Sardine 2011 DEPM – ICES areas IXa and VIIIc

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1. Background

The DEPM for estimation of sardine spawning biomass within the Atlanto-Iberian stock area is conducted every three years by IPIMAR (Instituto de Investigação das Pescas e do Mar, Portugal) and IEO (Instituto Español de Oceanografía, Spain) in an internationally coordinated survey. In 2011, the Portuguese survey took place in February/March covering the Atlantic waters from the entrance of the Strait of Gibraltar to the northern border of Portugal, while the Spanish survey took place in March/April covering the northern stock area from the river Minho to the south of the Armorican shelf (in French waters). The Portuguese DEPM survey was carried out from the 10th February to the 08th March onboard RV Noruega, while the Spanish survey was undertaken using two vessels, from the 25th March to the 10th of April onboard RV Cornide de Saavedra (for plankton sampling mainly) and from 26 March to 22 April using RV Thalassa to carry out the fishing hauls (Table 1).

2. Environmental data, SST distribution

In the Portuguese survey, records of water temperature, salinity and fluorescence were obtained for surface waters by the CTF probes associated with the CUFES system; the CTDF profiler usually used together with the vertical nets was not operational for the 2011 survey. A CTD (Sea Bird 37) profiler (Temperature and Salinity) was carried out at each CalVET station in the Spanish survey. Moreover a CTD (Sea Bird-25, higher resolution and accuracy) was used in each transect head and in alternate stations along the transects.

Surface temperature and salinity distributions are presented in Figure 3. Temperature values ranged from 12.5 to 16.9 ºC and the distribution patterns were similar to observations from previous years; the highest temperature values were observed in the southern area and the lowest values registered for the Cantabrian Sea. The winter/spring conditions in the Atlanto-Iberian region were very unstable and much severe than in 2008. During the first quarter of 2011 heavy rain and strong winds were frequent.

For the area covered by the Portuguese survey the temperature data used for egg ageing was registered underway at 3m depth (CTF probes). During the Spanish survey the data were extracted from the SBE-25 and SBE-37 records.

3. Egg data

During surveying vertical plankton hauls were carried out following a pre-defined grid of sampling stations along transects perpendicular to the coast and spaced 8 nmiles (Figure 1). The inshore limit of the transects was dependent on bottom depth (as close to the shore as possible), while the offshore extension was decided adaptively. The main sampler for the DEPM is the PairoVET net that collects eggs through the water column at point stations. The PairoVET sampler (=double CalVET) includes 2 nets (Ø 25cm) with 150 µm mesh size and a CTDF probe; sampling covered the water column from bottom, or 150m (100 m for IEO) (beyond the 150 isobath) depth, to the surface. PairoVET samples were taken every 3 nm in the inner shelf (up to 200 m depth or 100 m where the platform is wider) and every 3 or 6 nmiles beyond the inner shelf, depending on egg presence in CUFES samples.CUFES was used as the auxiliary egg sampler, helping in defining vertical hauls density and offshore extension of the transects. The outer limit of a transect was reached when two consecutive CUFES samples were negative beyond the 200 m depth.

All plankton samples were preserved in formalin at 4% in distilled water and the 2 samples from each net stored in separate containers. For IPIMAR both nets were used for egg density estimates while IEO used 1 net (the other being used for plankton dried mass calculations) (Table 2). IEO counted total number of eggs from the CUFES onboard in order to obtain a preliminary data of sardine egg abundance and distribution. In the laboratory, all sardine eggs were sorted from PairoVET and CUFES samples. The eggs from the vertical hauls (2 nets – IPIMAR, 1 net –IEO) were all counted and staged according to the 11 stages of development classification (adapted from Gamulin and Hure, 1955). For IPIMAR, the eggs from the CUFES sampler were all counted and a sub-sample, of a minimum of 100, was staged per sample.

All calculations for area delimitation, egg ageing and model fitting for egg production (P_0) estimation were carried out using the R packages (*geofun, eggsplore and shachar*) available within the open source project *ichthyoanalysis* (http://sourceforge.net/projects/ichthyoanalysis). Some routines of the R packages used were updated since the 2008 versions. To avoid high and low extreme values in the area represented by each of the sampled stations, this values were forced to the minimum and maximum values of 25 and 175 respectively (the extreme values usually occur on the borders of the survey area and therefore do not affect the estimation of the positive area). The range 25-175 was selected to be a mean interval suitable according to the distance between transect and stations (fixed to be 8 nmiles between transects and 3 between stations along the transects).

The model of egg development with temperature was derived from the incubation experiment data available within the *egg* R library. Egg ageing was achieved by a multinomial Bayesian approach described by Bernal *et al*. (2008) and using *in situ* SST. Distribution of the daily spawning cycle was assumed as a normal (Gaussian) distribution, with a peak at 21:00 h GMT and a standard deviation of 3 h (spawning period from 21-6 h to 21+6 hours). It is assumed that 0 time is at midnight and days are 24 hours long. The upper age cutting limit was determined using a maximum age for the strata considered and it is not dependent on the individual stations (upper.age=F). Older cohorts are dropped if their mean age plus 2* stdev hours is over the critical age at which less than 5% of the eggs are expected to be still unhatched (how.complete=95%). The lower age cutting excluded the first cohort of stations in which the sampling time is included within the daily spawning period (lower.age=T).

The strata used for estimations were defined according to biological/ecological (geographical) reasons (see Bernal *et al*., 2007), they represent the current view of the different nuclei of the Atlanto-Iberian stock (Bernal *et al*., 2007, Silva, 2007), and also consider the timing of the surveys (IPIMAR and IEO surveys are on average 1 month apart). Three strata (Stratum) were used: South, encompassing from the strait of Gibraltar to Cape St. Vicente; West, from Cape St. Vicente to the northern limit between the Spain and Portugal; and North, between the Spanish-Portuguese northern limit and the Spanish-French Atlantic limit. The maximun age and temperature was calculated for the different strata described previously.

The exponential model: $E[P] = P0\, e^{-Z\ age}$ was fitted by a Generalized Linear Model (GLM), assuming a negative binomial distribution. Finally, the total egg production was calculated multiplying the daily egg production by the positive area (area with eggs defined by an automated procedure using the spatstat library).

The different options to obtain coherent estimates of mortality and egg production and the discussion on the choice of the GLM models are presented in section 5.1 of WGACEGG 2011 report and section 6.1 of WGACEGG 2012 report.

The model used here to estimate mortality and egg production is the one considering 3 strata for egg production (P0) and 1 mortality (age) for the whole area covered (Figure 4, corresponding to model 2 of the referred sections):

<u>3 strata P0 (Stratum) and 1 mortality (age)</u>
glm.nb(cohort ~ offset(log(Efarea)) -1 + Stratum+ age, weights=Rel.area, data=aged.data)

Details on the methodologies used on board, during laboratorial work and for data analyses are summarized in Table 2.

In total 224 PairoVET hauls and 829 CUFES samples were obtained (Table 1), during the Portuguese survey the number of CUFES stations was reduced in circa 20% due to irreparable damage to the system. The percentage of stations with sardine eggs was 27% for the vertical tows and 33% for the surface samples. Considering only one of the PairoVET nets 3300 sardine eggs were gathered in total, of which more than half came from the northern region, around a third from the south and less than 15% from W Portugal. In the positive stratum, the highest egg abundance per haul was 4950 (egg/m^2) reached in the South (Cadiz), while in the West coast the maximum density per haul was 2970 (egg/m^2) and in the Northern stratum 1537 (egg/m^2). Sardine egg distribution, obtained from the PairoVET and CUFES systems for the whole area are presented in Figure 1. The egg distribution pattern derived from the observations from the two samplers is similar and it is evident that the area occupied by eggs was much smaller than in 2008, this is particularly clear for the West coast of Portugal. Spots of higher egg densities were observed in the eastern regions of the Gulf of Cadiz, south of Cabo Carvoeiro, off Cabo Mondego and in the Cantabrian Sea.

The surveys covered a total area of 83508 km2 of which 23745 km^2 (28.4 %) were considered the spawning area (Table 3). The northern stratum represented 52.5 % of the spawning area while 27.5 % were in the southern coast and 20.3 % in the western shores. On the whole, the spawning area estimates for 2011 were lower in all the strata compared to 2008 (ICES, 2009).

Daily egg production per m^2 was highest for the southern region (438.9 eggs/m^2/day). Total egg production estimated for the Atlanto-Iberian stock was 7.74x10^{12} eggs/day, highest for the northern region (4.04 x10^{12} eggs/day). In all regions, it was lower in 2011 than in 2008.

4. Adult data

Fishing hauls were conducted by either pelagic or bottom trawling following sardine schools detection by the echo-sounder. The number of samples and its spatial distribution was organized to ensure good and homogeneous coverage of the survey area (Figure 2). In the Portuguese survey, the samples collected by the RV were complemented with samples obtained from commercial purse-seiners at Olhão, Portimão, Sines Setúbal, Peniche, Figueira da Foz and Matosinhos. Samples from the fishing fleet were acquired within 1-2 weeks of the surveying by RV Noruega in each area, except for 5 trawls: 2 samples from Matosinhos collected with 4 weeks lag and 1 sample from Portimão collected with 3 weeks lag, both during the survey period; 1 sample from Portimão and 1 sample from Peniche obtained 2 and 3 weeks after the survey was ended, respectively. The fish from the 3 first samples were included in the calculation of the estimates whereas the 2 last ones were used only for the measures of batch fecundity with hydrated females.

Onboard the RV, and for each haul, a minimum of 60 sardines were randomly selected and biologically sampled. These were, in some occasions, also complemented by additional fish in order to achieve a minimum of 30 females per haul for histology, and/or to obtain extra hydrated females for the fecundity estimations. Individual biological information (length, total weight, sex, maturity state, gonad weight) was recorded for all fish, the ovaries were preserved for histology (with a 4% formaldehyde solution diluted in distilled water and buffered with sodium phosphate) and the otoliths removed (only from females for IPIMAR) for age determination. The biological sampling and ovaries preservation were always carried out in fresh material, with the exception of 5 commercial samples for which the ovaries were removed from the fish body and preserved immediately after the fish were landed, while the remaining body of the fish was frozen for posterior biological sampling in laboratory.

The preserved ovaries were weighed in laboratory and the obtained weights corrected by a conversion factor (between fresh and formaldehyde fixed material) established previously. These ovaries were then processed for histology: they were embedded in either resin (IEO) or paraffin (IPIMAR), the histological sections were stained with haematoxylin and eosin, and the slides examined and scored for their maturity state (most advanced oocyte batch) and POF presence and age (Hunter and Macewicz 1985, Pérez et al. 1992a, Ganias et al. 2004, Ganias et al. 2007). Prior to fecundity estimation, hydrated ovaries were also processed histologically in order to check for POF presence and thus avoid underestimating fecundity (Pérez et al. 1992b). The individual batch fecundity was then measured, by means of the gravimetric method applied to the hydrated oocytes, on 1-3 whole mount sub-samples per ovary, weighing on average 50-150 mg (Hunter et al. 1985). Additional batch fecundity measures (IPIMAR) were obtained by means of the methodology developed by Ganias et al. (2010) which applies the gravimetric method also to non hydrated ovaries (oocytes at the migratory nucleus stage) using automatic particle counting.

The adult parameters estimated for each fishing haul considered only the mature fraction of the population (determined by the fish macroscopic maturity data) and was based on the biological data collected from both surveys and commercial samples. Before the estimation of the mean female weight per haul (W), the individual total weight (Wt) of the hydrated females was corrected by a linear regression between the total weight of non-hydrated females and their corresponding gonad-free weight (Wnov). The sex ratio (R) in weight per haul was obtained as the quotient between the total weight of females on the total weight of males and females. The expected individual batch fecundity (Fexp) for all mature females (hydrated and non-hydrated) was estimated by modelling the individual batch fecundity observed (Fobs) in the sampled females and their gonad-free weight (Wnov) by a GLM (Normal errors distribution and identity link). In case a geographical variability was observed in individual batch fecundity, a posterior post-stratification was carried out, Fobs being modelled against the Wnov and the Stratum (second and third sets of strata used for the egg data analysis). The fraction of females spawning per day (S) was determined, for each haul, as the average number of females with Day-1 or Day-2 POF, divided by the total number of mature females (the number of females with Day-0 POF was corrected by the average number of females with Day-1 or Day-2 POF, and the hydrated females were not included) (Pérez et al. 1992a, Ganias et al. 2007). The mean and variance of the adult parameters for all the samples collected was then obtained using the methodology from Picquelle and Stauffer 1985 (weighted means and variances). All estimations and statistical analysis were performed using the R software. Final adult parameters include individual estimates for the Southern, Western and Northern areas, with three independent estimates.

Details on the methodologies used on board, during laboratorial work and for data analyses are summarized in Table 2.

For the 2011 survey an effort was made to guarantee the level of sampling already achieved in the 2002, 2005 and 2008 surveys, however a high percentage of fishing hauls (48 %) over the total, resulted negative for sardine. On the whole, 34 fishing hauls which caught sardines were performed during the surveys covering the whole area, complemented by 24 samples obtained from the Portuguese purse-seine fleet (Figure 2)**.** On the whole, around 3760 sardines were sampled (Table 1), more than 1450 ovaries were collected, preserved and analysed histologically and *ca.* 1070 otoliths were removed for age determination. A total of 72 hydrated females were caught for batch fecundity estimation (much lower than in 2008), with final 61 being effectively used (see also discussion ahead on batch fecundity for S and W)

Data were analysed and the parameters estimated for the two surveys jointly:

- The same linear regression between the non-hydrated females Wt and their corresponding Wnov was used for the whole surveyed area (Wt = 1.063 * Wnov - 0.988, R^2 = 0.995).
- The geographical distribution of female weight (Figure 5) and mean batch fecundity (F = 17157, 11838 and 40844 eggs/female, respectively, for South, West and North strata, Table 3) suggest the need for a spatial stratification in view of the parameters estimation. The above estimates were obtained by modelling Fobs against the Wnov and the Stratum, and though a relatively small number of hydrated females were collected per stratum (n = 11, 19 and 31), the model considering the three strata separately was statistically significant and thus selected to estimate F for the three areas (Figure 6).

The minimum mean weights by haul were observed in the North of Portugal and in the Gulf of Cadiz (Figure 5). Mean female weight (W) was similar for the whole Portuguese and Cadiz coasts (54.3 and 50.1 g for strata 1 and 2, respectively) and considerably higher for the Northern Spanish coast (85.8 g for stratum 3). Compared to previous surveys, mean female weight for the whole area surveyed was similar to the values estimated for the 2008 survey, the two latest surveys presenting the highest values of the historical series.

The mode of individuals age distribution off the Northern Spanish coast is 3 years-old, these fish representing almost half of the individuals for which otoliths were sampled. On the contrary, female age distribution is bimodal off the Western Portuguese and Southern coasts, with sardines aged 1 and 6 and over being the most abundant in the samples representing respectively, about one third and one quarter of the females for which otoliths were collected (the latter likely still corresponding to the 2004 strong recruitment) (Figure 5).

Mean batch fecundity estimates (F) were considerably lower (about one third) off the Portuguese and Cadiz than off the Northern Spanish coasts (Table 3). The latter presented the highest estimate of the historical series, though similar to the ones obtained for the 2005 and 2008 surveys. On the contrary, for the Southern and Western strata, although mean female weights were similar to the ones obtained during the 2008 survey, F estimates were among the lowest of the time series (especially off the West coast), only comparable to the batch fecundities obtained in the South for the 2002 and 2005 surveys.

Regarding spawning fraction (S), estimate for the Northern Spanish coast was higher than the one obtained during the 2005 and 2008 surveys (0.114 *vs.* 0.078 and 0.090, respectively). For the South and West strata, the S estimates obtained are very low (0.015 and 0.019, respectively) and likely unrealistic taking into account the females mean weight and the egg production in these 2 strata. These two S values would better not be included in the estimation of the SSB, and alternative estimates for this parameter were considered, using a non-parametric bootstrap approach: a bootstrap was performed using mean spawning fraction by each haul obtained along the whole series (between 1997 and 2008) and considering a single haul as the basic sampling unit. Hauls were resampled for each strata with replacement from the original data set, leading to a new, artificial sample that was then used to estimate S parameter. By repeating this procedure an adequate number of times (1000 in this application), we obtained an empirical probability distribution for the S parameter.

5. SSB estimate

Spawning stock biomass was estimated taking into consideration: the model for mortality and egg production with 3 P0 and 1 z, and the spawning fraction estimates for the S and W obtained from non-parametric bootstrap. The SSB estimate for the Atlanto-Iberian stock was 484.8 x10^3 tons This estimate is lower than the one obtained in 2008, but is the second highest biomass estimate of the historical series for the whole Iberian stock. The SSB estimate for the S area in 2011 was similar to the one in 2008, whereas the SSBs off the W and N coasts decreased substantially (to about half for the W).

6. Results summary

Despite the fact that the 2008 DEPM results for egg production and SSB were the highest of the historical series no strong recruitment has been identified in the past six years. In fact other sources such as acoustics surveying have been noticing a decline in the Iberian sardine since 2006 (ICES, 2011). During the 2011 survey the difficulty in obtaining positive hauls for sardine, even though considerable fishing effort was undertaken, suggests that the species was much less available than it was in 2008.

The spawning area estimates for 2011 were lower in all the strata compared to 2008 (ICES, 2009). The spawning area of sardine in the western and northern areas was much smaller than in 2008, around 75 and 50 % respectively. On the whole the total positive area was reduced to about 55%.

Total egg production estimates in all areas are lower than in 2008; however the decrease was less accentuated in the Southern area.

Mean female weights obtained for all strata were similar to the ones estimated in 2008, the values calculated for the N and NW coasts of Spain being higher than for the West and South strata.

As in previous years, batch fecundity estimates in 2011 were considerably higher for the North than for the West and South strata. Moreover, mean batch fecundity for the West and South strata were among the lowest values of the historical series (only comparable to 2002 and 2005), though mean female weights in these areas were among the highest.

The spawning fraction for the North strata in 2011 was higher than in the two previous surveys. As for the West and South coasts, the obtained S estimates were very low and considered unrealistic, they can thus not be reliably included in the calculation of the SSB; alternative estimates (obtained from non-parametric bootstrap) were considered for these two areas. Furthermore, these unexpected estimates are indicative that spawning activity during the 2011 survey period may have presented some unusual features, which deserve being further investigated (cf. section 7).

The SSB estimate for 2011 using 3 strata for egg and adult parameter is lower than the one obtained in 2008. The decrease was more accentuated for the W and N strata while for the S the value was close to the previous estimate.

In short :

- spawning area in 2011 reduced compared to 2008
- spawning area in the western area much smaller than in 2008 (only around 20% of the total spawning area in 2011)
- the southern region showed the highest daily egg production per m_2 (eggs/m2/day)
- total egg production in all regions lower than in 2008 but higher than in 2002
- total egg production for 2011 was higher than in 2005 for S but lower for W and N
- main differences in total egg production between 2011 and 2008 were related to spawning area differences; reduced in all regions
- mean female weights similar to the ones obtained in 2008, higher for the North than for the West and South strata
- mean batch fecundity considerably higher for the North than for the West and South strata in 2011
- mean batch fecundity estimated for the West and South strata among the lowest of the historical series (though mean female weights among the highest)
- spawning fraction for the North strata in 2011 higher than in the two previous surveys; estimates obtained for the South and West strata in 2011 considered unrealistic and replaced by values estimated by non-parametric bootstrap as alternativethe SSB estimated indicate a decrease of ca. 25% compared to 2008 for the whole Iberian stock, this decrease being considerably marked for the West and North coasts.

- the unexpected values obtained for some of the adult parameters in the S and W coasts suggest that spawning activity during the 2011 survey period may have presented some unusual features (cf. section 7).

7. The 2010-2011 reproductive season in the south and western strata

On account of the uncommon estimates obtained for the spawning fraction off the South and West strata, it was decided to explore all biological data available attempting to identify any unusual pattern in the sardine reproductive dynamics during the 2010-2011 spawning season, and in particular during the survey period.

Seasonal data from the commercial fleet

First, macroscopic data obtained from the samples collected regularly (monthly) within the framework of the Data Collection Regulation off the Portuguese Northwest (NW: Matosinhos, Póvoa de Varzim), Southwest (SW: Peniche), and South (S: Portimão) coasts were used. These included gonads macroscopic maturity stage, gonado-somatic index (GSI) and condition factor (relative weight).

The females' macroscopic maturity stages during the 2010-2011 reproductive season showed a common seasonal pattern (active females being present in the samples from September until April or June, depending on the areas). During the survey period, these macroscopic data were globally in line with the observations obtained during the survey. In February 2011, all females sampled were at maturity stage 3 (active) (exception: ~90% for the SW area). No females were available in March for the NW and S (temporary closure of the purse-seine activity), in the SW about 1/4 of the females were in March at maturity stage 5 (post-spawning). In the whole area considered, there is no evidence from the available information that reproductive season might have ended for most of the individuals during and/or soon after the DEPM survey (Figure 7). Similarly, the seasonal pattern observed for the GSI of mature females during the 2010-2011 spawning season was not uncommon, compared to other recent years (maximum values in December-January, minimum values in July-August). During the 2011 survey period, the data showed a slight increase of the GSI in February 2011 in the NW, whereas the GSI in the SW started decreasing from February 2011 onwards and the GSI in the S remained stable in January and February 2011 (Figure 8). The seasonal pattern observed for the condition factor during the 2010-2011 spawning season was also similar to the one obtained in other recent years (maximum values in August-October, minimum values in February-March). Minimum values were observed in January for the NW and in February for the SW and S areas (Figure 9).

Survey data: spawning activity

The adult data include all the samples obtained within the context of the 2011 DEPM survey (48 hauls), i.e. the samples collected onboard the RV as well as the samples provided by the commercial fleet during that period. The analyses were carried out taking into account both spatial and temporal factors. Four areas were considered: Cadiz (Spanish waters), South (Algarve), Southwest (from Sagres to Nazaré), and Northwest (from Nazaré to Caminha). The studied period was divided into weeks:

week 1: 8-14 Feb 2011, period during which most Cadiz area was covered by the survey;
week 2: 15-21 Feb 2011, period during which most Algarve area was covered by the survey;
week 3: 22-28 Feb 2011, period during which most SW area was covered by the survey;
week 4: 1-7 Mar 2011, period during which most NW area was covered by the survey.
week 5: 8-14 Mar 2011, just after the survey, only commercial samples

Both macroscopic (proportion of active females, n = 1472 females) and histological information (proportion of females with vitellogenic oocytes, proportion of mature females with POFs or with massive atresia, n = 1371 females) were considered for the analysis.

The reproductive activity was evaluated both macroscopically (active females with mature stages 3 and 4) and microscopically (females with oocytes at stage >= 3). Globally, macroscopic and histological data were in accordance (Figure 10). In the south, Cadiz and Algarve, activity was maximal at the beginning of the survey, then it seems to have decreased during weeks 2 to 4, and increased again after the survey was ended (week 5). In the SW, the pattern is more difficult to assess from the available information, whereas in the NW activity seems to have decreased from week 3 onward, although the data do not provide any evidence that reproductive season might have ended for most of the individuals towards the end or soon after the DEPM survey. Namely, 2 to 3 weeks after the end of the survey, hydrated females were obtained from commercial samples off both S and W coasts (hydrated ovaries used to model batch fecundity, see previously).

The gonado-somatic index (GSI) and condition factor (relative weight) mean values did not vary significantly over the weeks considered (not shown).

The prevalence of mature females with ovaries containing more than 50% of vitellogenic atretic oocytes (alpha atresia) was assessed based on the histological analysis (the percentage of atretic oocytes was not measured accurately but evaluated roughly from visual observation of the histological slide). Massive atresia increased in weeks 3 and 4 in the W coast up to about 20% of the mature females; however, its prevalence decreased again at the end of the survey, mainly in the SW coast (Figure 11).

Females with signs of recent spawning activity (POFs) were observed during the first week of the survey (at least, in the areas sampled during that period, i.e., Cadiz, Lisbon area and the NW coast) (Figures 12 and 13). Plankton sampling during the survey collected eggs of all ages in the Cadiz area; egg abundances were high in that area; eggs were also found all along the southern Spanish coast (no fishing was possible in the region between the Guadalquivir and Cabo St[a] Maria) (Figure 13). Following an interruption of 3 days, due to bad weather, the survey resumed off Cabo St[a] Maria (week 2): at the restart few (older) eggs were collected, however further to the west, in the region off Portimão-Sagres eggs of all stages were again observed. POFs were also present in adult samples during week 2. Egg production per unit area was higher in the south than in the west and northern strata (similar results had occurred in previous DEPM surveys). Several fishing hauls were undertaken but in general sardine schools were scarce and in some hauls none were caught.

From the microscopic ovary observations there was any or nearly any signs of spawning activity during weeks 2 and 3 in both S and W coasts. Egg abundance in the W coast south of Lisbon was nearly zero with only a small patch of eggs over Setubal canyon (weeks 2-3). However spawning activity was noted in commercial samples in the region Lisbon-Sines 2 weeks after surveying.

POFs were again present in ovaries sampled in weeks 4 and 5 (at least, in the areas sampled during that period, i.e., the S and SW coasts). POFs were present but very scarce in the samples from the NW area, simultaneously with the survey coverage of that area, and only a few hydrated females were collected by the RV in the N during week 4. But recently spawned eggs and subsequent ages were obtained in the region from Porto to Cabo Mondego in particular over the middle and outer shelf (due to the wind regime during the survey, advection offshore may account for the apparent mismatch between sardine schools and egg distributions). Fishing for sardine in the NW coast was more successful than in other areas, however sardine distribution was quite patchy and the RV operated mainly in the same areas where the purse-seiners were fishing.

During the 2011 survey the CTDF profiler that operates together with the CalVET net was not operational and therefore no data on the water column structure is available. However, in situ SST showed that the temperature distribution patterns were the typical for a late winter situation. Despite the fact that the temperature was slightly lower than in 2008, it was, for all areas, within the range registered for the DEPM surveying series and can not therefore explain the uncommon biological observations discussed.

Summary and work in progress

The above preliminary results, as well as the information on the size/age composition of the samples collected suggest:

- Cadiz area: the area was sampled only during the first week of the survey, there is no information for the remaining weeks. During week 1, nearly all females were active and actively spawning, though the presence of POFs in the ovaries is lower than in other years. Egg abundances were in accordance to results from other years, although lower than in 2008. Recently spawned eggs as well as eggs from days 1, 2 and 3 cohorts were collected.
- Off the South and Southwest coasts: the macroscopic data (from both surveys and regular DCF sampling) do not indicate that the reproductive season had ended or been interrupted during the period considered, since most females had active ovaries with vitellogenic oocytes (though the percentage was lower than the one observed in the Cadiz area). Eggs of all ages were observed in the Algarve during week 2. But only very few were collected in the west region south of Lisbon (weeks 2/3) and even over Promontório da Estremadura (Cabo Raso-Cabo Carvoeiro), where abundances are regularly high. Adults collected during weeks 2 and 3 of the survey did not present evidence of spawning activity (presence of POFs), but resumed spawning the 4th week onwards. The prevalence of massive atresia was slightly higher (~20%) than observed in other years at the same period, it was observed mainly in females aged 3 and above, but atresia could difficultly be considered the main cause for the nearly absence of spawning during that period. The above observations leads to the hypothesis that for reasons not yet identified (environmental, physiological) during a period of *ca.* 2-3 weeks, the sardines of the S and SW coasts could have skipped the full maturation and ovulation of one batch of oocytes.
- In the Northwest area: Though the available information does not allow a full understanding of what happened in this area, both macroscopic and histological data seem to indicate that the scenario might have been in part different from the S and SW. Most females sampled in this area were young individuals (~60% sized under 16 cm, ~ 70% aged 1 and 2), the prevalence of 19% of massive atresia in week 4 corresponded exclusively to fish aged 1 or 2, and in weeks 4 and 5, spawning activity (presence of POFs) kept on being scarce in the NW area. However the egg results indicate spawning activity, eggs of all ages, including very young ones (< 8h), were collected in the NW area, though not at the coastal stations. It is known that the duration of the reproductive season is shorter for younger sardines (Silva et al. 2006, Stratoudakis et al. 2007). The working hypothesis concerning the NW area is that perhaps a considerable fraction of the individuals (young first-time spawners) in this area would have been ending their reproductive season during the last week of the survey.
- The investigation on the eventual causes for the unusual observations related to the spawning activity during part (temporal or spatial) of the survey period is still in progress. Further analyses on the environmental data, from remote sources and operational models, will be explored in order to assess potential relationships between the biological observations and the environmental scenario.

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Table 1. General Sampling DEPM 2011

Institute	IPIMAR	IPIMAR	IEO
Survey area	**South**	**West**	**NW & N Spain**
SURVEY EGGS			
R/V	Noruega	Noruega	Cornide de Saavedra
Date	10/02-20/02	20/02-08/03	25/03-10/04
Transects	21	36	56
PairoVET stations	170	309	337
Positive stations	54	40	130
Tot. Eggs	2208	803	1794 (1 net)
Max eggs/m2	4950	2970	1537
Temp (ºC) min/mean/max	14.6/16/16.9	13.5/14.7/16.1	12.5/13.4/14.6
Max age	56.8	66	70.9
CUFES stations	183	309	337
Positive CUFES stations	60	54	163
Tot. Eggs CUFES	4607	479 (inc. area)	34438
Max eggs/m3	81.73	22.13	97.26
Hydrographic stations	NA	NA	337
SURVEY ADULTS			
Number Hauls R/V (total)	11	23	53
- Pelagic Trawls	10	20	53
- Bottom trawls	1	3	-
Numer Hauls Commercial vessel	7	17	-
Number (+) trawls	16	32	10
Date	10.02-20.02	20.02-08.03	12/04-20/04
Depth range (m)	33-107	25-116	61-185
Time range	During the whole day		07:00-20:00
Total sardine sampled	975	2065	718
Length range (mm)	115-266	120-246	162-256
Weight range (g)	11-89	12-98	26.8-130.8
Female for histology	397	827	230
Hydrated females	11	30	31
Otoliths	235	429	409
Female Ages Range	1-10	1-10	1-11

Table 2. Surveying, processing and analyses for eggs and adults

DEPM Surveys	Portugal (IPIMAR)	Spain (IEO)
Survey	**PT-DEPM11-PIL**	**SAREVA0411**
Survey area	South-West	NW & N Spain
SURVEY EGGS		
Sampling grid	8 (transect) x 3(station)	8 (transect) x 3(station)
PairofVET Eggs staged (n egg) (stages from Gamulin and Hure, 1955)	All (2 nets)	All (1 net)
Sampling maximum depth (m)	150	100
Temperature for egg ageing	10 m	
Peak spawning hour	(PDF 21 ± 2 * 3)	
Egg ageing	Bayesian (Bernal et al, 2008)	
Strata	No strata/Stratum (South,West,North)/StratumI (South+West/North)	
Egg production	GLM	
CUFES, mesh 335	3nm (sample unit)	3 nm (sample unit)
CUFES Eggs counted	All	All
CUFES Eggs staged (stages from Gamulin and Hure, 1955)	Subsampled of a minimun of 100	No
Hydrographic sensor	CTDF (FSI)	CTD (SBE 37) CTD SBE 25
Flowmeter	Y	Y
Clinometer	Y	Y
Environmental data	Fluorescence, Temperature, Salinity	Fluorescence (surface only), Temperature, Salinity
SURVEY ADULTS		
Biological sampling:	On fresh material, onboard the R/V or in laboratory; on frozen material for certain commercial samples (ovaries removed before)	On fresh material, on board of the R/V
Sample size	60 indiv randomly ; extra if needed (30 females min for histology) and if hydrated females found	60 indiv randomly (30 mature female); extra if needed and if hydrated found
Sampling for age	Otoliths from the same females sampled for histology	Otoliths from random males and females
Fixation	Buffered formaldehyde 4% (distilled water)	Buffered formaldehyde 4% (distilled water)
Preservation	Formalin	Formalin
Histology:		
- Embedding material	Paraffin	Resin
- Stain	Haematoxilin-Eosin	Haematoxilin-Eosin
S estimation	Day 1 and Day 2 POFs (according to Pérez et al. 1992a and Ganias et al. 2007)	Day 1 and Day 2 POFs (according to Pérez et al. 1992a and Ganias et al. 2007)
R estimation	The observed weight fraction of the females	The observed weight fraction of the females
F estimation	On hydrated females (without POFs), according to Pérez et al. 1992b and Ganias et al. 2010	On hydrated females (without POFs), according to Pérez et al. 1992b

Table 3. Results DEPM 2011

Institute	IPIMAR	IPIMAR	IEO	TOTAL
Area	**South**	**West**	**NW & N Spain**	
Survey area (Km^2)	17578	32098	33832	83508
Positive area (Km^2)	6524	4817	12405	23746
Z ($hour^{-1}$) (CV%):	-0.047***(13)			
P0 (eggs/m2/day) (CV%):	438.9(27)	174.4(29)	326.0(24)	
Daily mortality rate (%):	67.8			
P0 tot (eggs/day) ($x10^{12}$) (CV%):	2.86(27)	0.84(29)	4.04(24)	7.74 (16)
Female Weight (g) (CV%)	54.25 (7)	50.07 (6)	85.85 (3)	
Batch Fecundity (CV%):	17157 (11)	11838 (9)	40844 (5)	
Sex Ratio (CV%)	0.498 (9)	0.496 (4)	0.487 (12)	
Spawning Fraction (CV%):	0.081 (9)	0.066 (8)	0.114 (26)	
Spawning Biomass (thousand tons) (CV%):	223.7(33)	108.1 (32)	152.9 (38)	484.8 (21)

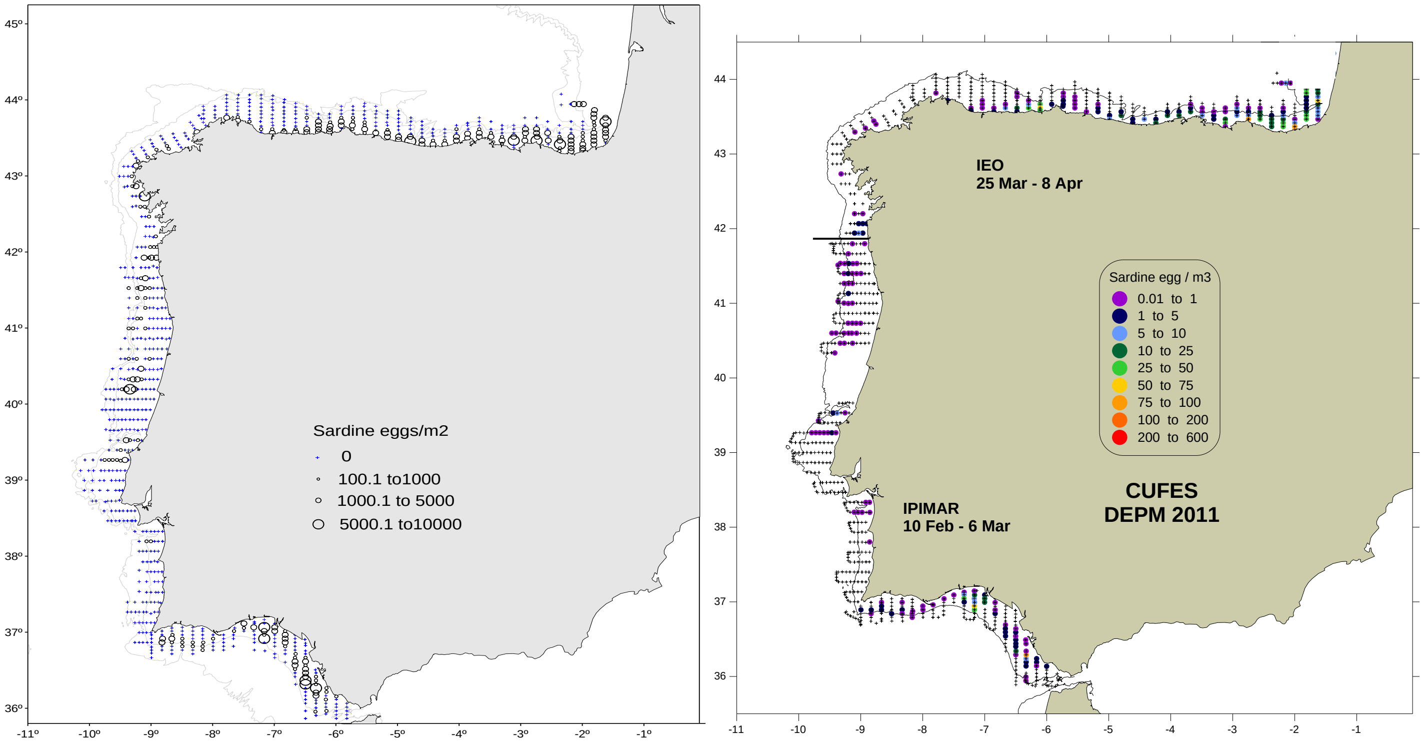

Figure 1. Sardine egg distribution. Left panel: Egg/m^2 from PairoVET sampling; Right panel: Egg/m^3 from CUFES sampling; (+, egg absence).

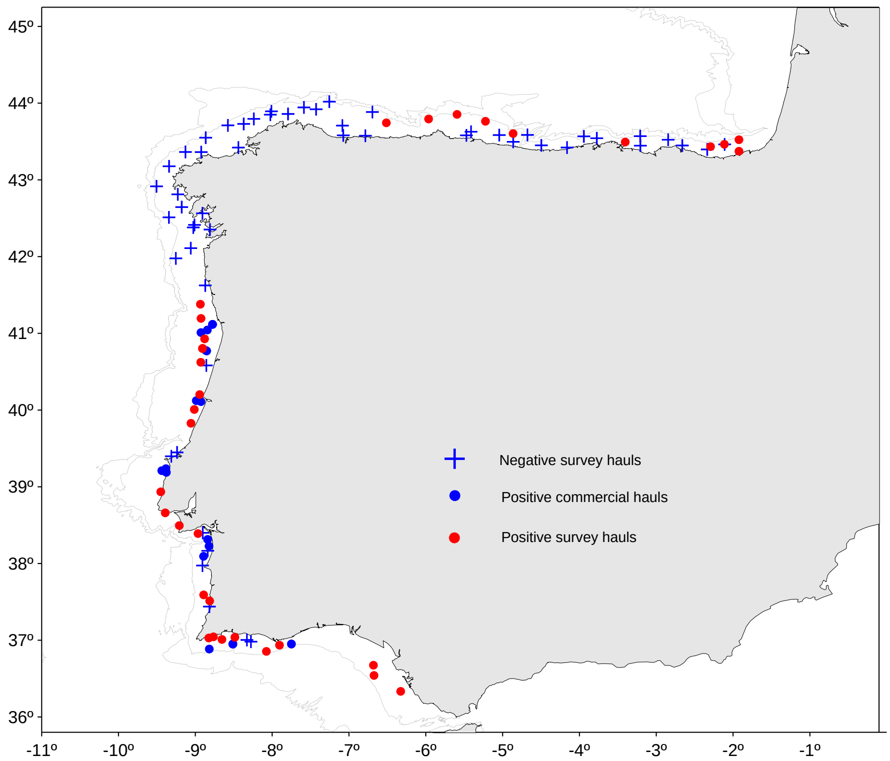

Figure 2. Spatial distribution of fishing hauls (+, hauls without sardine presence)

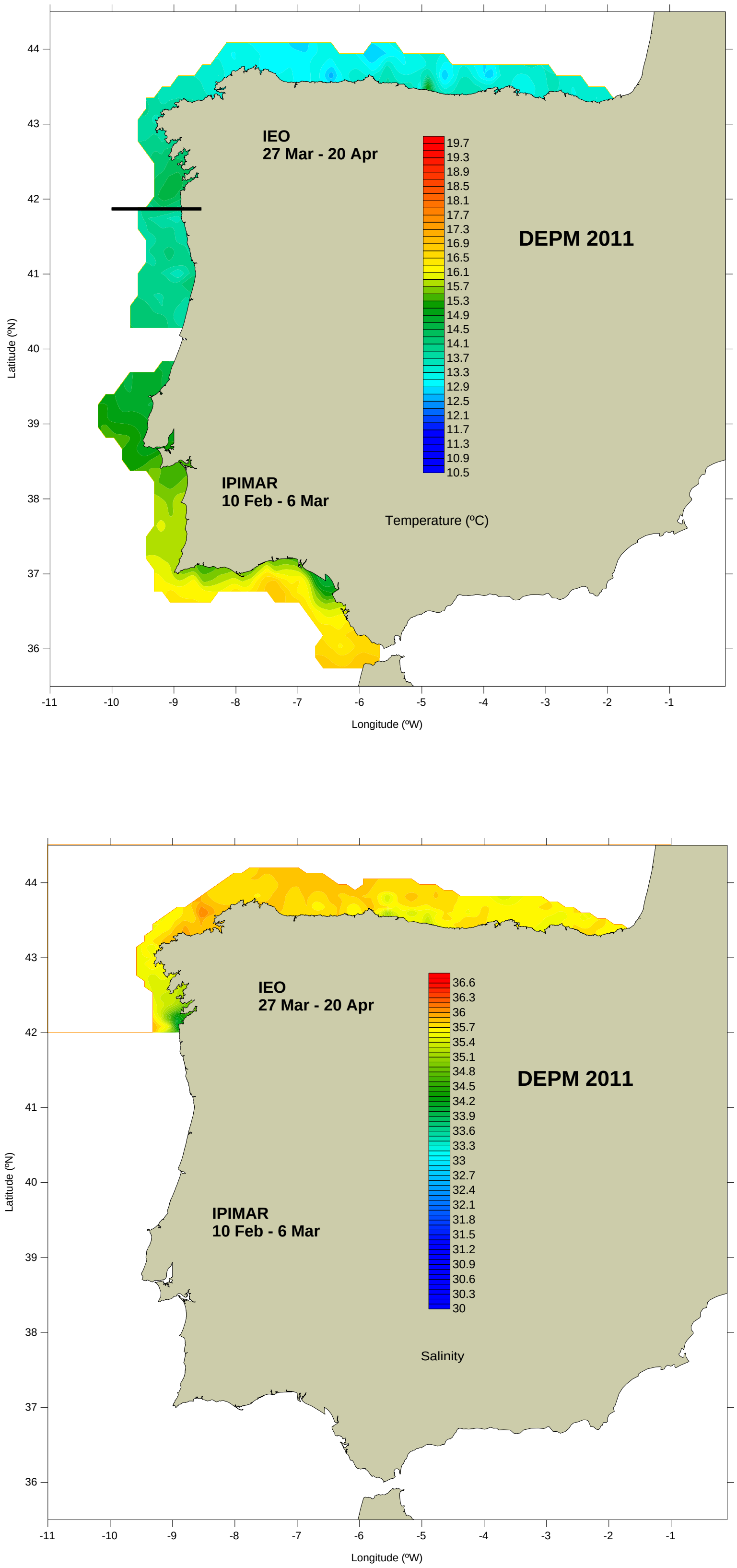

Figure 3. Distribution of sea surface temperature (above) and salinity (below).

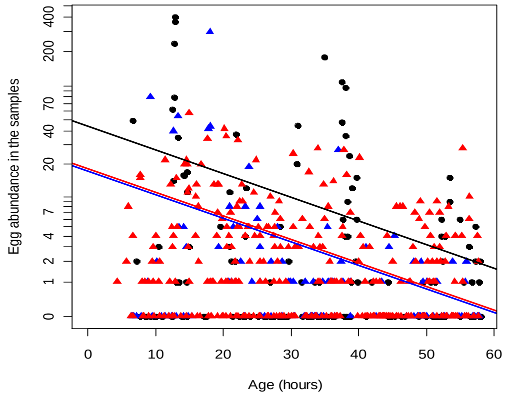

Figure 4. Abundance by age of eggs in the different spatial strata (black = south, blue = west, red = north) and its corresponding fitted mortality curve. Note that southern, western and northern mortality curves were forced to have a common slope (mortality) in this model (Model 2).

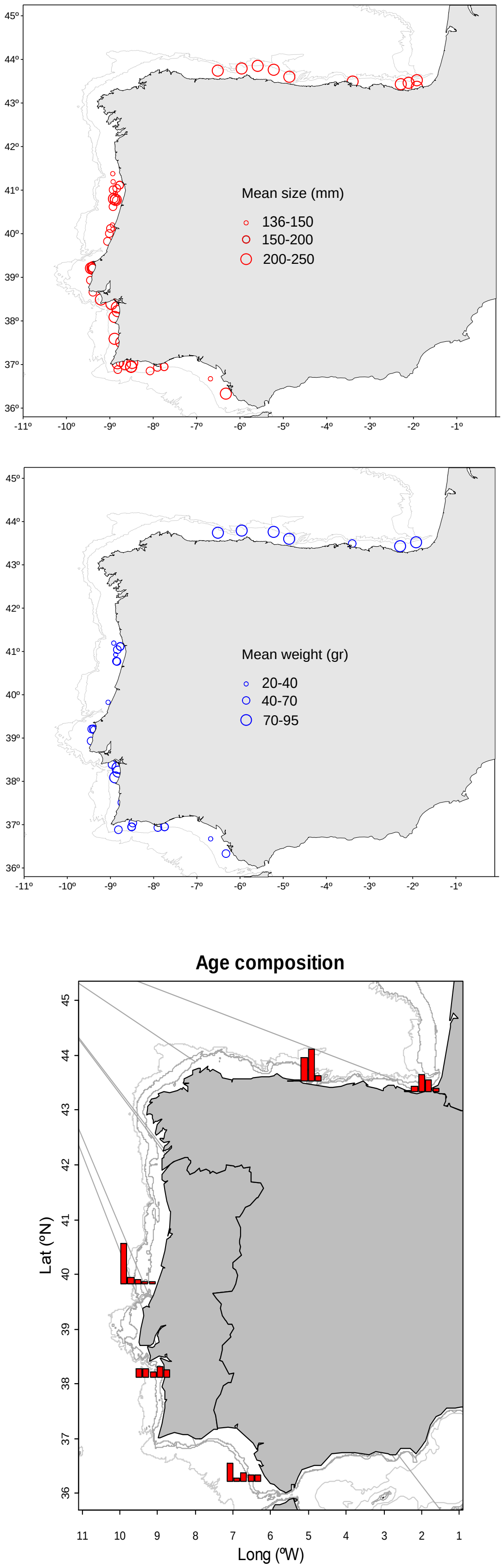

Figure 5. Spatial distribution of the mean size (above), mean weight for mature females, and age composition for the South, West and North areas.

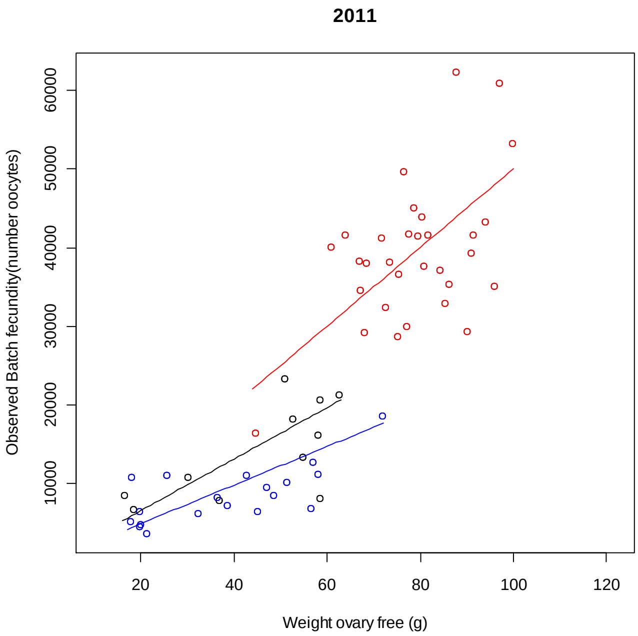

Figure 6. Observed batch fecundity vs. gonad free weight of the corresponding females and regression line of the model for the three geographical areas (black: South stratum, blue: West stratum, red: North stratum).

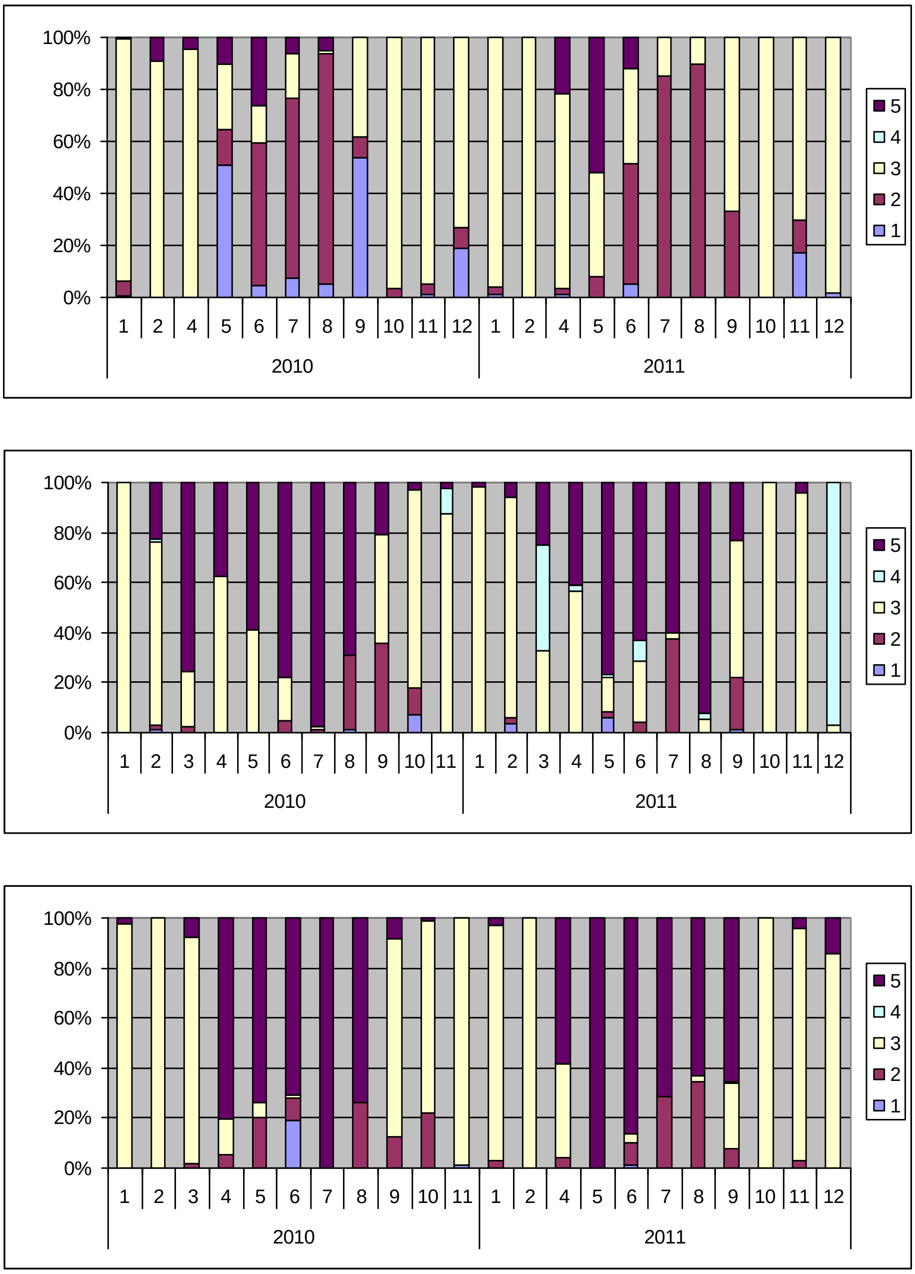

Figure 7: Monthly evolution of the relative proportion of female gonad macroscopic maturity stages (1 to 5) in the samples obtained regularly from the commercial fleet within the framework of the Data Collection Regulation off the Portuguese Northwest (upper panel), Southwest (middle panel) and South (lower panel) coasts during the period January 2010-December 2011.

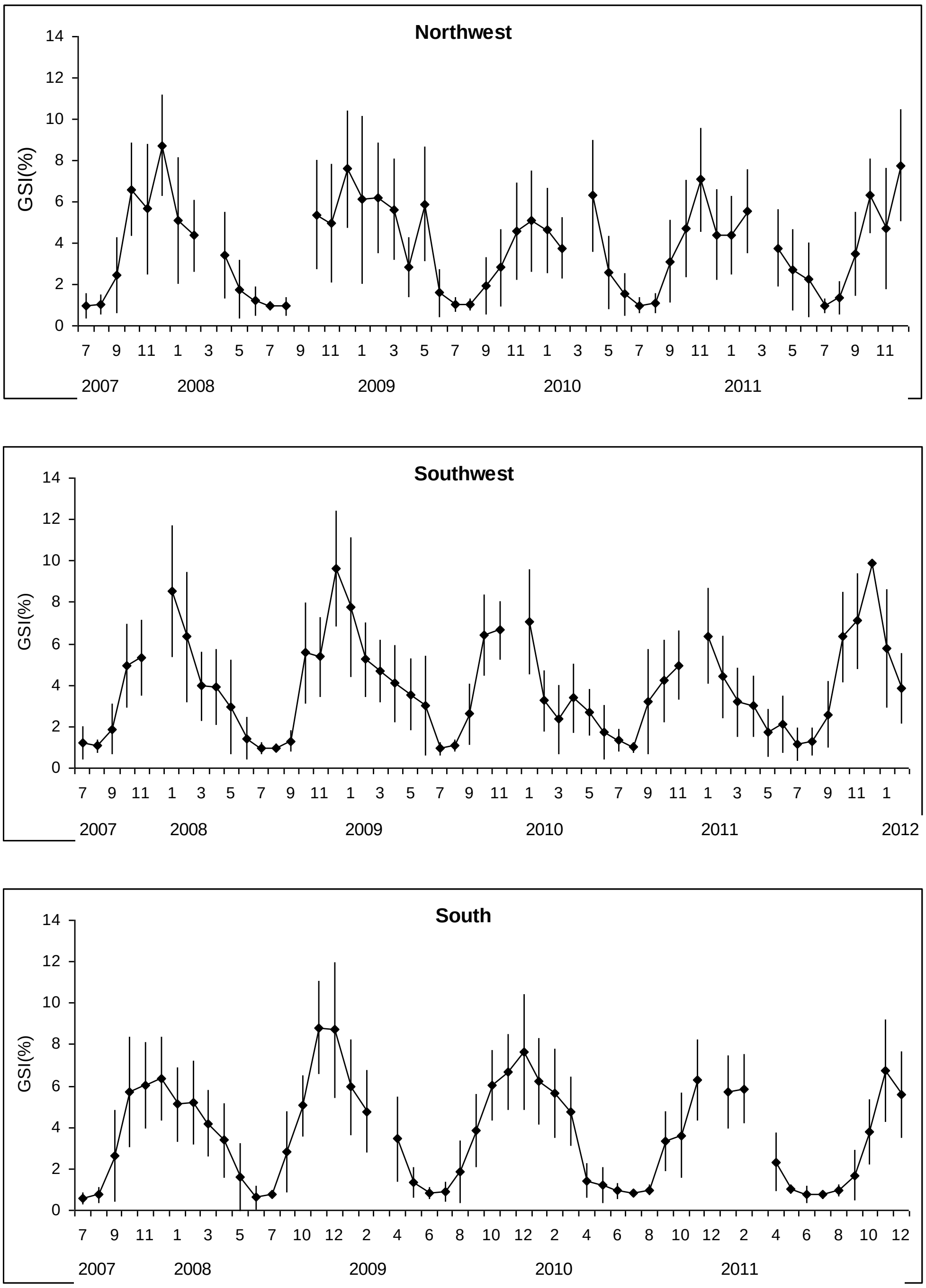

Figure 8: Monthly evolution of the mean (± standard-deviation) gonado-somatic index (GSI) of mature females from the samples obtained regularly from the commercial fleet within the framework of the Data Collection Regulation off the Portuguese Northwest (upper panel), Southwest (middle panel) and South (lower panel) coasts during the period July 2007-December 2011.

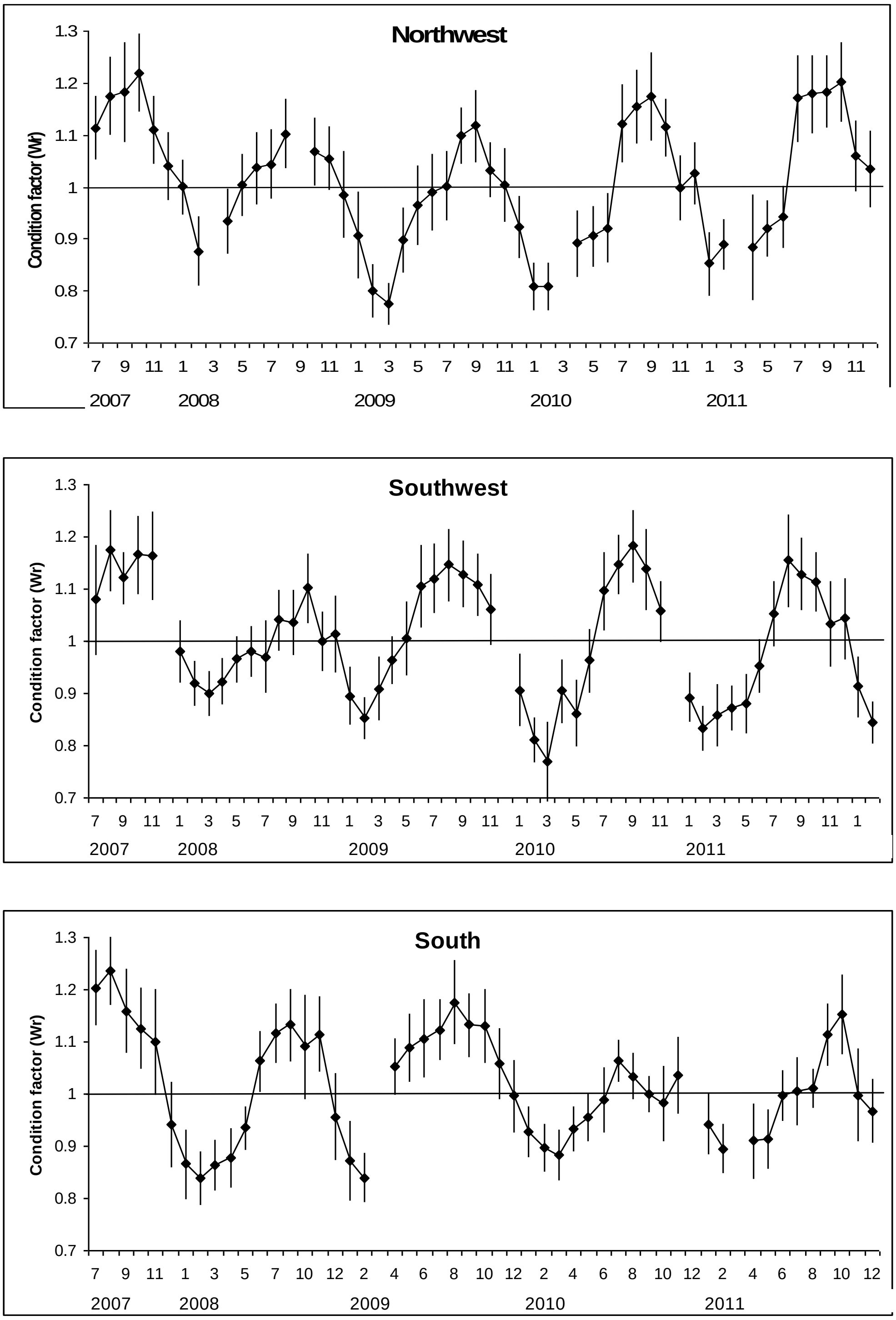

Figure 9: Monthly evolution of the mean (± standard-deviation) condition factor (relative weight Wr) of mature females from the samples obtained regularly from the commercial fleet within the framework of the Data Collection Regulation off the Portuguese Northwest (upper panel), Southwest (middle panel) and South (lower panel) coasts during the period July 2007-December 2011.

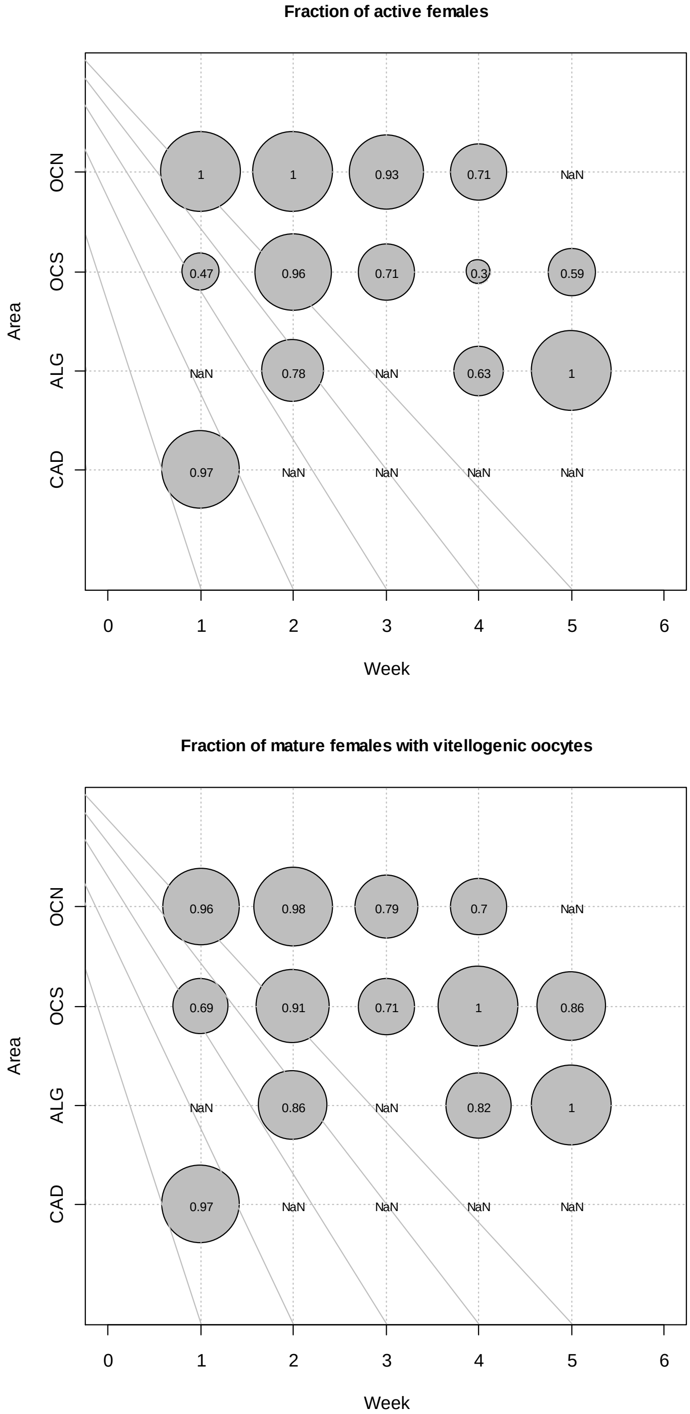

Figure 10: Bubble plots representing the proportion of active females (i.e., the females with ovary macroscopical mature stages 3 and 4) (upper panel) and the proportion of mature females with ovaries containing vitellogenic oocytes (i.e., oocyte stages ≥ 3) (lower panel) sampled in each geographical area (CAD: Cadiz Spanish waters, ALG: Portuguese South, OCS: Portuguese Southwest, and OCN: Portuguese Northwest coasts) and for each week (weeks 1 to 4: period of the DEPM survey coverage, corresponding to samples collected by both R/V and the commercial fleet; week 5: the week after the completion of the survey, corresponding only to adult commercial samples); the figures inside the bullets indicate the corresponding proportion values.

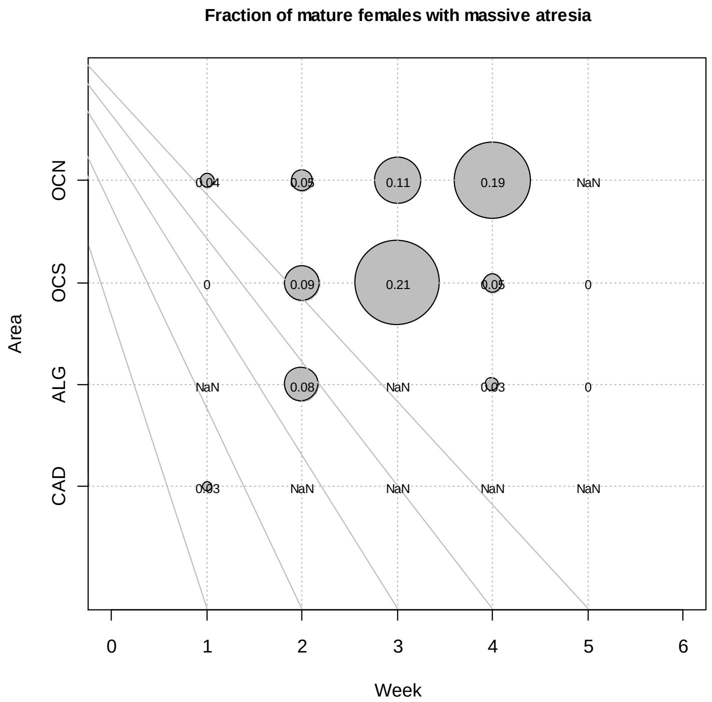

Figure 11: Bubble plot representing the proportion of mature females with ovaries containing more than 50% of the vitellogenic oocytes with alpha atresia, sampled in each geographical area (CAD: Cadiz Spanish waters, ALG: Portuguese South, OCS: Portuguese Southwest, and OCN: Portuguese Northwest coasts) and for each week (weeks 1 to 4: period of the DEPM survey coverage, corresponding to samples collected by both R/V and the commercial fleet; week 5: the week after the completion of the survey, corresponding only to adult commercial samples); the figures inside the bullets indicate the corresponding proportion values.

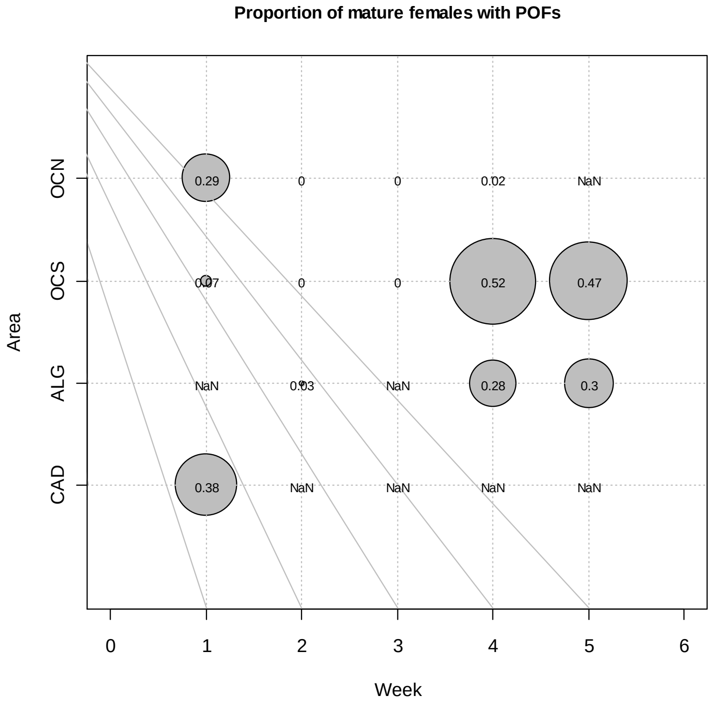

Figure 12: Bubble plot representing the proportion of mature females with ovaries containing post-ovulatory follicles (POFs), sampled in each geographical area (CAD: Cadiz Spanish waters, ALG: Portuguese South, OCS: Portuguese Southwest, and OCN: Portuguese Northwest coasts) and for each week (weeks 1 to 4: period of the DEPM survey coverage, corresponding to samples collected by both R/V and the commercial fleet; week 5: the week after the completion of the survey, corresponding only to adult commercial samples); the figures inside the bullets indicate the corresponding proportion values.

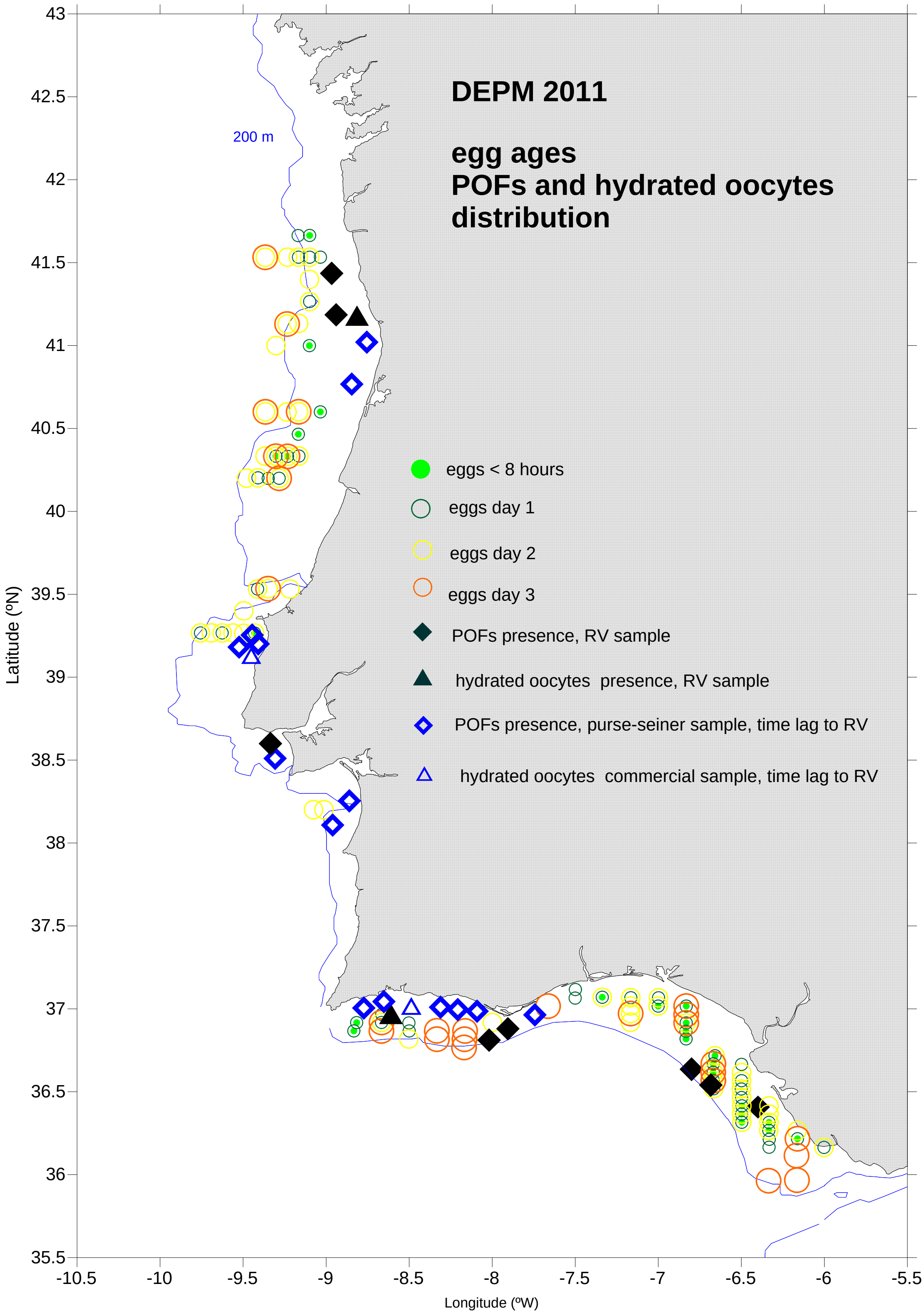

Figure 13: Geographical distribution of eggs (of different ages) from the plankton samples, and of females with signs of spawning activity (hydrated ovaries and ovaries with post-ovulatory follicles, POFs) collected by both the R/V and the commercial vessels (some fishing hauls were not taken simultaneous to the plankton surveying).