000 and 2001 in the Bay of Biscay. In all graphs the 1-1 line is represented with a dashed line.

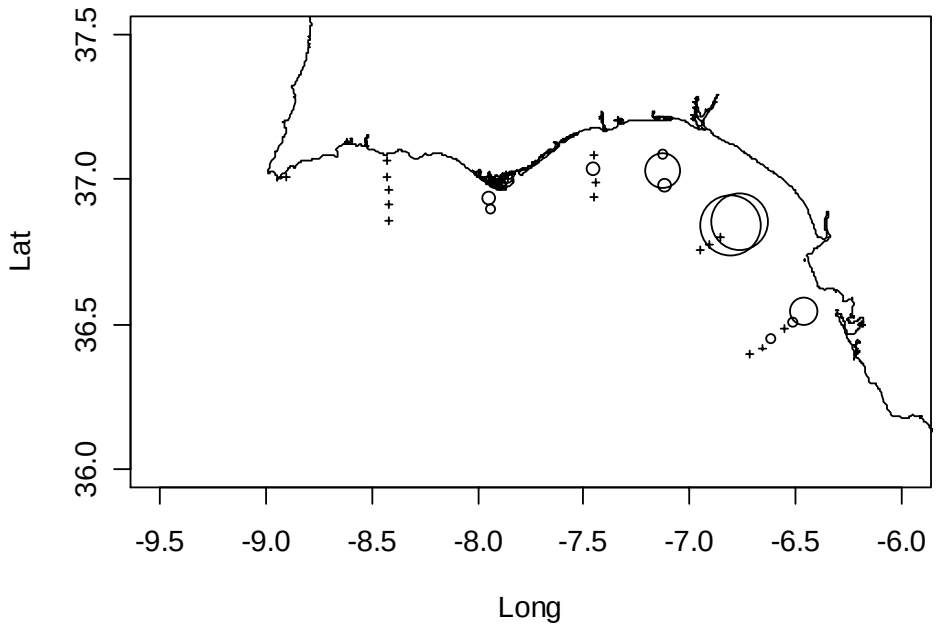


Figure 5.3.2.1: BOCADEVA-0604. Anchovy eggs abundance (density in nº/m2) in the Gulf of Cadiz (PAIROVET).

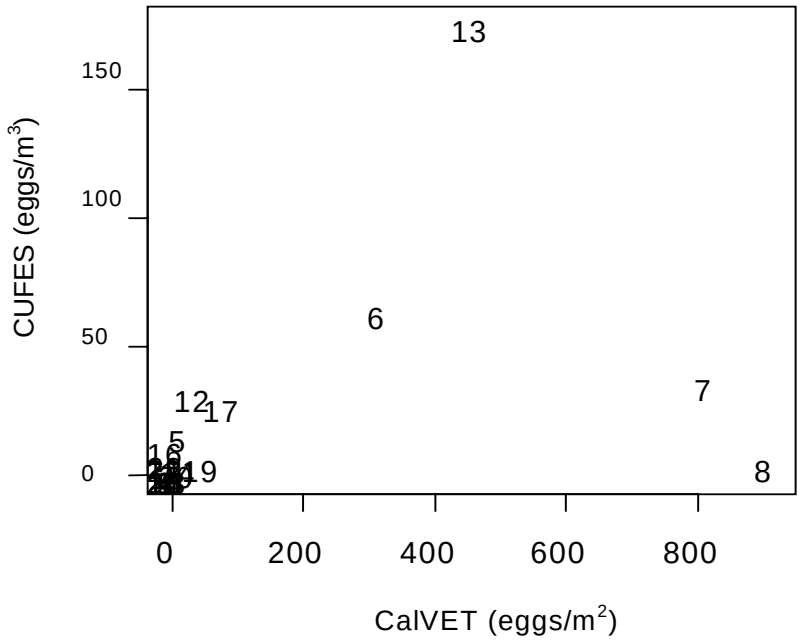


Figure 5.3.2.2: BOCADEVA-0604. Lineal relationship among the densities of anchovy eggs in the Gulf of Cadiz, obtained by PAIROVET and CUFES.

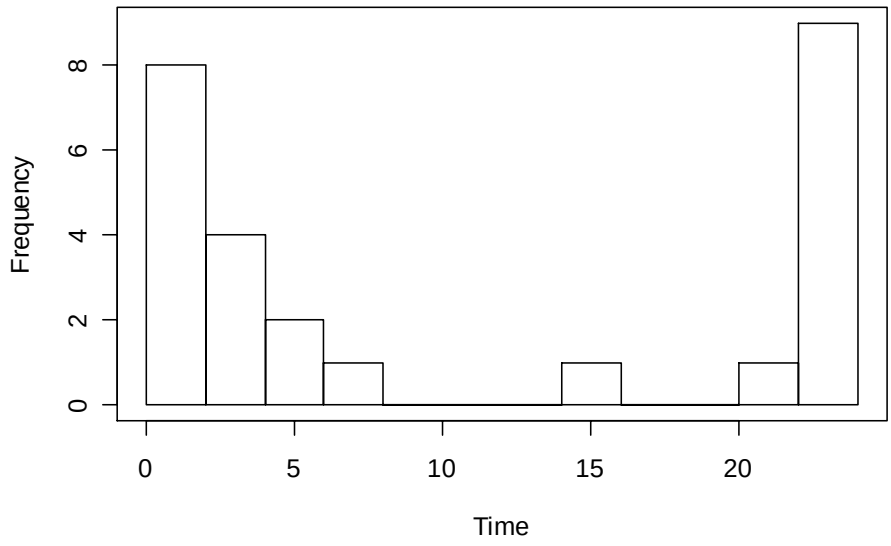


Figure 5.3.2.3: Hourly distribution of the CUFES validation samples in the Gulf of Cadiz.

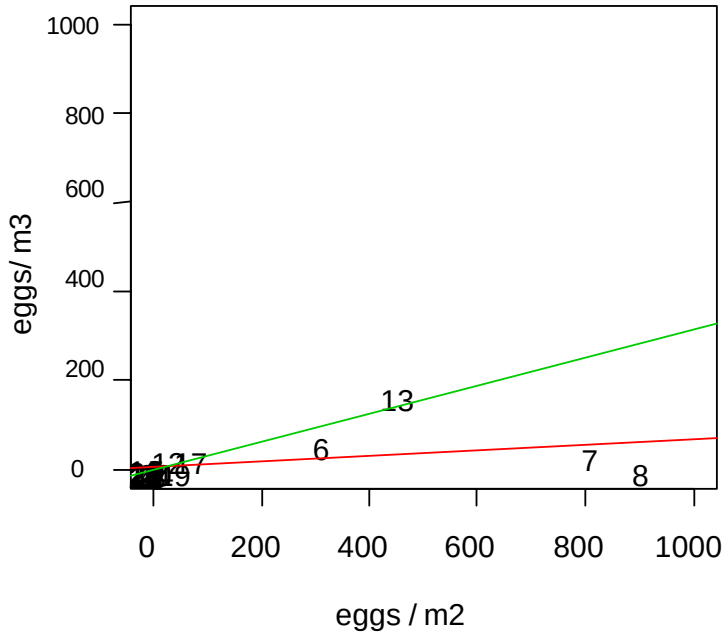


Figure 5.3.2.4: BOCADEVA-0604. Results of the lineal relationship among the densities of anchovy eggs in the Gulf of Cadiz, obtained among CUFES (eggs/m3) and PAIROVET (eggs/m2). Model 1 in red; Model 2 in green.

6 Summary and Conclusions

The summary and main conclusions of the SG meeting in relation to the terms of reference listed at the start of the report (Section 1.1) are:

- Planning the 2005 DEPM surveys (ToR a): The DEPM surveys for sardine and anchovy that will take place in 2005 will follow the same general design and differences between institutes and species are minimal to non-existent. Some effort was placed to devise clear rules for the use of CUFES in adaptive decisions during the ichthyoplankton survey and to propose a common format for the storing of DEPM data for future analysis. Finally, some initial ideas to enhance the efficiency and international co-ordination of all egg surveys in European waters were launched, but further discussion/decisions is beyond the remit of this SG.

- DEPM estimation and comparison between traditional and GAM estimators (ToR b): The thorough comparison between traditional and GAM-based estimators was aiming to allow decisions on whether GAMs can be recommended as the standard methodology for routine DEPM estimation. However, in the last year consensus has been built up among SG members that this question is, in fact, misleading and cannot be answered universally. Instead, the emphasis was placed in obtaining software that can allow the use of a range of options of increasing complexity for DEPM estimation, and in identifying the situations where each solution is more adequate in the light of the data available and the spatial complexity of the population under study. Following this rationale, existing DEPM software was considerably extended and used to perform comparisons between ageing and estimation methods. Along this process, a series of important decisions/assumptions affecting egg production estimation were identified using simulations and exploration of existing survey data. Given that these issues can seriously affect both traditional and GAM-based estimation, it was considered important to invest in their clarification and resolution prior to taking final decisions on the comparison between traditional and GAM-based estimation for the existing sardine and anchovy surveys.

- Spatial mapping of DEPM variables (ToR c): The change in research direction described for ToR b above also resulted in a delay in the production of spatial maps for the two species. However, average spatial maps of adult parameters from pooled survey were produced, providing useful insight into the average spatial distribution (and the degree of temporal stability) of sardine and anchovy reproductive parameters important for DEPM estimation.

- Reproductive parameters and spawning dynamics (ToR d): Sardine and anchovy POF stages were revised based on existing histological observations in order to base description exclusively on the morphological characteristics of POF degradation. The process of independent ageing of POF stages was initiated based on existing laboratory experiments (anchovy) and the daily evolution of stages in the samples (sardine and anchovy). In the case of sardine, additional work has refined the microscopic maturation scale and identified aspects of the macroscopic/microscopic comparisons with relevance to DEPM estimation. Finally, existing data from the Iberian Peninsula and the eastern Mediterranean were analysed and discussed in order to describe the main factors influencing the seasonality of maturation and spawning for sardine.

- CUFES updates (ToR e): The results from a series of experiments performed by AZTI demonstrated that egg extrusion is a serious problem for CUFES operating with 500 micron mesh net, and that retention rate is lower when the flushing rate of

the pump is higher (at least for anchovy). Substituting by a 335 micron net in the concentrator improves considerably the retention rate both for anchovy and sardine. The extrusion problem has probably over-emphasized the differences between CUFES and PAIROVET in previous calibration exercises, although new comparisons with the LHPR show that part of the problem is still related to the poor performance of the selected vertical egg distribution model under low salinity conditions. Existing models of vertical egg distribution were briefly summarized and discussed and their importance for improving the performance of CUFES as a quantitative egg sampler in the water column was highlighted.

7 Recommendations

In relation to future DEPM work, the SG recommends to:

- Continue the investment on a summer survey for anchovy in the Gulf of Cadiz. If possible, a DEPM survey should take place in the summer of 2005, making every effort to increase the inshore coverage at depths below 30 m.
- Increase the interaction between IPIMAR and IEO scientists participating in the respective national surveys in the Gulf of Cadiz.
- Explore the possibility to use the 2005 anchovy DEPM survey in Biscay to obtain egg and adult data for sardine. If possible, extend northwards the spatial coverage, accounting for the wider sardine distribution in the area.
- Conclude the analysis on the appropriate delimitation of cohort age limits for egg production estimation and identify the impact on existing estimates for sardine and anchovy.
- Continue field and modelling studies to describe better the vertical distribution of eggs in order to allow CUFES sampling to be used as an estimator of total egg abundance in the water column.
- Advance the preparation of DEPM software (and the associated manual) and make them available to the wider scientific community.
- Use random samples of all females for microscopical examination of sardine gonads using the template provided in Annex 10.3 and registering all possible biological information (on POFs, oocytes and atresia).
- Use resin for the histological preparation of all gonads with POFs. Classify POFs into morphological stages (using the scales presented for sardine and anchovy) but providing simultaneously an indication of the possible elapsed time from spawning.
- Conduct new experiments for sardine and anchovy to describe POF age as a function of morphological stage and temperature.
- Provide final estimates of sardine DEPM surveys for the Iberian Peninsula (including the 2005 survey) by the summer of 2006 in order to be used in the benchmark assessment of ICES in September 2006.

Given that SGSBSA has now concluded its life cycle, the ICES forum for reporting DEPM-related work in the future will be the recently created WGACEGGS.

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9 Working Documents

During the Study Group meeting, the following 10 working documents were delivered in text and presented orally:

- Alday A, Martin I, Martinez U, Santos M: Codes and criteria for the identification of anchovy ovaries: traditional and newly proposed classification key. 11 pp.
- Alday A, Uriarte A, Santos M, Martin I, Martinez U, Motos L: Post-ovulatory follicle degeneration of anchovy ovaries in warm waters. 22 pp.
- Bernal M, Stratoudakis Y, Ibaibarriaga L: Using R to obtain estimates of fish Daily Egg Production (v. 0.0.2). 39 pp.
- Ganias K, Somarakis S, Koutsikopoulos K, Machias A: Factors affecting the reproductive period of sardine. 20 pp.
- Jiménez MP, Bernal M, Romero Z: BOCADEVA-0604 - egg survey preliminary results. 15 pp.
- Millán M, Vila Y, Ramos F: Sampling of anchovy DEPM-adult parameters during the BOCADEVA 0604 Spanish pilot survey (June 2004, ICES Subdivision IXa South): a progress report. 13 pp.
- Rueda L, Santos M, Uriarte A, Boyra G: Checking the efficiency of CUFES as indicator of egg abundance. 27 pp.
- Silva A, Nunes C: A (very) short note on sardine maturity: macro-micro classification of gonads and maturity ogives. 9 pp.
- Stratoudakis Y, Nunes C, Angelico MM, Silva A: Planning the 2005 Portuguese DEPM survey for sardine. 6 pp.
- Stratoudakis Y, Bernal M: Unfinished DEPM business (sardine estimation and general methods). 12 pp.

Annex 1: Anchovy POF stages

Stage 1: New post ovulatory follicles showing no signs of degeneration. Immediately after ovulation, the follicle cell layers, appearing cord-like, form loose folds or loops. The granulosa cells, which have been extensively stretched during hydration, appear elongated and extremely narrow. The lumen contains eosinophilic granules of uncertain origin. The underlying theca layer is thin, not very noticeable and is not adhered to the granulosa. After spawning, the fully collapsed postovulatory follicle is a much more tightly folded structure. It is relatively large, irregular in shape, with an irregular lumen. The granulosa cells are characteristically columnar or cuboidal and in some cases have hypertrophied slightly; these cells are arranged orderly along the edge of the lumen with their cell walls usually evident and possessing evident nuclei. The nucleus of the granulosa cells may be located at either the apex or base of the cell. The thecal cell layer is more clearly defined and adheres closely to the granulose layer.

Stage 2: The first signs of deterioration start to appear. The postovulatory follicle still presents a quite tightly folded structure although is slightly shrunken. The lumen is easily visible and contains some granular material. The granulosa cells of the follicle conserve the orderly alignment characteristics although not as arranged as they were in the stage 1. The first vacuoles start to appear in some of the granulose cells cytoplasm. The majority of the cell walls remain intact. The underlying thecal layer conserves his integrity closely adhered to the granulosa.

Stage 3: The degeneration is pronounced in this stage. The postovulatory follicle is more shrunken but the lumen is but still visible and it may still contain some eosinophilic granules. The main characteristic is the presence of a great number of vacuoles affecting to at least one half of the granulosa cells some of whom loose their walls. Some of the granulosa cells nuclei become pycnotic. Some pattern in arrangement of cells can still be seen. The thecal layer becomes thinner and is closer to the granulosa. The nuclei of their cells could be pycnotic.

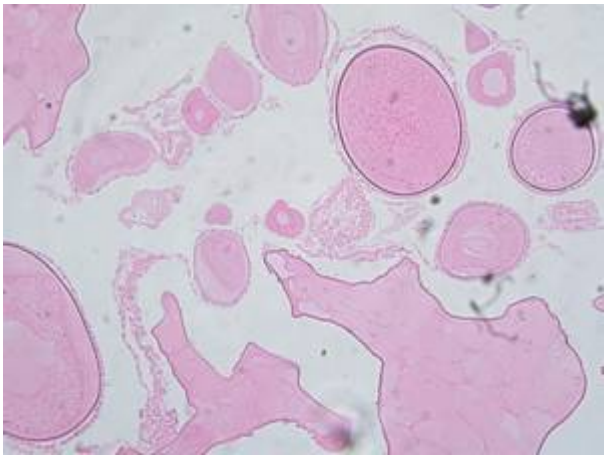
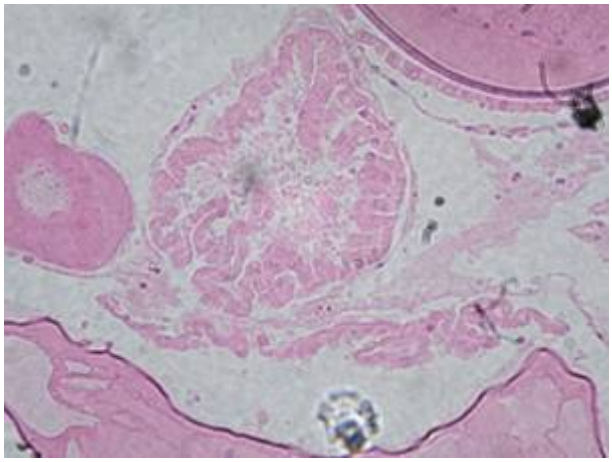
Stage 4: The regressing follicle has few folds in this stage acquiring hence, a more regular form. Initially, the lumen is greatly reduced and no eosinophilic granules are present. The presence of big vacuoles is massive in the granulosa cells occupying all their cytoplasm. It cannot be seen any arrangement pattern of the cells. The theca layer is present although his cells are less distinct.

Stage 5: This stage would be a late form of the stage 4 differenced by the absence of the lumen. The vacuoles growth produces the tear of the cell walls. All of the granulose cell nuclei have become pycnotic.

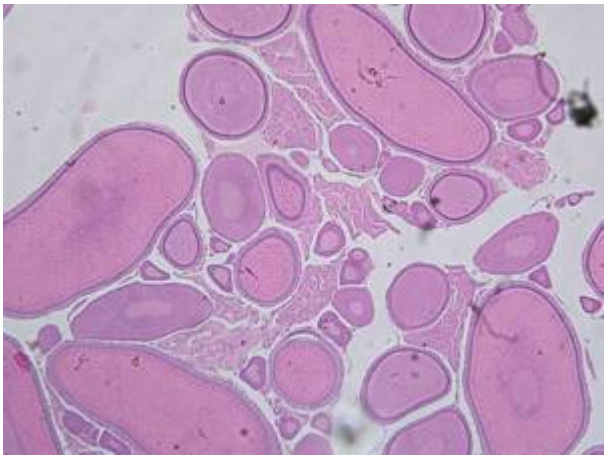
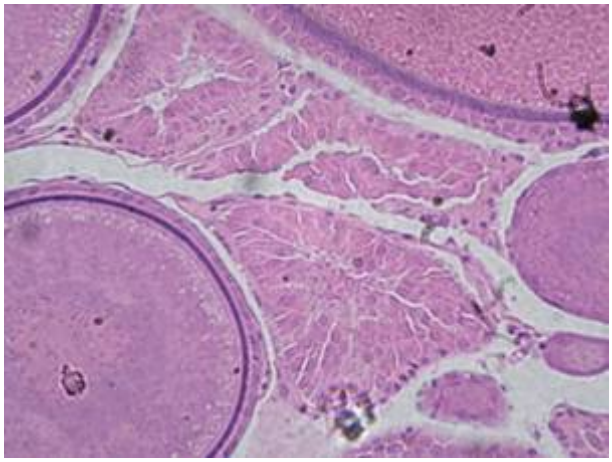
Stage 6: The postovulatory follicle size is greatly reduced. Cell walls are absent in the remaining granulosa layer tissue, and few vacuoles or pycnotic nuclei may be seen. The theca is present but is often indistinct as it becomes incorporated into the ovarian connective tissue stroma. The number of postovulatory follicles in the ovary section is much reduced.

Stage 7 POFs classified as stage 7 are all the polyhedron shaped tissue remains that can be founded occupying space between the oocytes. They still present some pycnotic nuclei but no cellular differentiation can be observed.

Stage 0: No FPOs are founded between the oocytes.

Stage 1 (Immediately after ovulation)

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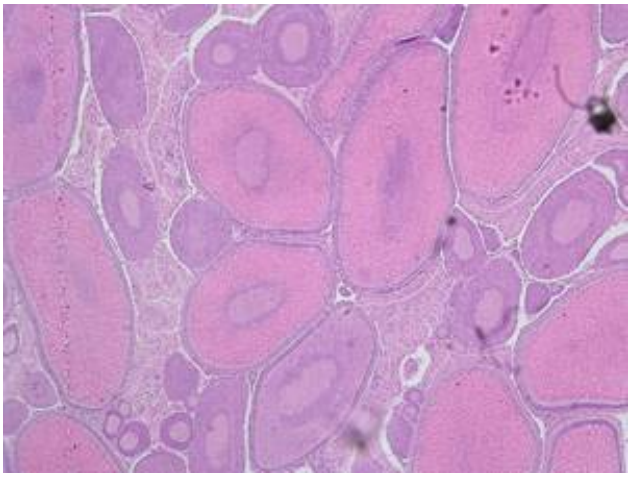
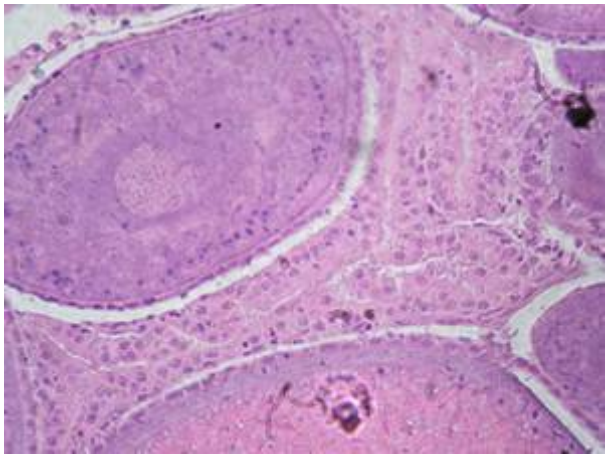

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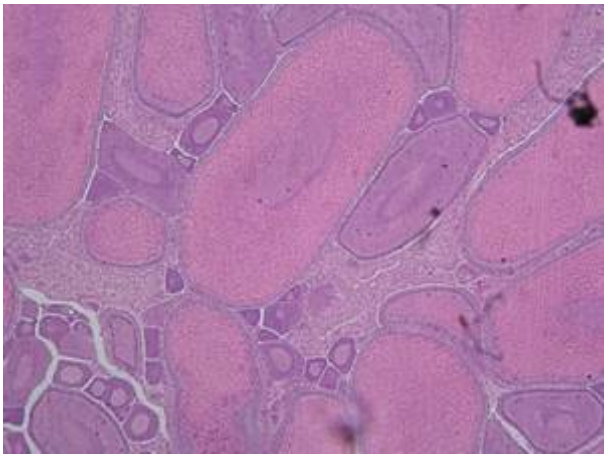
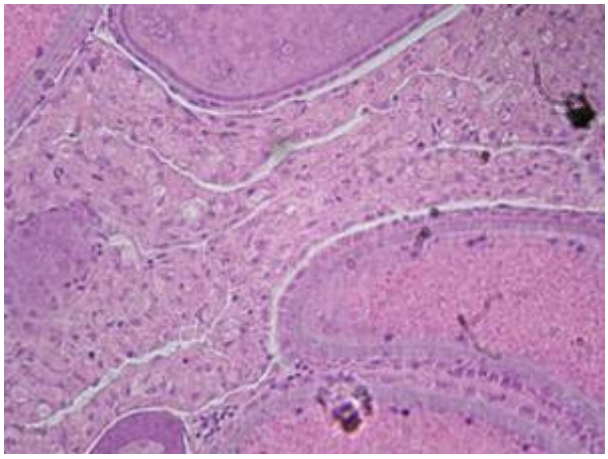

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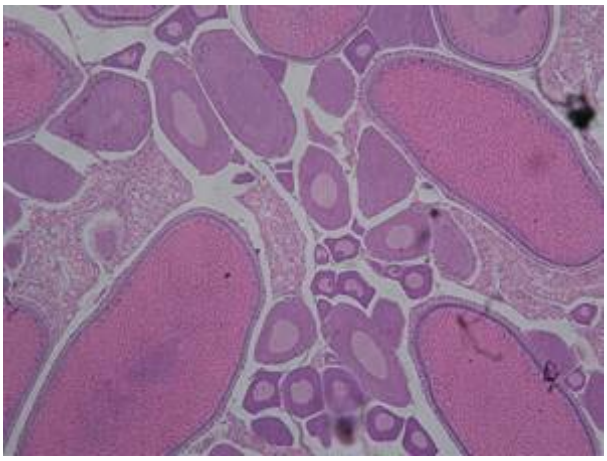
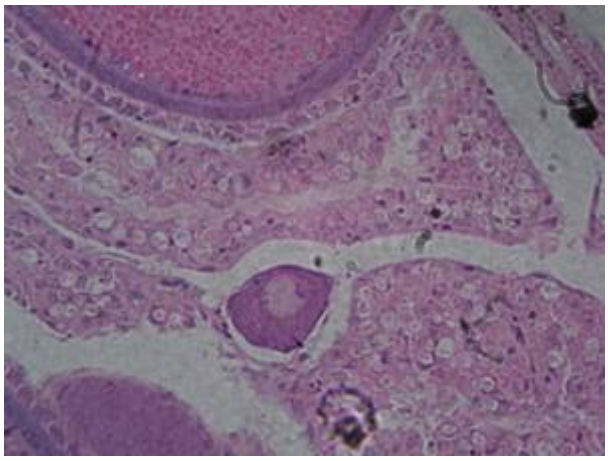

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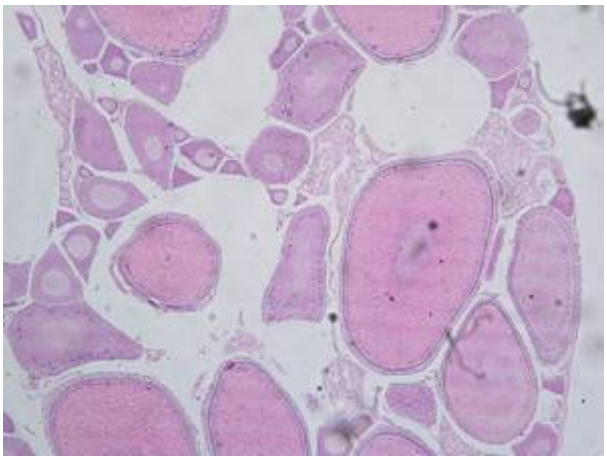
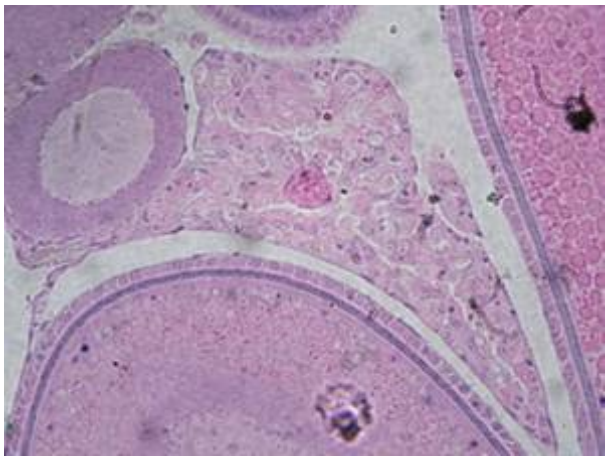
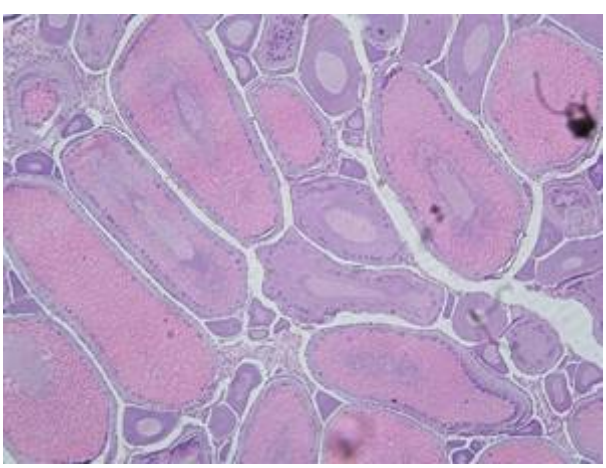
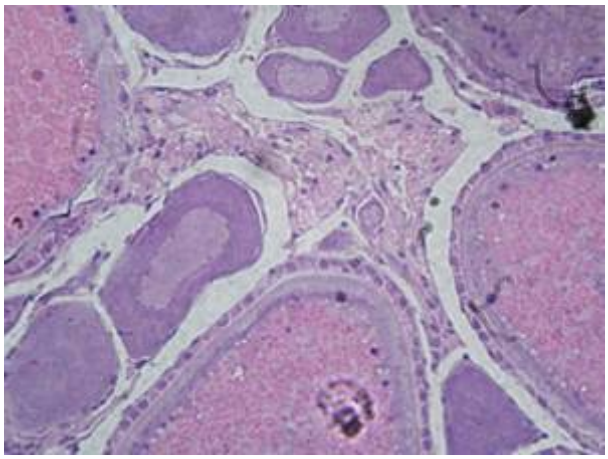
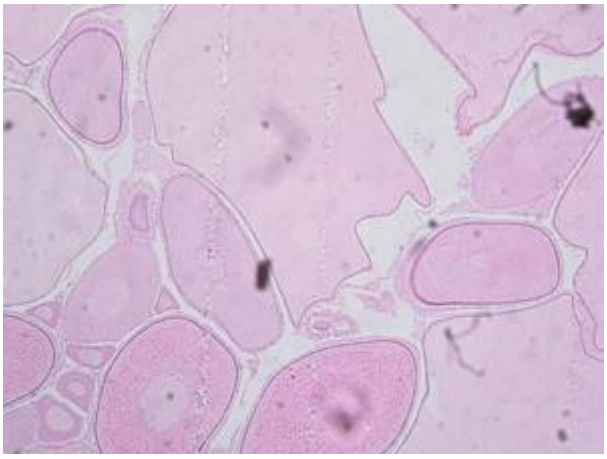
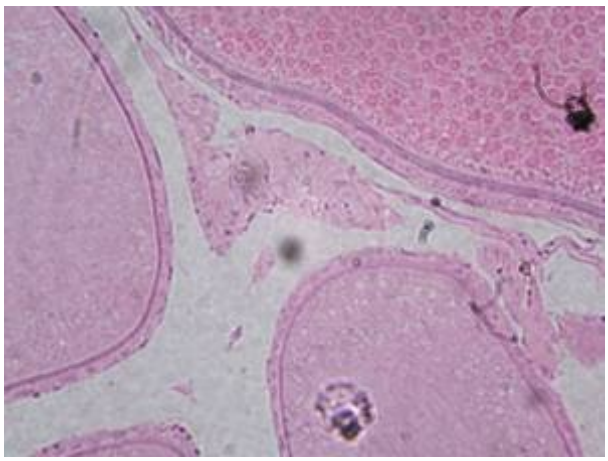



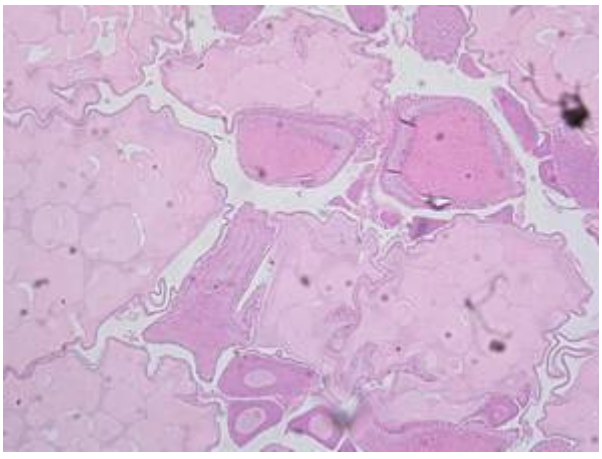
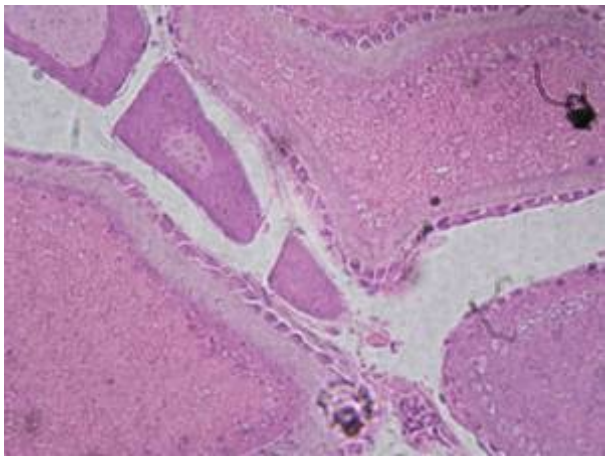
Stage 5

Stage 6

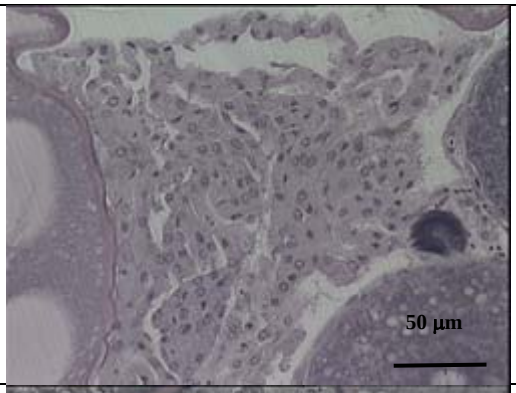
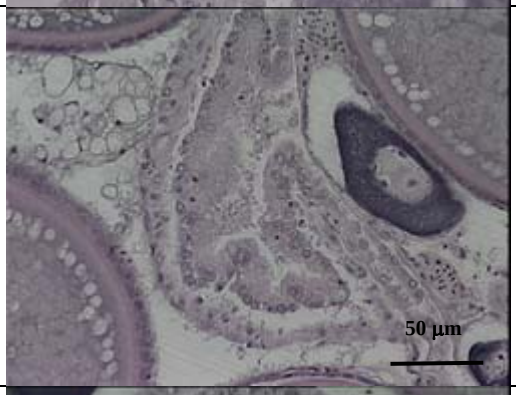



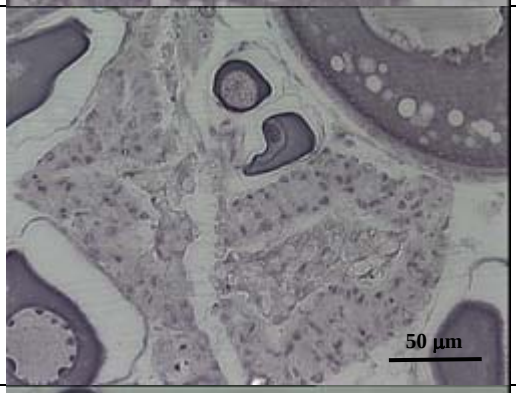
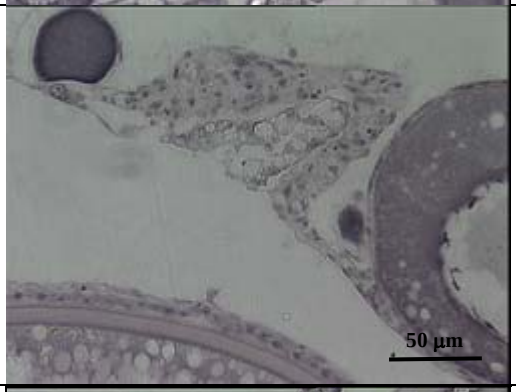
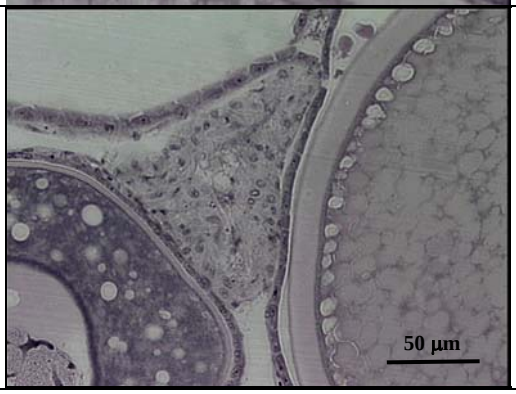
Stage 7

Stage 0

Annex 2: Sardine POF stages

Stage 1 POF Day-0		Newly-formed POF, often still in simultaneous with hydrated ovocytes Large, irregular and convoluted (loops and folds) in shape The cells of the granulosa layer clearly distinct, arranged linearly, with a central or apical nucleus The theca layer still very stretched and difficultly distinguishable from the granulosa one No signs of degeneration
Stage 2 POF Day-0		POF with still a convoluted shape; the loops delimiting a clear empty space (lumen) The cells of the granulosa layer still arranged linearly, with all the nuclei in a basal position The theca layer already clearly distinct from the granulosa one No or very few signs of degeneration
Stage 3 POF Day-0		POF with still a convoluted shape Presence of granular material in the lumen (dead cells?) The cells of the granulosa still arranged linearly but cell walls less evident Clear signs of degeneration: Pycnotic nuclei in the granulosa cells Oval structures (hypertrophied resorption cells?) in the granulosa layer The theca layer still distinct from the granulosa one
Stage 4 POF Day-1		POF less convoluted and irregular in shape (reduction of the folds) The lumen reduced in size The cells of the granulosa arranged less orderly, with pycnotic nuclei, cell walls more difficultly evident The oval structures very common in the granulosa layer The theca layer still distinct from the granulosa one

Stage 5 POF Day-1		The convoluted shape of the POF difficultly distinct The lumen almost or totally inexistent A continuous mass of granulosa elements: fewer intact cells and nucleus; pycnotic nuclei, oval structures The theca layer less clearly separated from the granulosa one
Stage 6 POF Day-2	 	POF of reduced size, with a frequent triangular shape The lumen absent The granulosa elements reduced to a few remaining oval structures and pycnotic nuclei The theca layer representing, proportionally, a higher fraction of the POF Attention: confusion with β-stage atresia
Stage 7 POF Day-3 (?)		POF reduced to almost only the theca layer

Annex 3: Template for data register during histological observations for DEPM purposes

CODE:

ANCHOVY HISTOLOGICAL READINGS

READER 1:	*DATE:*

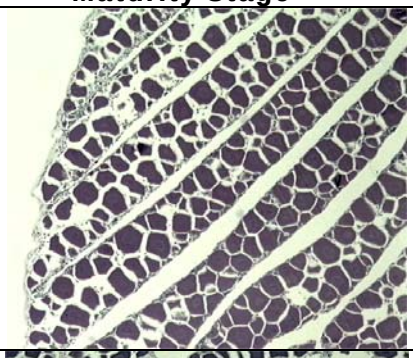
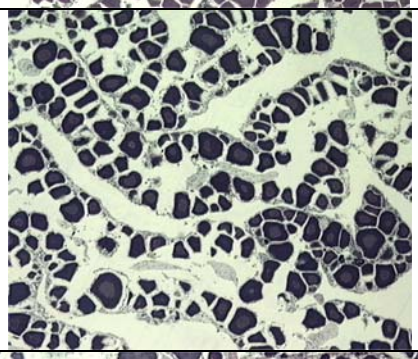
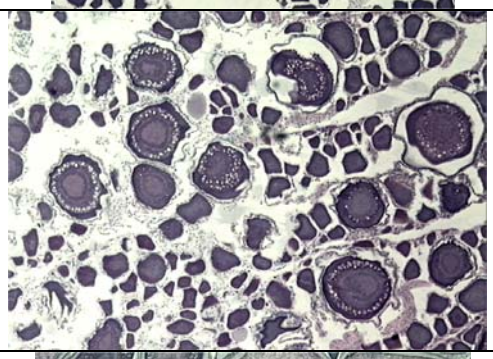
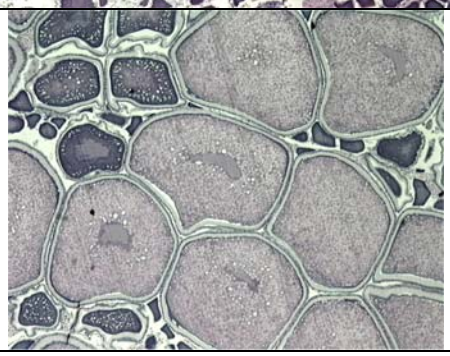
READER 2:	*DATE:*

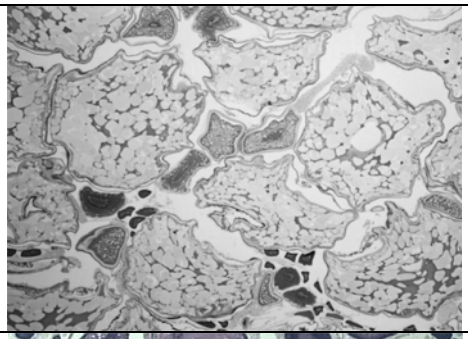
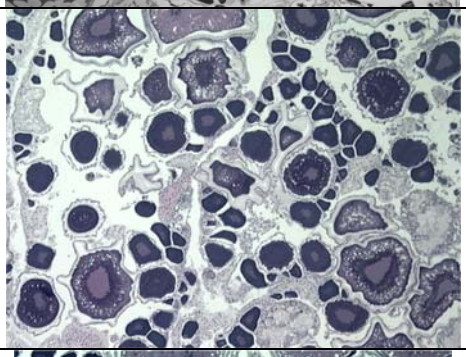
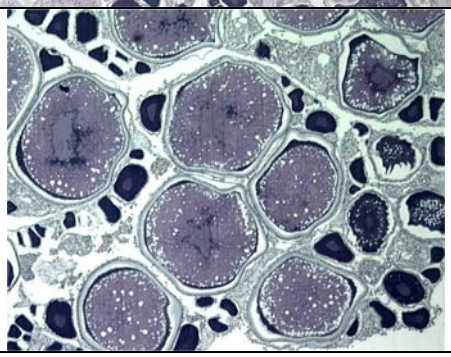
VALIDATION :	*DATE :*

SAMPLE CODE:	*SURVEY:*	*VESSEL NAME:*	*TIME GMT:*	*SAMPLE READING TIME:*

	oocyte type								postspawning stage							atresia					comments
nº fish	NV **1**	PV **2**	V<50 **3**	V>50 **4**	MIn **5**	MAv **6**	MAp **7**	hid **8**	FR **1**	FDI **2**	FDP **3**	FRG **4**	FMR **5**	RF **6**	no F **0**	no vit **%**	part vit **%**	vit **%**	beta **%**	**Total %**	

Annex 4: Sardine microscopic maturity scale

Maturity Stage		Microscopic Description
Virgin		Oocytes well organised in individualized ovarian lamellae Only unyolked ooocytes (pre-vitellogenic: chromatin nucleolar, perinuclear stages) No signs of degenerating/reabsorbing material
I		Oocytes well organised in individualized ovarian lamellae Only unyolked oocytes (pre-vitellogenic: chromatin nucleolar, perinuclear stages) No signs of degenerating/reabsorbing material Presence of blood vessels
II		Ovarian lamellae becoming adjacent The most advanced batch of oocytes: partially yolked oocytes (cortical alveoli) Few or no degenerating oocytes (atretic)
III		Ovarian lamellae no longer separated The most advanced batch of oocytes: yolked oocytes (vitellogenic: lipid droplets, yolk globules, nuclear migration) Few or no degenerating oocytes (atretic)

IV		Ovarian lamellae no longer separated The most advanced batch of oocytes: mature or hydrated oocytes (hydration, fusion of yolk globules) Few or no degenerating oocytes (atretic) Possibility of presence of recent post-ovulatory follicles
V		Ovary less clearly organized Presence of all stages of oocytes; dominance of the early stages; the most part of the more advanced stages with signs of degeneration (atresia) Signs of previous spawnings (empty spaces, atresia, reabsorbing material, post-ovulatory follicles) Presence of blood vessels
VI		Ovarian lamellae no longer separated The most advanced batch of oocytes: yolked oocytes Signs of previous spawnings (empty spaces, atresia, reabsorbing material, post-ovulatory follicles)