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Application of the daily egg production method (DEMP) for sardine (*Sardina pilchardus*) in the inner of the Bay of Biscay from 1997 until 2011.

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Introduction

In order to provide an estimate of the spawning stock biomass of the Atlantic-Iberian sardine, different DEPM surveys have taken place covering the area from the Gulf of Cadiz to the inner part of the Bay of Biscay (Atlantic-Iberian stock). The Daily Egg Production Method (DEPM) for sardine has been applied by Instituto Español de Oceanografía (IEO) to estimate the spawning stock biomass of the North Atlanto-Iberian sardine stock since 1988 (García et al., 1992) and then repeated in 1990, 1997, 1999, 2002, 2005, 2008 and 2011. From 2000 onwards the surveys have been planned and conducted within the framework of ICES, on a triennial basis. Since 2002 extra effort was put in place in order to standardize methodologies for surveying, laboratorial and data analyses. These objectives were possible due to methodological developments and effective coordination undertaken first by the SGSBSA (2002-2004) and later by the WGACEGG (Stratoudakis *et al*., 2004, Stratoudakis *et al*., 2006, ICES 2009, ICES 2010, ICES 2011).

Spring surveys for the application of the DEPM, consisting of ichthyoplankton, adults and hydrographic sampling, and since 1997 the sampling area was extended in order to reach the 45 degrees latitude North, covering the region from the northwestern (border Minho River), north Iberian Peninsula (north Spanish Atlantic and Cantabrian waters, ICES division IXa North and VIIIc) and the inner part of the Bay of Biscay (from 42 ºN to 45°N, ICES division VIIIb). The region from the Gulf of Cadiz to the northern Portugal/Spain border (Miño River) was surveyed by IPIMAR (Instituto de Investigação das Pescas e do Mar, Portugal).

This working document provides for the first time a description of the sampling, laboratory analysis and estimation procedures used to obtain the spawning stock biomass estimate for the application of DEPM conducted by IEO from 1997 to present in the inner of the Bay of Biscay (ICES Division VIIIb). Estimations for area delimitation (surveyed & spawning), egg ageing, mortality and model fitting for egg production (P0) are presented. Results from adults fishing sampling are showed and parameters from the mature fraction of the population (mean females weight, sex ratio, batch fecundity and spawning fraction) are calculated.

Preliminary estimates were based on procedures and software adapted and developed during the WKRESTIM- 2009 and modifications carried out subsequently for the revision of the sardine DEPM historical series (1988-2011) in divisions IXa and VIIIc. Several issues that arose from data analysis, namely the absence of data to model batch fecundity, the unreliable egg mortality estimated in 1999 and the absence of data to obtain spawning fraction in the same year, are planned for discussion at the WGACEGG.

Material and Methods

Surveying

The Spanish surveys were undertaken using two vessels, RV Cornide de Saavedra for plankton sampling mainly and RV Thalassa to carry out the fishing hauls (in 2008 and 2011 some fishing hauls carried out on RV Cornide de Saavedra). The surveys were designed to obtain en adequate spatial and temporal coverage during the spawning peak of sardine in the area. Vertical plankton hauls were carried out following a pre-defined grid of sampling stations along transects perpendicular to the coast and spaced 8 miles from 2005 onwards (Figure 1). The inshore limit of the transects is determined by bottom depth (as close to shore as possible) while the offshore extension was decided adaptively, based on the presence of eggs.

The main egg 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; sampling covered the water column from bottom, or 100 m (beyond the 100 isobath) depth, to the surface. PairoVET samples were taken according to the designed grid and covering the extension of the platform to the 200m isobath. From 2002, CUFES was used as an auxiliary egg sampler, helping in defining the offshore extension of the transects. Due to the bad weather, in 2005 was not possible to complete the plankton sampling coverage, so no data is presented in this work.

All plankton samples were preserved in formalin at 4% in distilled water and the 2 samples from each net stored in separate containers. Sample for one net was used for egg density estimates and the other being used for plankton dried mass calculations. The total numbers of eggs from both plankton samplers, CalVET and CUFES, were counted onboard in order to obtain a preliminary data of sardine egg abundance and distribution.

From 1997 to 2005, a CTD (Sea Bird-25) profile (Temperature and Salinity) was carried out in each CalVET station. From 2008 to 2011 the Sea Bird-25 was used in each transect head and in alternate stations along the transects, meanwhile a CTD (Sea Bird-37) was coupled to the CalVET sampler.

Fishing hauls were conducted by pelagic trawling following sardine schools detection by the echo-sounder (for RV Thalassa). The number of samples and its spatial distribution was organized to ensure good and homogeneous coverage of the survey area (Figure 2) in order to obtain a representation of the sardine population.

Onboard the RV, and for each haul, a minimum of 60 sardines were randomly selected and biologically sampled. These could also be 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. The biological sampling was always carried out in fresh material, and ovaries were immediately collected and preserved in a formaldehyde buffered solution (4% diluted in distilled water) for posterior histological processing and analysis at the laboratory. Moreover, otoliths were extracted to obtain the age composition per sample.

Laboratorial analysis

In the laboratory, all sardine eggs were sorted from PairoVET and CUFES samples. The eggs from the vertical hauls were all counted and staged according to the 11 stages of development classification (adapted from Gamulin and Hure, 1955).

The preserved ovaries were weighted in laboratory and the obtained weights corrected by a conversion factor (between fresh and formaldehyde fixed material) established previously. These ovaries were processed for histology, first, they were embedded in resin (paraffin before 2005), the histological sections were stained with haematoxylin and eosin, and then the slides examined and scored for their maturity state, POF presence and age assignment (Hunter and Macewicz 1985, Pérez et al. 1992a, 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, weighting on average 50-150 mg (Hunter et al. 1985).

Data bases with date, time, position, bottom depth and other variables registered during the sampling on board and in the laboratory, were merged in a common standardised dataset (eggs and adults data separately) and include all surveys undertaken in the period from 1997 to 2011. The updated data set for eggs and adults include minor corrections (*eg.* wrong geographical coordinates, duplicated points, ovary and total weights data, etc.), that were observed as mistakes in a first exploration data.

Data analysis

Egg data

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 extremes values detected 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 model of egg development with temperature was derived from the incubation experiment data available within the *sardata* 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 spawning period between 15:00 and 03:00 h GMT. The upper age cutting limit was determined using a maximum age for the entire area considered and it is not dependent on the individual stations (upper.age=F). Older cohorts are dropped if their mean age plus 2* st-dev 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 exponential model: $E[P] = P0\ e^{-Z\ age}$ was fitted as a Generalized Linear Model (GLM) with negative binomial distribution and log link. Weights proportional to the relative area represented by each station (estimated using the dirichlet tessellation and divided by the mean area represented by a station) were used to account for increased sampling in areas of expected high egg densities. Finally, the total egg production is calculated multiplying the daily egg production ratio (eggs per m^2 and day) by the positive area (in m^2). For 1999 survey a model without mortality was applied since an estimate for mortality led to non coherent mortality.

Fish data

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 surveys. For the present estimations, a minimum sample criterion (n = 30) was introduced: a few hauls containing less than 30 fish sampled were excluded from the mean and variance calculations. All estimations and statistical analysis were performed using the R software.

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 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).

In 1999 no histology samples were available to estimate S and a non- parametric bootstrap approach (Melia *et al*., 2012) was performed using mean spawning fraction by each haul obtained along the all series and considering a single haul as the basic sampling unit. Hauls were resampling 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.

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 hydrated females and their gonad-free weight (Wnov) by a GLM (with a negative binomial error distribution and an identity link). In 1999, 2002 and 2008, no hydrated o very few hydrated females were collected off the Inner of the Bay of Biscay (no one in 1999 and 2002, and n = 3 in 2008). For these years, F was modelled polling data from the inner Bay of Biscay and North Spanish coast, but F estimates were nevertheless calculated for the two areas separately.

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).

Details on the methodologies used on board, during laboratorial work and for data analyses are summarized in Table 1.

Results

Eggs

Total transects and PairoVET stations that were sampled along the years are summarised on Table 2. In 1997 and 2011 the number of samples performed was higher than others years and 1999 was the year with less stations sampled. The percentage of stations with sardine eggs was higher than 63 % for all years and has been increasing from the first survey (1997) until the last one (2011), reaching a 85% in 2011. In total 6667 were sorted, staged and counted for the vertical tows in the area studied, of which 2764 were caught in 2011, around 1100 in 1997, 2002 and 2008, and 586 in 1999. The highest egg abundances per haul were 2332.1 (eggs/m^2) and 2321.7 (eggs/m^2) reached in 2008 and 2011 respectively. The lowest egg abundance per haul was 1185.4 (eggs/m^2) in 1999 and with values ranged from 1185.5 to 1669.6 (eggs/m^2) for 2002 and 1997 respectively.

Along the survey series an increasing in the number of stations with high egg abundance (eggs/m2) have been observed (Figure 3).

For all the surveys, 99.2 % of the sardine eggs have been classified into 11 stages according to the degree of embryonic development. It has been found sardine eggs in all the described stages (except stage I in 1999 and 2002). The abundance of eggs and numbers per stage and year is showed in figure 4. The most abundant development stages were II, V and VI. Very few eggs of stage I and XI (right after and before the spawning and hatching respectively) were found along the series.

Sardine egg distribution, obtained from the PairoVET sampler, for the whole area is presented in figure 1. Almost the entire shelf (from coast to slope) was occupied by sardine eggs.

For some years (1997, 2008 and 2011), two areas of spots with higher density occurred along the coast and offshore, namely in waters along the end of the continental slope (200 m depth), meanwhile some zones of weaker density in the distribution were observed between both, coast and offshore waters.

The oceanographic setting during the period of the surveys for the region was showed in figure 1 and table 2. Minimum, mean and maximum measured SST ranged from 12.1 to 15.3 ºC. The highest temperature values were observed in 1997 and 2011; meanwhile the lowest one was registered in 2002.

The largest area sampled was reached in 1997, covering a total of 20149 km^2 (Table 3), while the smallest one was 6793 km^2 in 1999. The spawning area was quite similar in 1997 and 2011 (12755 km^2 and 12400 km^2 respectively), smaller in 2002 and 2008 (9154 km^2 and 8167 km^2) and the lowest value was obtained in 1999 (5724 km2). The percentage of spawning area over the sampling area was all the years greater than 60 %, reaching the 80% in 1999, 2008 and 2011.

Table 3 shows the mortality values obtained as described above. Mortality values for the period between 2002 and 20011 are much higher than for the 1997 one. Mortality calculated for each one of the years surveyed (except 1999) shows negative and significantly different from zero values and was considered acceptable for egg production estimation. For 1999 survey a model without mortality was applied since an estimate for mortality led to non coherent (positive) mortality.

Final egg production model (Table 3 and Figure 5) includes individual egg production and mortality estimates by year. Daily egg production per m^2 (eggs/m^2/day) in 2011 (219) is the highest in the series, meanwhile the lowest (78.7) corresponds to 1999. Total egg production (eggs/day) estimated for years varies from 0.45x10^{12} (1999) to 2.72x10^{12} (2011). Egg production estimates (figure 5) in 2011, 2008, 2002 and 1997 were higher than in the 1999 as a result of high egg production density (eggs per day per square metre).

Adults

On the whole DEPM series, 22 fishing hauls which caught sardines were performed during the surveys using pelagic trawling (Figure 2). The fishing effort and its spatial distribution were made to guarantee good and homogeneous level of sampling for the survey area.

In total, almost 1759 sardines were sampled (Table 2) and more than 500 ovaries were collected, preserved and analysed histologically. On the whole, a total of 749 otoliths were removed for age determination in 1999, 2002, 2008 and 2011. A total of 71 hydrated females were caught for batch fecundity estimation, although ovaries from hydrated females caught in 1999 (12) and 2002 (2) were not preserved for histological analysis on the laboratory and not number of oocytes was obtained to estimate batch fecundity.

Length and age distribution of sardine is showed in figure 7. Sardine shows a bimodal length distribution in 1999 and 2008, with the first mode about 15 and 17 cm respectively and the second about 21 and 20 cm. In 1999 the size range is the wider for the whole historical series, with a minimum of size measured of 12.3 cm and a maximum of 26 cm. The age structure of the sampled population is different by year, and it must be noticed that the number of individuals, especially between 1 and 3 ages were really important in all years which otholits were collected.

Estimates of the mean female weight (W), batch fecundity (F), sex ratio (R), spawning frequency (S) and spawning stock biomass (SSB) with their CVs are given in Table 3 and the variability, both intra annual and from year to year for all adults parameter estimates by haul is showed in figure 8.

The minimum mean weights by haul were observed in 1999 and the maximum 1997. The linear regression model between gonad-free-weight and total weight fitted to non-hydrated females by year is given in Table 4. Mean female weight (W) was similar for 1999, 2002 and 2011(63.6, 62.9 and 61.3, respectively) and considerably higher in 1997 (74.5). Mean females weights in 2008 survey present the lowest value of the historical series (38.1). Concerning sex ratio estimates, mean values are quite homogeneous across the whole surveys.

The relationship between the Fobs and the female Wnov is shown in figure 7. Considering that few hydrated females (n=3) were collected in 2008 and no hydrated females were available in 1999 and 2002, the data from these three years were pooled with data from North Atlantic Spanish coast, for the modelling of batch fecundity. Mean batch fecundity estimate (F) was considerably lower (15849 number of oocytes, 286 oocytes/gr) in 2008 according to the mean female weight estimated. On the contrary the first two surveys (1997 and 1999) presented the highest estimates (32269, 433 oocytes/gr and 32704, 514 oocytes/gr) of the historical series, though similar to the one obtained for the 2011(30383, 495 oocytes/gr) survey. In particular, for 2002, although mean female weight was similar to the ones obtained during the 1999 and 2011 surveys, batch fecundity estimate was reduced to 24577 (390 oocytes/gr) when compared to the values obtained these years.

Bootstrapped estimate of spawning fraction for 1999 was 0.124. Mean Spawning fraction estimate for 2011 survey was among the lowest (0.066) of the time series. For the remaining surveyed years the values are generally quite high and homogeneous (between 0.124 and 0.137).

Mean adult parameters by haul showed a quite remarkable variability intra annually (female weight and batch fecundity in 1997 - 1999, and sex ratio in 1999 - 2002). Spawning fraction is the adult parameter subject to less fluctuations in terms of inter annual variability

<u>SSB estimate</u>

The whole survey series DEPM-based SSB estimate is showed in table 3. SSB in 2011 is the highest estimate of the time series (162930 tons), while 1999 is among the lowest of the time series (13200 tons). In 1997 and 2002 estimates are comparable (60332 and 60720 tons respectively) and in 2008 an increase in relation to the previous surveyed years was found (73942 tons).

Brief discussion

The lowest and highest SSB estimates found in 1999 and 2011 respectively are related to the egg production. Egg production estimate in the 1999 survey is the lowest of the time series, probably due to the egg survey period has not covered the amount of spawning peak activity. By the contrary the large egg production estimate in 2011 is sustained by a combination of high egg production density (in eggs per day per square meter) and large spawning area. Moreover, the contribution of the lowest spawning fraction value (0.066) estimated in 2011 on the equation applied to estimate SSB, has largely increased the SSB value.

The estimates presented from DEPM application in the inner of the Bay of Biscay, are a priori considered provisionally as several issues that arose from data analysis, namely the absence of data to model batch fecundity, the unreliable egg mortality estimated in 1999 or no data at all available to estimate spawning fraction in 1999, are planned for discussion at the WGACEGG. The way to obtain batch fecundity estimates for 1999, 2002 and 2008, modelling together with data from the North Atlantic Spanish coast, prevents to consider these preliminary results as definitely ones. To solve the unreliable egg mortality estimated in 1999 an aggregated model similar to that used by Bernal *et al*., 2011, could be tried. All these issues require further analysis in terms of implications for the best estimation procedures and reliability of the results.

Despite the preliminary nature of results, this WD presents the first essay of applying the DEPM method to estimate the spawning biomass in the inner of the Bay of Biscay since 1997 until 2011. Taking into account that only a part of the area has been surveyed for the estimations (the inner part of VIIIb until 45º N), the document could be considered as a contribution for the next ICES benchmark assessment for the sardine stock in VIIIabd and VII in order to discuss the use of the DEPM SSB estimates for assessment purposes in a modeling approach.

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Table 1. Surveying, processing and analysis methodology for eggs and adults.

DEPM Surveys	**IEO 1997-2011**
Survey area	VIIIb (until 45º N)
SURVEY EGGS	
Sampling grid	15 (transect) x 3(station) (1997-1999) 6 (transect) x 6(station) (2002) 8 (transect) x 3(station) (2005-2011)
PairofVET Eggs staged (n egg)	All (1 net)
Sampling maximum depth (m)	100
Temperature for egg ageing	10 m
Peak spawning hour	(PDF 21 ± 2 * 3)
Egg ageing	Bayesian (Bernal et al, 2008)
Egg production	GLM
CUFES, mesh 335	3 nm (sample unit)
CUFES Eggs counted	All
CUFES Eggs staged	No
Hydrographic sensor	CTD SBE 25 (1997-2011) CTD SBE 37 (2008-2011)
Flowmeter	Y
Clinometer	Y
Environmental data	Fluorescence (surface only), Temperature, Salinity
SURVEY ADULTS	
Biological sampling:	On fresh material, on board of the R/V
Sample size	60 indiv randomly (30 mature female); extra if needed and if hydrated found
Sampling for age	Otoliths from random males and females
Fixation	Buffered formaldehyde 4% (distilled water)
Histology:	
- Embedding material	Resin (from 2004) before Parafine
- Stain	Haematoxilin-Eosin
S estimation	Day 1 and Day 2 POFs
R estimation	The observed weight fraction of the females
F estimation	On hydrated females (without POFs)

Table 2. General sampling by years.

SURVEY EGGS	1997	1999	2002	2008	2011
R/V	Cornide de Saavedra				
Date	27/03-02/04	03/04-05/04	06/04-12/04	20/04-24/04	09/04-15/04
Transects	12	11	10	8	10
PairoVET stations	140	48	75	97	134
Positive stations	89 (63.6)	37 (77.1)	55 (73.3)	74 (76.3)	114 (85.1)
Tot. Eggs	1123	586	1090	1104	2764
Max eggs/m2	1669.6	1185.4	1220.1	2332.1	2321.7
Temp (ºC) min/mean/max	12.8/14.1/15.3	12.5/12.7/13.3	12.1/12.9/13.9	12.6/13.1/13.9	13/14/14.7
CUFES stations	-	-	130	95	137
Positive CUFES stat.	-	-	88(67.7)	84 (88.4)	124 (90.5)
Tot. Eggs CUFES	-	-	7108	13837	39798
Max eggs/m3	-	-	83.6	215.5	97.3
SURVEY ADULTS	**1997**	**1999**	**2002**	**2008**	**2011**
R/V	Thalassa	Thalassa	Thalassa	Thalassa/ Cornide de Saavedra	Cornide de Saavedra
Number positive hauls	4	6	4	5	3
Date	29/03-31/03	06/03-10/03	29/03-31/03	21/04-24/04	13/04-15/04
Time range	07:00-20:00				
Total sardine sampled	239	516	199	503	302
Total males	104	241	106	280	150
Total females (% Mature)	135 (100)	271 (98)	93 (100)	223 (100)	152 (100)
Length range (mm)	180-255	123-260	152-244	154-250	175-243
Weight range (g)	45-144	13-152	23-104	25-114	41-102
Oocyte stage ovaries	68	50	20	164	127
Hydrated females (Batch fecundity)	42	12		3	14
Females for spawning	68		20	161	124
Otoliths	NA	328	195	97	129
Ages Range		1-10	1-8	1-9	1-9

Table 3. Results for egg and adults by years.

Parameter	**Year**				
Eggs	**1997**	**1999**	**2002**	**2008**	**2011**
Survey area (Km^2)	20149	6793	11888	10187	14091
Positive area (Km^2) (%)	12755(63)	5724(84)	9154(77)	8167(80)	12400(88)
Z ($hour^{-1}$)(CV%)	-0.012(41)	-0.006(89)	-0.022(18)	-0.019(26)	-0.018(22)
Max age (hours)	66.8	81.6	81.6	78.6	68.8
Daily mortality rate (%)	25.3	13.7	41.7	37.3	35.6
P0 (eggs/m2/day)(CV%)	136.6(20)	78.7(13)	182.3(19)	171.4(23)	219.1(16)
P0 tot (eggs/day) ($x10^{12}$) (CV%)	1.74(20)	0.45(13)	1.67(18)	1.4(23)	2.72(16)
Adults					
Female Weight (g) (CV%)	74.5(11.8)	63.6(12.7)	62.9(5.6)	55.4(11.1)	61.3(9)
Batch Fecundity (CV%)	32269(17)	32704(45)	24577	15849(29)	30383(4)
Sex Ratio (CV%)	0.508(8.1)	0.535(10.7)	0.492(22.9)	0.483(8.9)	0.51(19.6)
Spawning Fraction (CV%)	0.131(9.7)	0.124(15.4)	0.143	0.137(24.4)	0.066(49.2)
Spawning Biomass (tons) (CV%)	**60332(31)**	**13200(52)**	**60720**	**73942(47)**	**162930(55)**

Table 4. Coefficients resulted from the linear regression model between gonad-free-weight and total weight fitted to non-hydrated females by year with their standard error and the P-Value

Year	Parameter	Estimate	Standard error	P-Value
2011	Intercept	1.3617	0.8846	0.127
	Slope	1.0488	0.0153	<2e-16***
2008	Intercept	-1.3285	0.4716	0.0054**
	Slope	1.0985	0.0084	<2e-16***
2002	Intercept	-9.3675	3.8776	0.0266*
	Slope	1.2250	0.0615	1.04e-13***
1999	Intercept	0.0391	8.2777	0.99653
	Slope	1.1052	0.1251	0.00306**
1997	Intercept	1.0237	0.8660	0.24
	Slope	1.0583	0.0122	<2e-16***

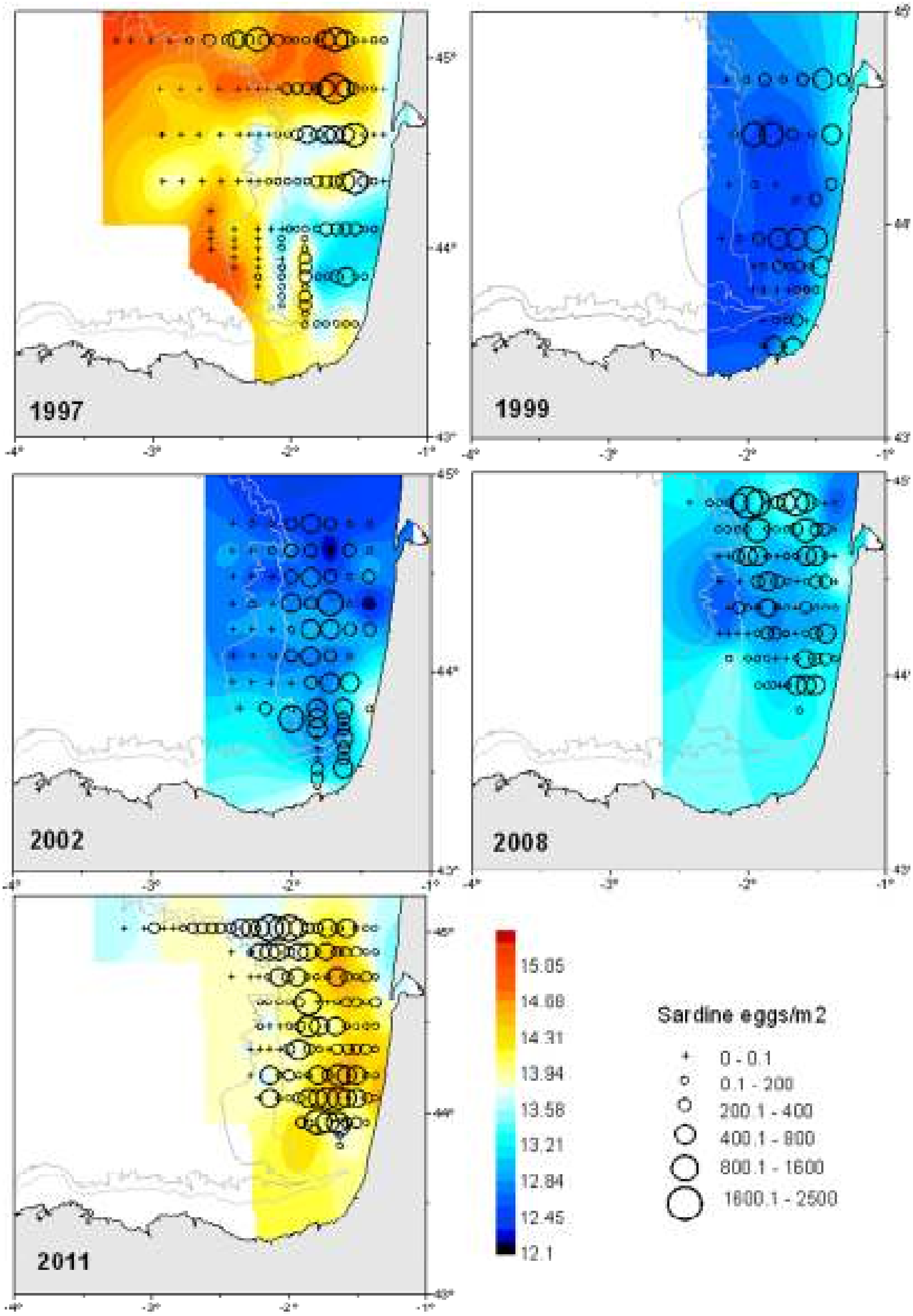

Figure 1. Sardine egg distribution (eggs/m2 from PairoVET sampler) and SST (ºC) by year in the inner of the Bay of Biscay.

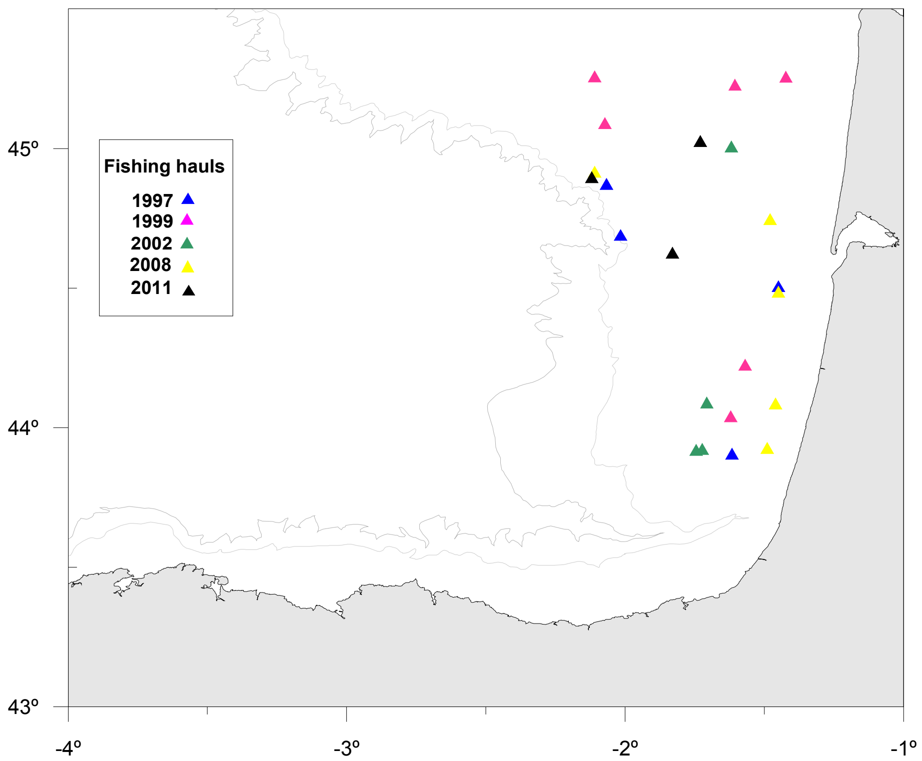

Figure 2. Spatial distribution of the positive fishing hauls by year.

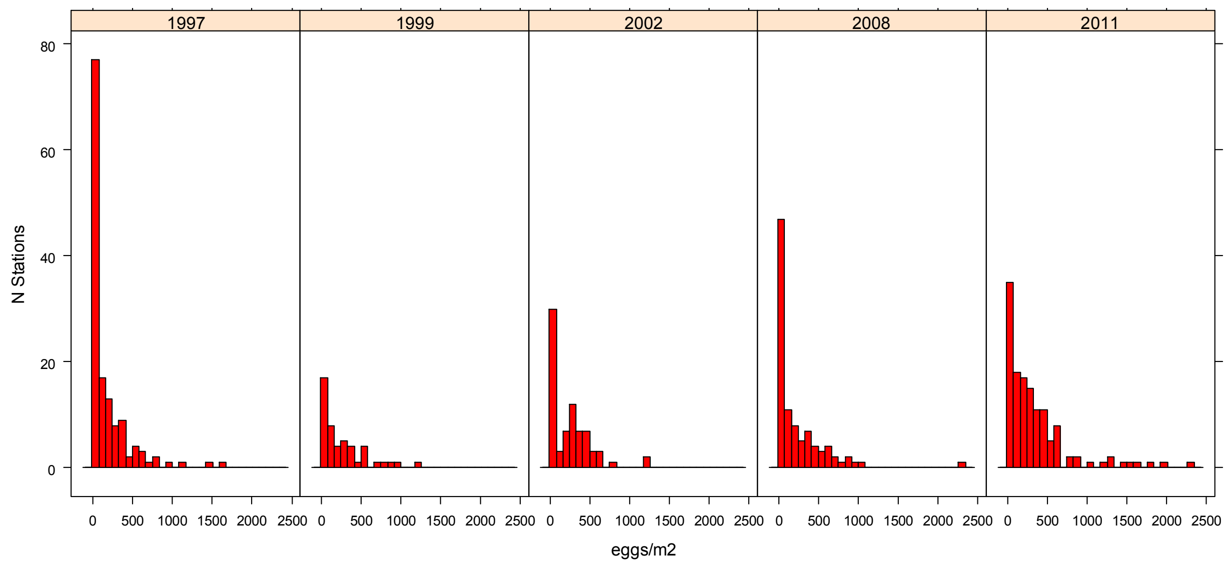

Figure 3. Number of stations and egg abundance (eggs/m^2) resulted from CalVET sampler by year.

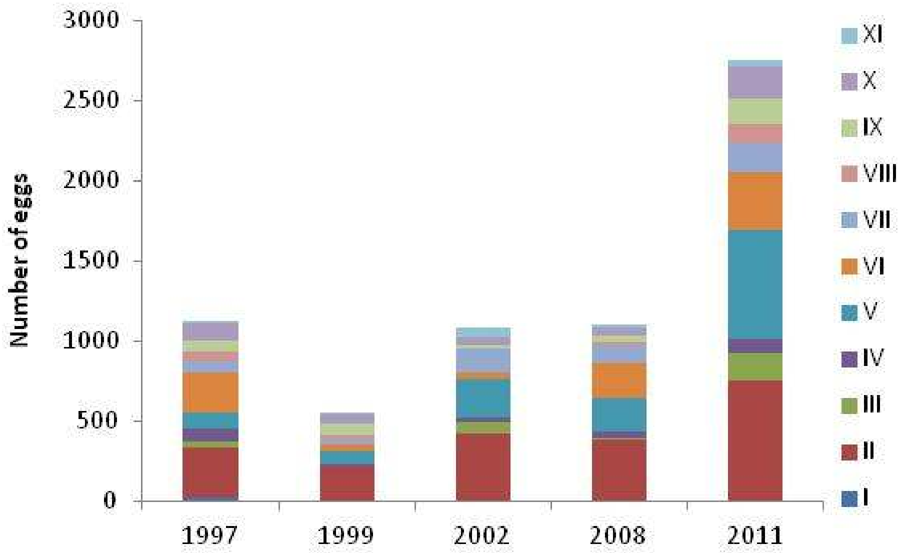

Figure 4. Number of sardine eggs, classified into different developmental stages from CalVET sampler and year.

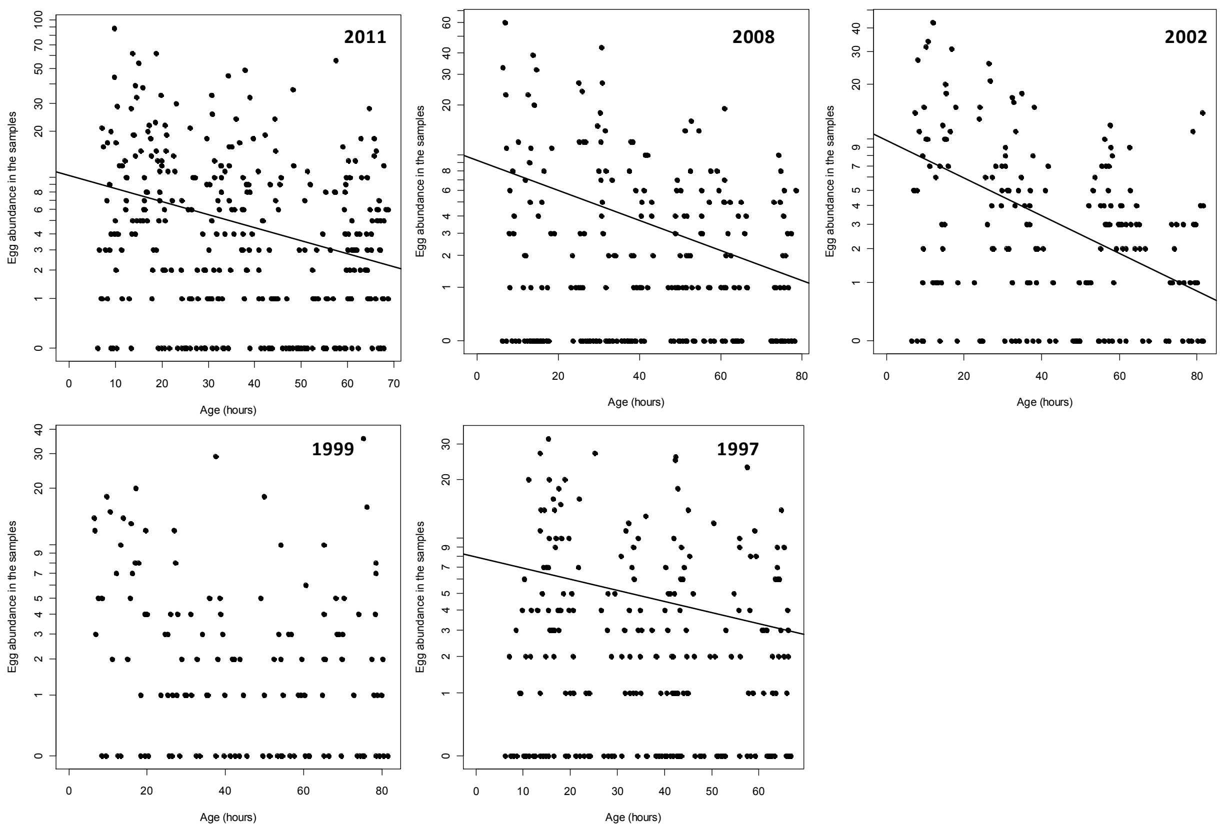

Figure 5. Abundance by age of eggs and its corresponding fitted mortality curve by year.

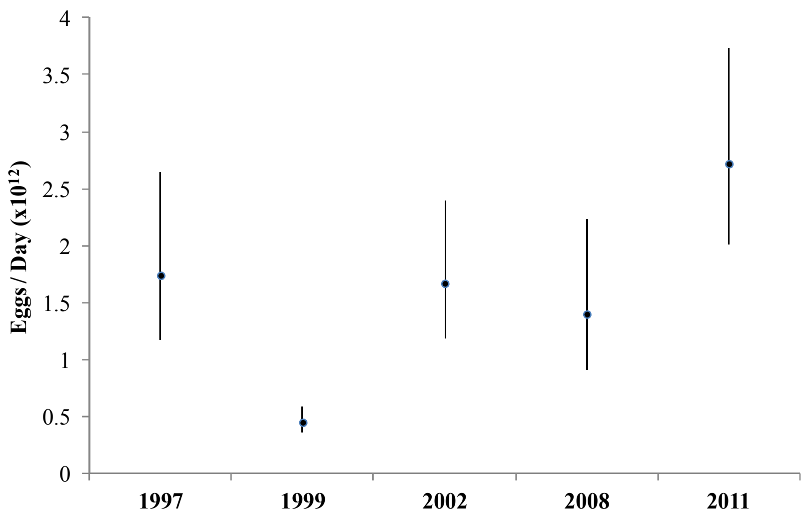

Figure 6. Time series of total egg production (eggs/day x 10^{12}) estimates in the inner of the Bay of Biscay. Vertical lines indicate confidence intervals.

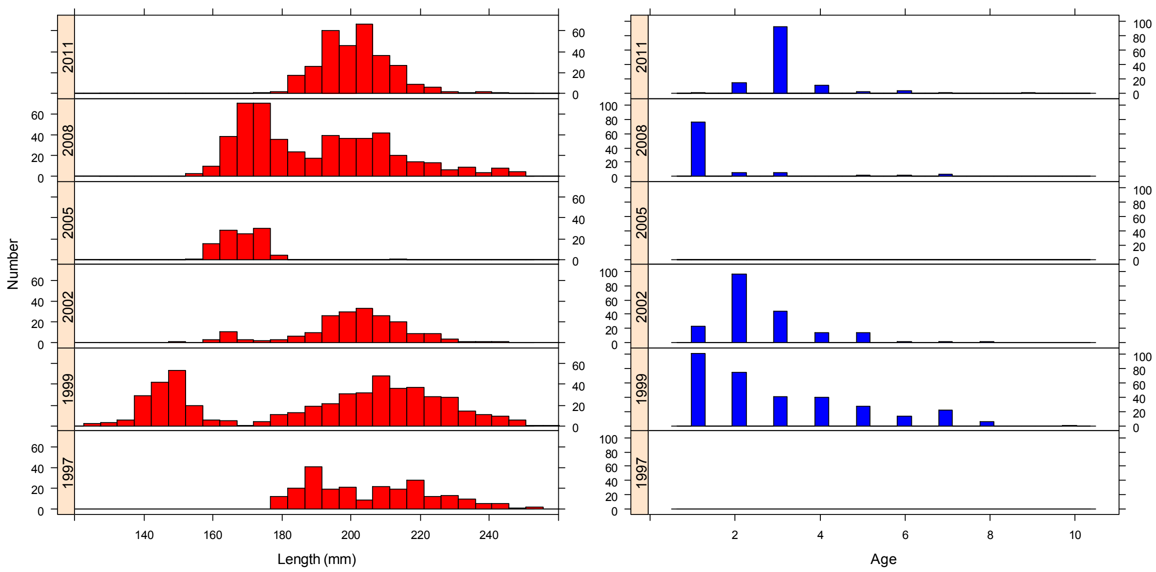

Figure 7. Length (mm) and age distribution of sardine by year. No otoliths for age reading were available in 1997.

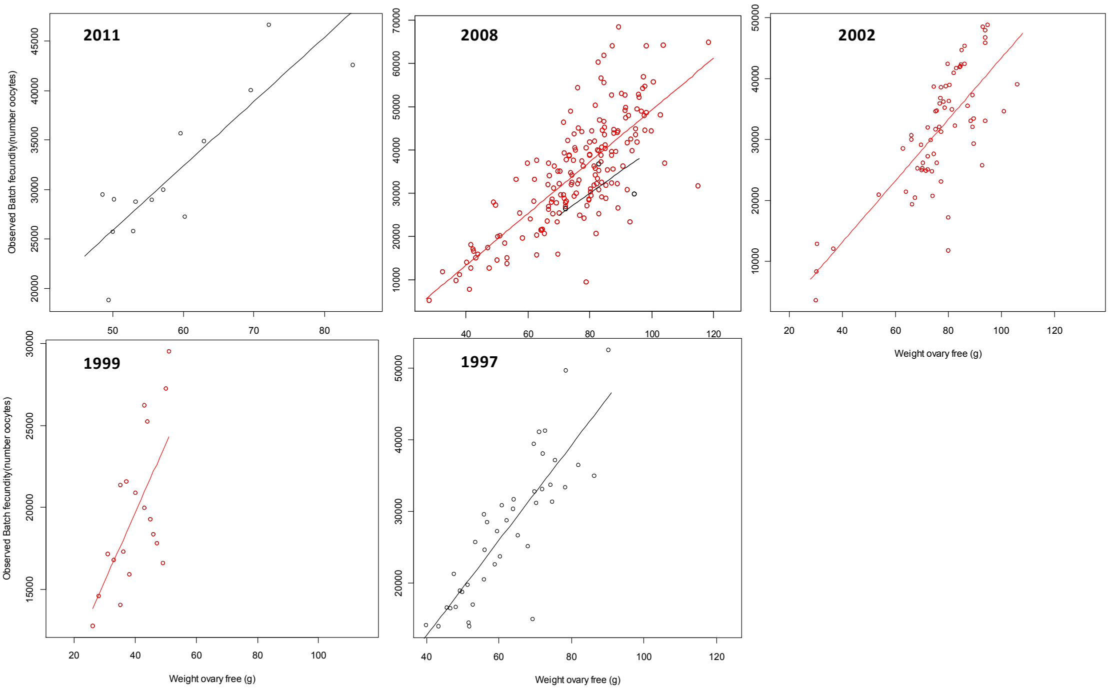

Figure 8. Observed batch fecundity vs gonad free weight of the hydrated females and regression line of the corresponding model per year. Red points correspond to data from the hydrated females collected in the North Spanish Atlantic coast surveys in 1999, 2002 and 2008.

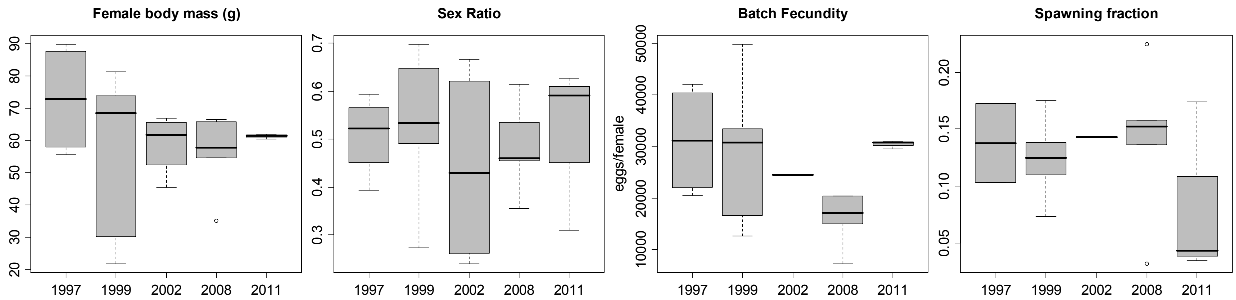

Figure 9. Box plots show interquartile ranges (boxes), median values (horizontal lines) and 90 % confidence intervals (whiskers) for adult parameters ((Females Weight, Sex Ratio, Batch Fecundity and Spawning Fraction) estimates by year. Distribution of spawning fraction in 1999 was obtained by a bootstrap approach.