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Sardine *(Sardina pilchardus)* spawning biomass estimation through the application of DEPM.

SAREVA 0321

ICES subdivision 9a North and division 8c

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1. Introduction

The Daily Egg Production Method (DEPM) for estimation of sardine (*Sardina pilchardus)* spawning biomass within the Atlanto-Iberian stock area (pil.27.8c9a) have been conducted every three years by Portugal (Instituto Portugués do Mar e da Atmosfera, IPMA; PT-DEMP-PIL) and Spain (Instituto Español de Oceanografía, IEO; SAREVA) since 1999 in an internationally coordinated survey, planned under the framework of ICES WGACEGGS. For this application based on standardized procedures and analytical methodologies (cf. ICES, 2004, 2005, 2006, 2017, Massé *et al.* 2018), the entire stock area (ICES divisions 8.c and 9.a -Cantabrian Sea and Atlantic Iberian waters) is surveyed from Cape Trafalgar in the Gulf of Cadiz to the north of the French/Spanish border in the Bay of Biscay. Plankton samples, along a grid of parallel transects perpendicular to the coast, are obtained for spawning area delimitation and daily egg production density estimation; concurrently, fishing hauls are undertaken for estimation of adult parameters (sex ratio, female weight, batch fecundity and spawning fraction) within the mature component of the population. The total spawning biomass from the two DEPM surveys is used in the assessment such as fishery independent index for the sardine stock pil.27.8c9a (ICES divisions 8.c and 9.a -Cantabrian Sea and Atlantic Iberian waters).

In 2020 both surveys were planned and coordinated under the framework of the ICES WGACEGG. The Portuguese survey was successfully conducted, however, the Spanish survey; SAREVA 0320, was cancelled due to the COVID-19 health crisis and the subsequent ‘state of alarm’ lockdown in Spain.

Due to the lack of data for the northern stratum (IEO sampling area; 9a North + 8c) the spawning stock biomass (SSB) estimate for the whole stock was achieved raising the SSB calculated to the southern (9a South) and western (9a West) areas by an estimated factor (1.3), to account for the north Spain area. The raising index was obtained by linear regression analysis of the Portuguese surveys SSB vs the whole stock SSB (Diaz *et al.* 2020).

However, to avoid a too large information gap in the historical series of sardine DEPM data from the northern Atlantic Iberian waters and the Cantabrian Sea (ICES divisions 9aN and 8c), the IEO decided to carried out the SAREVA 0321 survey to estimate the sardine spawning biomass through the area.

This working document provides a brief description of the SAREVA 0321 survey, laboratory analysis and estimation procedures used to obtain the spawning stock biomass estimate for the 2021 DEPM application to the northern and north-western waters of the Iberian Peninsula (Cantabrian Sea and Atlantic Iberian waters) sardine stock. The laboratory tasks for processing adult samples are still underway, and therefore estimates presented for the batch fecundity and spawning fraction are preliminary at this meeting.

2. Material and Methods

2.1 Surveying

The sardine DEPM survey (SAREVA0321) was undertaken using two vessels; RV Vizconde de Eza (from 29th March to 22th April), for ichthyoplankton and hydrographic sampling mainly and RV Miguel Oliver for adult samples which were collected during the acoustics survey (PELACUS 0321) from 27th March to 18th April. Both surveys were coordinated and carried out concurrently.

The survey was carried out from West to East, starting in the radial 1- station 1, located close to the Spanish-Portuguese northern border (Figure 1) and covered northern and northwestern waters of the Iberian Peninsula (ICES divisions 8c and 9.a North, Cantabrian Sea and Atlantic Iberian waters).

For plankton sampling, vertical plankton hauls were carried out following a pre-defined grid of sampling stations along 54 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 included 2 nets (Ø 25cm) with 150 µm mesh size; sampling covered the water column from bottom, or 100 m depth (beyond the 100 isobath), to the surface. PairoVET samples are taken every 3 nm in the inner shelf (up to 200 m depth) and every 3 or 6 nm beyond the inner shelf, depending on the egg results of the CUFES sampler. 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 is reached when two consecutive CUFES samples are negative beyond the 200 m depth.

All plankton samples were preserved in a solution of buffered 4% formaldehyd in distilled water and the 2 samples from each net stored in separate containers. Onboard procedures include the analyses of the CUFES and PairoVET samples for obtaining preliminary data of sardine, anchovy, mackerel and horse mackerel eggs abundance and distribution.

The water column structure (temperature, salinity and fluorescence) is surveyed by CTD cast (using a Sea Bird-25) in each PairoVET station. The surface water layer (5 m depth) was sampled continuously with a Sea Bird-45 associated to the CUFES water pump recording surface temperature and salinity. Other environmental variables such as winds and currents were recorded and used for general environmental characterization.

Fishing hauls for estimation of adult parameters were undertaken during PELACUS acoustic survey which was carried out concurrently with SAREVA. Fishing hauls were conducted by pelagic trawling following sardine schools detection by the echo-sounder. The number of samples and its spatial distribution was scheduled to ensure a good and homogeneous coverage of the survey area.

Onboard the RV, and for each haul with sardine catches, a minimum of 60 sardines (males and females) were randomly selected and biologically sampled. For reproductive parameters, a minimum of 30 females per haul was required, thus, in some occasions; the random sampling was complemented with additional directed sampling in order to get females enough for histological analysis, and/or fecundity estimations. Individual biological information (length, total weight, sex, maturity state and gonad weight) was recorded for all fish, the ovaries were preserved for histology (with a 4% buffered formaldehyde) and the otoliths removed for age determination. The biological sampling and ovaries fixation were always carried out in fresh material.

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

2.2 Laboratorial analyses

The samples from PairoVET and CUFES are revised at the laboratoriy to assess the quality of the identification and quantification of eggs made onboard the RV. The sardine eggs from the vertical hauls (PariroVET-1 net) were all counted and staged according to the 11 stages of development classification (adapted from Gamulin and Hure, 1955). The eggs from the CUFES sampling were all counted and a sub-sample of a minimum of 100 eggs was staged per sample in 3 phases (No Embryo- NE: Includes stages I, II and III, Early Embryo- EE: Includes stages IV, V and VI, Late Embryo-LE: Includes stages VII to XI).

For the first time, anchovy eggs (all or a sub-sample of a minimum of 100 eggs) were staged following an adapted version of the 11-stage development scale of Moser and Alshtrom (1985).

At the laboratory, the fixed ovaries were weighted and corrected to fresh weights by a conversion factor (based on the fresh and fixed ovary weight relationship). These ovaries were then processed for histology: they were embedded in resin, the histological sections were stained with haematoxylin and eosin, and the slides examined and scored for their maturity state, POF presence was checked and they were aged (Hunter and Macewicz 1985, Pérez et al. 1992a, Ganias et al. 2007). Batch fecundity (number of hydrated oocytes in the gonad) was estimated in hydrated ovaries without POFs (Pérez et al. 1992b) by using the gravimetric method, on 1-3 whole mount sub-samples per ovary of 50-150 mg each (Hunter et al. 1985).

2.3 Data analysis

Egg data

All calculations for area delimitation, egg ageing and model fitting for egg production (P_0) estimation are 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.

Area delimitation (both surveyed and spawning) is attained through an automated procedure included in the spatstat library and by each sampling station limits are set (25-175 km^2) in order to prevent very extreme values. The range 25-175 was selected to be a mean interval suitable according to the distance between transect and stations (fixed to be 8 nm between transects and 3 between stations along the transects).

The strata defined, according to biological/ecological (geographical) reasons (Bernal *et al*., 2007) used in the analyses for mortality and egg production estimation are: (i) Stratum 1 - South (9a S), encompassing from the strait of Gibraltar to Cape St. Vicente; Stratum (ii) 2 - West (9a W), from Cape St. Vicente to the border between northern Portugal and Spain and (iii) Stratum 3 - North (9a N & 8c), between the Spanish-Portuguese northern border and the Spanish-French Atlantic limit.

Since the revision adopted by WGACEGG in 2016 the mortality estimation (Z) is obtained following the procedure described by Bernal et al (2011a). An updated version of the model published, including data from the whole historic series, is now being used to insure coherent mortality estimates for each of the three strata. After, daily egg production (P0) is calculated per statrum using the average mortality estimates previously obtained. The exponential model: $E[P] = P0\, e^{-Z\, age}$ is fitted by a Generalized Linear Model (GLM), assuming a negative binomial distribution. Finally, the total daily egg production (Ptot) is calculated by multiplying the daily egg production by the positive area.

Egg ageing is achieved using the egg development multinomial model presented in Bernal *et al*. (2008) and the Bayesian approach described in Ibaibarriaga *et al.* (2007). The model of egg development with temperature in use was derived from the incubation experiment data available within the *sardata* R library. A lognormal distribution (offset= 12 h, equivalent mean= 21 h, equivalent s.d.= 4 h) is assumed for the daily spawning cycle Bernal *et al* (2011a); peak spawning time is used to define the daily cohorts, their abundance and mean age for all stations. The spawning curve is considered in order to be conservative and allow an extended (reallistic) spawning period and therefore few eggs are excluded from the analyses (how.complete=0.95).

Then the mortality curve is fitted to the estimates of abundance-by-cohort. The expected number of eggs for a cohort with a given age resulting from egg production rate and mortality are described by the general model:

$E[Na] = g^{-1}(\text{offset}(\log(Efarea)) + \log(D0) - ma)$ (Eq 1)

E [Na] = expected number of eggs in a cohort of mean age a
D0 = the rate of egg prouction
m = the mortality rate
g^{1} = the inverse of the link function that relates the linear predictor and the response

The general model (eq 1) is then expanded to allow both egg production and mortality to be a function of the spatial and temporal strata and also temperature, and the respective first-order interactions. Terms in which age is involved indicate mortality terms, and the rest of the terms affect egg production. The model selection is carried out using backward stepwise approach (at each step, the term with least significance (<5%) is dropped, and the procedure repeated until dropping terms leads to no improvement). Model fiitting is accomplished by an iterative procedure. During the model selection procedure comparisons using the Akaike information criterion (AIC) are also performed. To avoid bias in the mortality model caused by the data extremes, lower and upper limits are set for the tails of the mortality curve. The lower age cutting excludes the first cohort for stations in which the sampling time is included within the daily spawning period. At the other end (upper tail) the age limit is considered by stratum, and eggs excluded when 5% of the eggs would already have hatched considering the temperature of the 95% quantile (per stratum).

Model development results in a model in which mortality is estimated by a general term and an interaction with temperature:

glm.nb(formula = cohort ~ offset(log(Efarea)) - 1 + Sstrata + Tstrata + Temp + Sstrata:Tstrata + Sstrata:Temp + Tstrata:Temp + age + Temp:age) (Eq 2)

The above model (Eq 2) was developed by Bernal et al (2011a), considering surveys until 2008, and it is now routinely updated (and reassessed) to include the data of each new survey (five more years: 2011, 2014, 2017, 2020 and 2021 have been added). Model running allows the estimation of coherent mortality values for each stratum for all surveys of the series.

To obtain P0, an egg production model that can accommodate mortality estimates external to the estimation procedure is now required. This model is developed in Bernal et al. (2011b) as follows:

glm.nb(formula = cohort ~ offset(log(Efarea) - death * age) - 1 + Sstrata, data, weights = Rel.area) (eq 3)

The general model is expanded to include weights to account for differences in station representative area. Model running allows the estimation of P0 for each stratum for all surveys of the series. Each update of the model with new data will also update (slightly) the series P0 estimates. Such small changes in the series values should only be considered during the course of a benchmark.

Finally, total egg production (P_{tot}) is calculated, per stratum for each survey, by multiplying the daily egg production (P0) estimates by the corresponding spawning area (A+) values.

$$P_{tot} = P_0 \cdot A+$$

Fish data

Adult parameters (mean female weight, sex ratio, batch fecundity and spawning fraction) are estimated independently for each fishing haul, using only the mature fraction of the population (macroscopic maturity stages 2-6) and based on the biological data collected from PELACUS survey. 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 based on the 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 is 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 is estimated by modelling the individual batch fecundity observed (Fobs) and gonad-free weight (Wnov) by a GLM (with an identity link function and a negative binomial distribution).

Histological process is still underway, thus, the POFs ageing is not available and therefore estimates presented for the batch fecundity and spawning fraction are preliminary at this meeting.

The preliminary daily spawning fraction of females (S) was determined, for each haul, as the ratio between hydrated females (macroscopically determined) and the total number of mature females from random samples. No histological correction (presence of recent POFs) was taking into account to estimate the preliminary spawning fraction.

$$S=\frac{\sum_0^i H}{\sum_0^i Mat}$$

Where H is the number of hydrated females in the haul (i), and Mat the number of mature females in the haul (i).

The mean and variance of the adult parameters for all the samples collected is then obtained using the methodology from Picquelle and Stauffer 1985 (weighed means and variances). All estimations and statistical analysis are performed using the R software.

Those hauls containing less than 30 fish sampled were excluded from the mean and variance calculations.

Spawning stock biomass (SSB) mean estimates are calculated based on equation:

$$SSB = Ptot * W/(R * F * S)$$

For the calculation of the coefficient of variation, variance is estimated using the Delta method (Seber 1982, as shown by Parker 1980 for the DEPM):

$$CV(B)^2 = CV(Ptot)^2 + CV(W)^2 + CV(R)^2 + CV(F)^2 + CV(S)^2$$

3. Results

3.1 Eggs

The Ichthyoplankton surveying undertaken in SAREVA 0321 survey obtained a total of 392 PairoVET hauls and 356 CUFES samples (Table 2). The percentage of stations with sardine eggs was 42 % and 57% for the vertical tows (PairoVET) and horizontal sampling (CUFES) respectively. Considering only one of the PairoVET nets, 1790 sardine eggs were counted in total and 9965 sardine eggs were captured with the surface samples (CUFES) in the area studied.

The number of eggs collected in 2021 for the vertical tows represented a considerable increase respect to those collected in 2014 and 2017 surveys (with 313 and 343 eggs respectively), almost quintupled the figure obtained in the 2017 survey and was similar to the observations from 2011. The same trend was observed with eggs collected with CUFES. 99% of sardine eggs were classified into 11 stages according to the degree of embryonic development (Figure 2). The most abundant stages were II, VII and VI (28, 13 and 11%, respectively)

Sardine egg distribution, obtained from the PairoVET and CUFES systems is presented in Figure 3. The egg distribution pattern derived from the observations from the two samplers is quite similar. Almost the entire shelf (from coast to slope) was occupied by sardine eggs. Some zones of weaker density or gaps in the distribution were nevertheless observed in the eastern part of the Cantabrian Sea. Spots of higher egg density were observed mainly in the Galician shelf. The highest egg abundance per haul was 2876 egg/m^2 reached in the South Galicia (Rias Baixas).

The survey covered a total area of 40532 km2 of which 17034 km^2 (42 % of the total surveyed area) were considered the spawning area. The total area occupied by eggs during the 2021 survey was more than double the observed in 2017 (7627 km2) and represented the second highest value of the historical survey series (Figure 4).

Since the 2016 WGACEGG revision, the procedure adopted to estimate mortality and egg production follows the approach published by Bernal et al 2011a, 2011b and described in the methods section. The results from the model update are shown in table 3 and the parameters estimated for the 2021 survey appear in table 4. The mortality estimated (-0.015 $hours^{-1}$, CV% 11.3) obtained considering survey temperatures and the data from the whole historic series, was within the range observed (-0.013 to -0.023 $hours^{-1}$) for the DEPM series in the northern and northwestern waters of the Iberian Peninsula and similar to the value obtained in 2017. Abundance by age of eggs in the surveyed area and its corresponding fitted mortality curve is shown in Figure 5.

The Daily Egg Production estimated during the survey was 101.9 (eggs/m2/day). The result represented an increase nearly 100% above respect to the values obtained in 2014 and 2017.

Total egg production (eggs/day) estimated for the surveyed area was 1.74 $x10^{12}$ which represented an increase considerably higher (330% above) in 2020 than in 2017 (0.4 $x10^{12}$) (Figure 6). In fact, the increase in total egg production was the highest registered for the past three DEPM surveys (the highest since 2011).

Total egg production estimate was the highest since 2011 as a result of high egg production density (eggs/m^2/day) and a more widespread distribution of eggs than in the previous three years of the series which have resulted on a large increase of the spawning area.

A summary of the general egg sampling for anchovy, mackerel and horse mackerel derived from PairoVET and CUFES is showed in table 5. Figure 7 shows egg abundance and distribution of these species.

A total of 1506 anchovy eggs were collected and 180 of the 392 stations carried out were positive for anchovy eggs, representing the 46% of positive stations. Two areas with anchovy eggs were well defined in the Galician coast (West area) and in the central and eastern part of Cantabrian coast. The eggs were absent between Gijón (Asturias) and the westernmost part of the Cantabrian Sea. The highest densities (more than 800 eggs/m2) were observed in Galicia (around Cape Finisterre).

Mackerel eggs were the more abundant and widely distributed along the surveyed area, with 164671 counted in 300 positive stations (76.5%), an average density of 865 eggs/m2 and a maximum density of 18614 eggs/m2. The highest abundances were found in the centre of the Cantabrian and northwest of Galician coast.

A total 187 of the 392 stations carried out were positive for horse mackerel eggs (48%). The number of eggs quantified was 980, with an average density of 108 eggs/m^2 and a maximum density estimated of 678 eggs/m2. Horse mackerel eggs were found widespread distributed although eggs were scarce on the eastern area of the Cantabrian Sea.

3.2 Temperature and salinity distributions

Distributions of sea temperature and salinity observed during the 2021 DEPM survey are shown in figure 8.

The sea surface temperatures registered varies in range 11.7 ºC to 14ºC as usually is observed during these early spring surveys and quite similar to the observations from the more recent surveys. The warmer waters were located in 9aN (Galician coast) and at the inner part of the Bay of Biscay, whereas the colder waters were associated to the central part of the Cantabrian Sea.

The salinity plumes observed in the Galician Rias in previous surveys were apparent during the 2021 survey. Itwas observed a trend in salinity from the western part, where the saltiest waters were registered, towards the eastern part (inner part of the Bay of Biscay), where the waters are in general less saltier. Influence of fresh waters from rivers could be significant.

3.3 Adults

In total, 25 fishing hauls positive for sardine were performed during the PELACUS survey (Figure 9). Fishing hauls were well distributed along the whole surveyed area and a total of 1706 sardines were sampled (Table 2), 625 ovaries were collected and fixed in 4% buffered formaldehyde for histological analysis and 1104 otoliths were removed for age determination. For batch fecundity estimation, 137 hydrated females were caught.

The length frequency distribution of the random and mature fish sampled ranged between 93 to 250 mm (Figure 10), with a mode around 170 and 180 mm. Individuals off the Northern Spanish coast were mostly aged 2 years old (Figure 11, about half of the fish for which otoliths were sampled).

Mean female weight (W) and sex ratio (R) were recorded in the total area (25 hauls) and ranged between 24.7-88 g and 0.23-0.92 respectively. Therefore, preliminary spawning fraction (S) was estimated based on the 11 hauls where hydrated females were found.

The linear regression used to estimate Wnov was Wt = 1.074 * Wnov - 0.582 (R^2 = 0.996, Table 5 and figure 12). The GLM model of Fobs modelled based on this Wnov is shown in Figure 13.

Minimum mean female weights by haul were observed in the eastern part of the Cantabrian Sea (24.7 to 33.8 g) and the maximum in Galician coast (66-88 g). Mean female weight (W) was 56.08 g (CV 7%) in the whole surveyed area, slightly higher than mean weight recorded in 2017 (51 g) but significantly lower than those values estimated between 1997 and 2011 (Figure 14).

Concerning the sex ratio, it was estimated in 0.492 (CV 8%) for 2021, almost identical to the one obtained in 2017 (0.505).

Mean batch fecundity (F) estimates obtained for the area 9a North + 8c in 2021 (21 456 eggs spawned per mature females per batch) was slightly higher than the value obtained in 2017 (20 698), nevertheless both values (mean female weight and mean batch fecundity) are among the lowest values of the historical series and significantly lower than those observed in the DEPM surveys delivered between 1997 and 2011 (Table 4 and Figure 14). In any case, F estimations are not definitive as values from hydrated females with POFs must be removed from the analysis.

Preliminary S (Figure 14) for the Northern Spanish coast (9a N + 8c) was 0.111, similar to those obtained during the 2017 survey (0.115). In any case, as preliminary S has been estimated based on macroscopically hydrated females, present results could over or underestimate S values. Thus, results have to be interpreted with caution until final estimates based on histological analysis be available.

SSB estimate

In the present document preliminary estimate of SSB in 2021 was calculated for the area 9a North + 8c at 84 067 tons (CV 17.2 %). The value highly increased for this area in comparison to the 2017 survey (16 129 tons, around 420% of increase), being the fourth highest value of the time series (Table 4 and Figure 15), but still within the historical range.

4. Brief discussion

In 2020 the SAREVA 0320 DEPM survey was cancelled because of the COVID-19 pandemia. In that year and for assessment purposes, the spawning stock biomass (SSB) was estimated for the whole stock (Cantabrian Sea and Atlantic Iberian waters, ICES divisions 8.c and 9.a) by raising the SSB calculated to the southern and western areas by an estimated factor (1.3), to account for the north Spain area. The raising index was obtained by linear regression analysis of the Portuguese surveys SSB vs the whole stock SSB along the series; details of these analyses are presented in Diaz et al. 2020. However, to avoid a large gap of information on sardine DEPM data, the IEO carried out the SAREVA survey in the present year (SAREVA-0321).

Despite DEPM-based SSB estimates for the northern area in 2021 are still preliminary, obtained values are within the range of historical values and follow similar trend to those observed in the acoustic and 2020 DEPM Portuguese surveys, thus, the perception of the stock provided by the DEPM in the area surveyed by SAREVA 0321 (9a North + 8c) is not expected to change greatly once definitive data be available.

The large total egg production estimated in the northern area in SAREVA 0321 is sustained by a high egg production density (in eggs per day per square meter) and a large spawning area.

As mentioned before, the estimate of egg production is in line with the estimates obtained from the DEPM carried out in 2020 in areas off the Portugal. However, spawning areas estimated in 9a South and 9a West did not increased in 2020 respect to 2017 as much as in the NW and N Spanish coast.

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Table 1. Surveying, processing and analyses for eggs and adults

Institute	**Spain (IEO-CSIC)**
Survey area	NW & N Spain (ICES 9a N + 8c)
SURVEY EGGS	**SAREVA 0321**
Sampling grid	8 (transect) x 3 (station)
PairofVET Eggs staged (Gamulin and Hure, 1955)	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)
Strata	No strata/Stratum (South-9aS,West-9aW,North-9aN+8c)
Egg production	GLM, negative binomial, log link
Egg mortality	External model using whole series and temperature. Update of Bernal et al 2011b model
CUFES, mesh 335	3 nm (sample unit)
CUFES Eggs counted	All
CUFES Eggs staged	Subsampled of a minimun of 100
Hydrographic sensor	CTD SBE 25 & TSG SBE 45
Environmental data	Fluorescence (surface only), Temperature, Salinity
SURVEY ADULTS	**PELACUS 0321**
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	4% Buffered formaldehyde
Preservation	4% Buffered formaldehyde
Histology:	
- Embedding material	Resin
- Stain	Haematoxilin-Eosin
Mean female weight (W)	Individual total weight of hydrated females corrected by a linear regression between total weight of non-hydrated females and their corresponding gonad-free weight
Spawning fraction (S) : preliminary estimation	Quotient between the total number of random hydrated females (macroscopically classified) and the total random mature females in the haul.
Sex ratio (R)	The observed weight fraction of the females
Batch fecundity (F): preliminary estimation	On hydrated females (without checking histologically POFs absence), according to Pérez et al. 1992b

Table 2. General Sampling for DEPM 2021, SAREVA 0321

Institute	**IEO-CSIC**
Survey area	NW & N Spain (**ICES 9a N + 8c)**
SURVEY EGGS	**SAREVA 0321**
R/V	Vizconde de Eza
Date	29/03-22/04
Transects	54
PairoVET stations	392
Positive stations	163
Tot. Eggs	1790
Max eggs/m^2	2875.9
Temp (ºC) min/mean/max	11.73/13.03/14.05
Max age	76.9
CUFES St.	356
Positive CUFES St.	203
Tot. Eggs CUFES	9965
Max eggs/m3	156.6
Hydrographic stations	378
SURVEY ADULTS	**PELACUS 0321**
R/V	Miguel Oliver
Date	27/03-17/04
Number Hauls R/V	
Number (+) trawls	25
Depth range (m)	42.5-139.4
Time range (hh:mm)	6:27-16:16 GMT
Total sardine sampled	1706
Length range (mm)	93-250
Weigth range (g)	5.4-126
Females for histology	625
Hydrated females	137
Otoliths	1104

Table 3. Fitted parameters of the final mortality model updated with 2011, 2014, 2017, 2020 and 2021 data.

2021	Estimate	Std. Error	z value	Pr(>|z|)	
Sstrata1	0.651	0.984	0.662	0.5078	
Sstrata2	6.834	0.780	8.759	< 2e-16	***
Sstrata3	-1.801	0.755	-2.386	0.017	*
Tstrata1	5.184	0.885	5.860	4.64E-09	***
Temp	0.508	0.055	9.265	< 2e-16	***
age	0.054	0.013	4.036	5.44E-05	***
Sstrata2:Tstrata1	-0.322	0.154	-2.088	0.0368	*
Sstrata1:Temp	-0.169	0.070	-2.416	0.0157	*
Sstrata2:Temp	-0.594	0.058	-10.207	< 2e-16	***
Tstrata1:Temp	-0.393	0.063	-6.188	6.11E-10	***
Temp:age	-0.005	0.001	-5.624	1.86E-08	***

The z-value indicates the value of the z-statistics used to test the significance, and Pr(>|z|) the propability of the null hypothesis (H0: parameter does not differ from zero). Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 4. DEPM parameters derived from 2021 SAREVA 0321 survey with their CV (%) in brackets in ICES 9a North and 8c areas. Surveyed and positive area (km^2), Mortality Z (hour^{-1}), Daily egg production P0 (eggs/m^2/day), Total egg production P0 tot (eggs/day) (x10^{12}), Females mean weight (g), Batch fecundity (number of eggs spawned per mature females per batch), Sex ratio (fraction of population that are mature females by weight), Spawning fraction (fraction of mature females spawning).

Parameters	**DEPM 9a N + 8c**
Eggs	
Survey area (Km^2)	40532
Spawning area (Km^2)	17034
Z (hour^{-1})(CV%)	-0.015 (11.3)
P0 (eggs/m^2/day)(CV%)	101.9 (10)
P0 tot (eggs/day) (x10^{12}) (CV%)	1.74 (10)
Adults	
Female Weight (g) (CV %)	56.85 (7)
Batch Fecundity (CV %)	21546 (8.8)
Sex Ratio (CV %)	0.492 (8.2)
Spawning Fraction (CV %)	0.111()
Spawning Biomass (tons) (CV%)	84067 (17.2)

Table 5. SAREVA 0321. Anchovy, Mackerel and Horse mackerel eggs (number and density) by PairoVET and CUFES.

	Anchovy	**Mackerel**	**Horse mackerel**
PairoVET			
Nb. St.	392	392	392
Positive st.	180	300	187
% Positive	45.9	76.5	47.7
Eggs Nb.	1506	16467	980
Max. (eggs/m^2)	2146.2	18614	678.1
Mean (eggs/m^2)	172.6	1130.5	107.7
CUFES			
Nb. St.	356	356	356
Positive st.	229	293	235
% Positive	64.3	82.3	66
Eggs Nb.	20315	70578	4290
Max. (eggs/m^3)	42.8	151.8	14.9
Mean (eggs/m^3)	7.1	16.1	1.5

Table 6. Coefficients resulted from the linear regression model between gonad-free-weight and total weight fitted to non-hydrated females with their standard error and the P-Value.

Parameter	**Estimate**	**Standard error**	**Pr(>|t|)**
Intercept	-0.58215	0.15395	0.000174 ***
Slope	1.07440	0.00278	<2e-16***

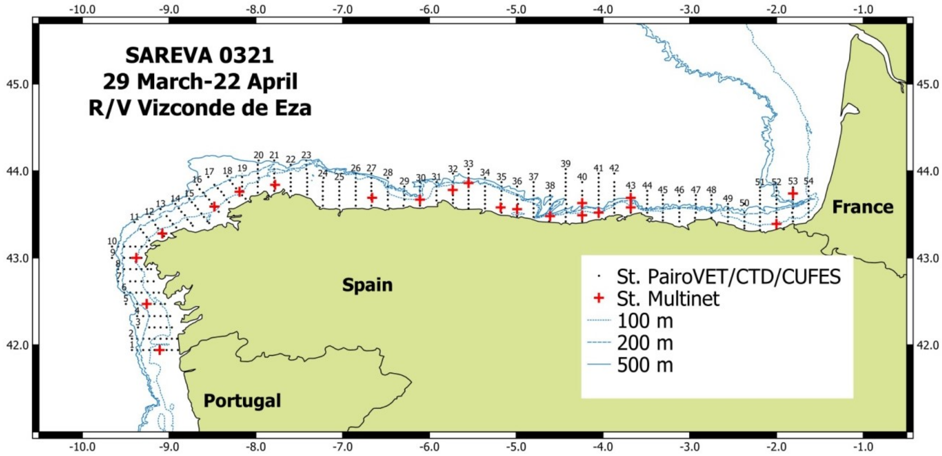


Figure 1. SAREVA 0321. PairoVET, CTD and Multinet stations.

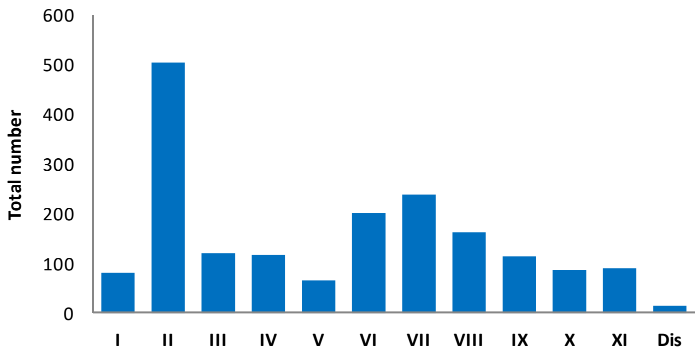


Figure 2. Number of sardine eggs (total eggs) counted from PairoVET sampler by development stages.

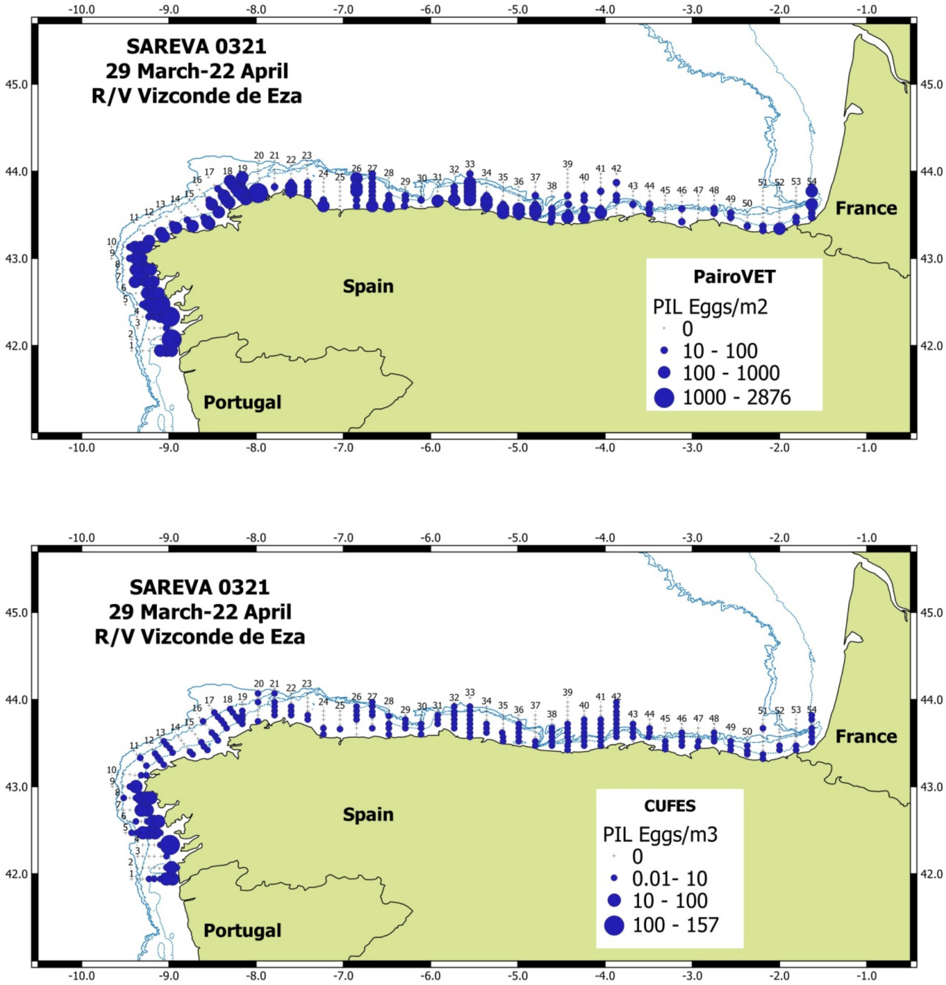


Figure 3. Sardine egg distribution. Upper panel: Eggs/m^2 from PairoVET sampling; lower panel: Eggs/m^3 from CUFES sampling; (+, egg absence).

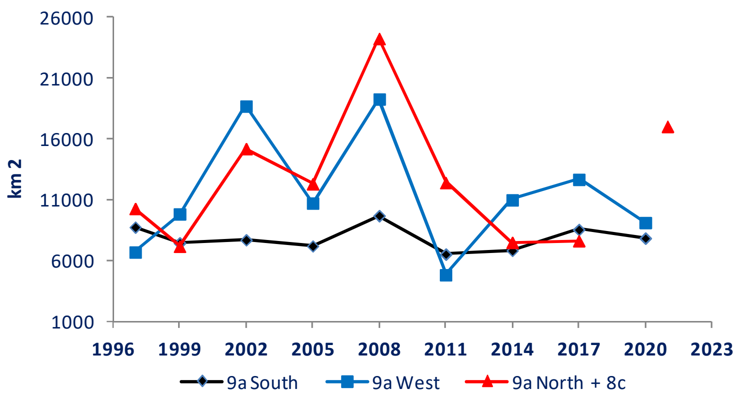


Figure 4. Spawning area (km^2) by spatial stratum (black = south, blue = west, red = north) for the historic series 1988-2021.

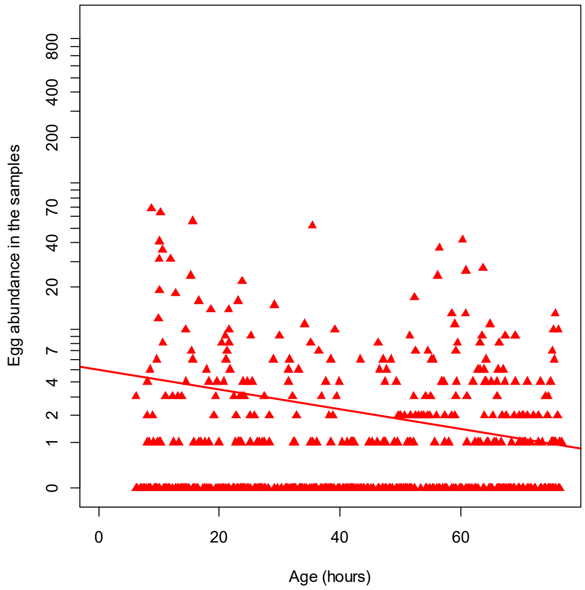


Figure 5. Abundance by age of eggs in the surveyed area (ICES 9aN+8c) and its corresponding fitted mortality curve.

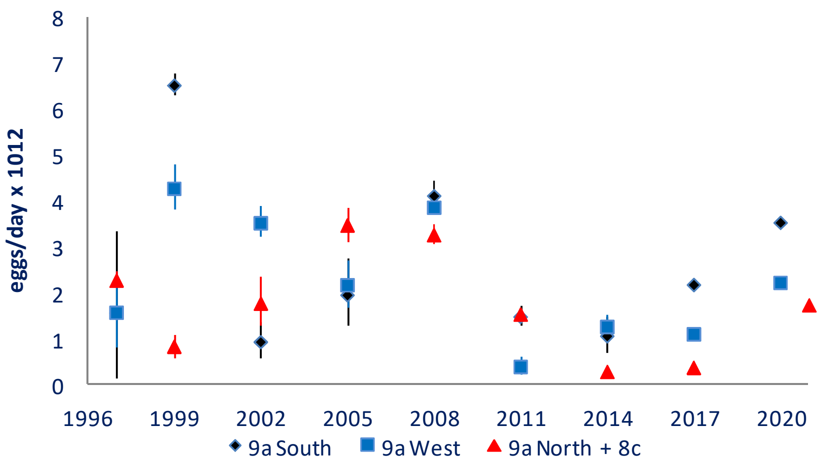


Figure 6. Total egg production (eggs/day*10^{12}) by spatial strata (9a South in black, 9a West in blue and 9a North + 8c in red). Dots and lines indicate the estimates of egg production and their confidence intervals.

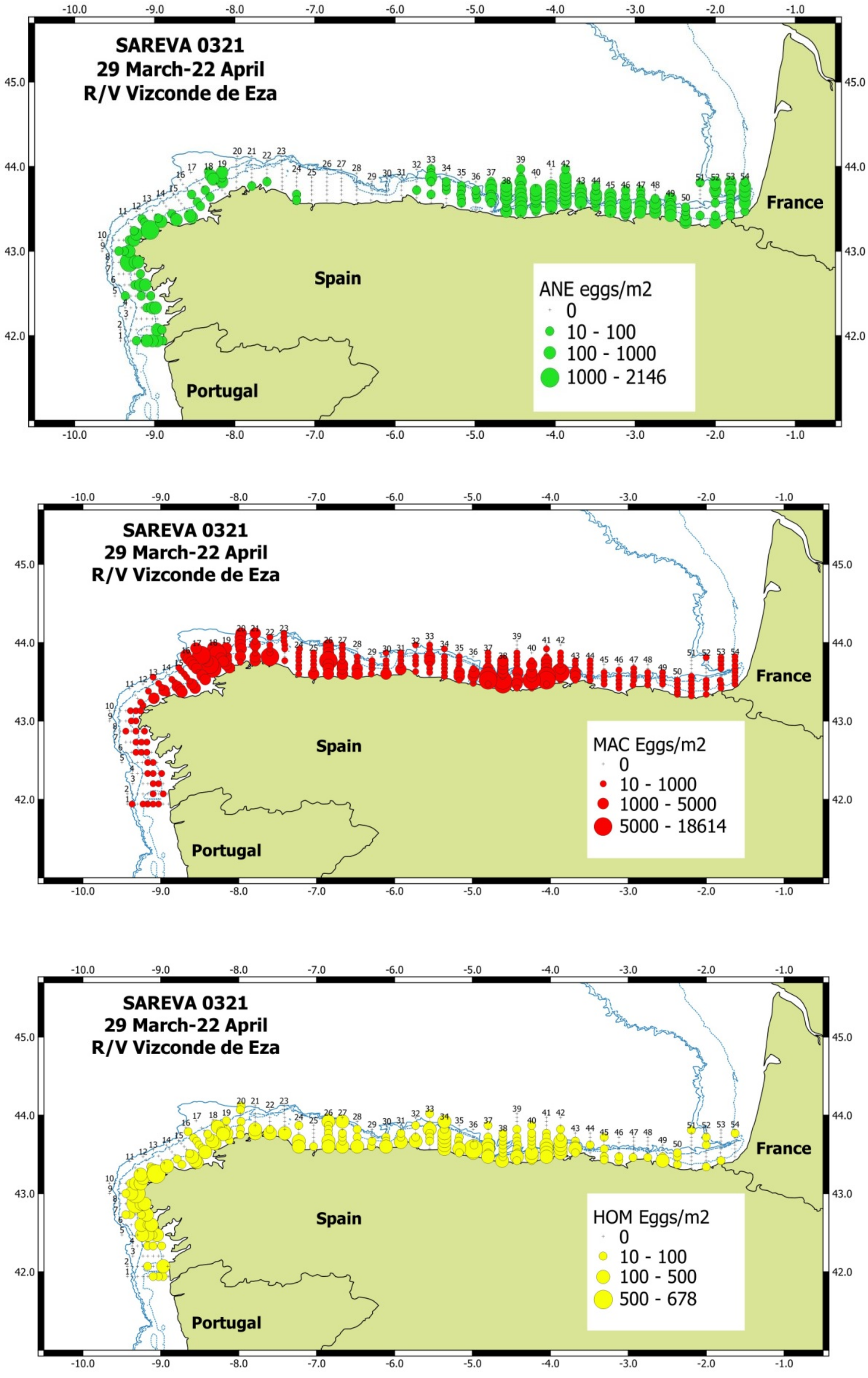
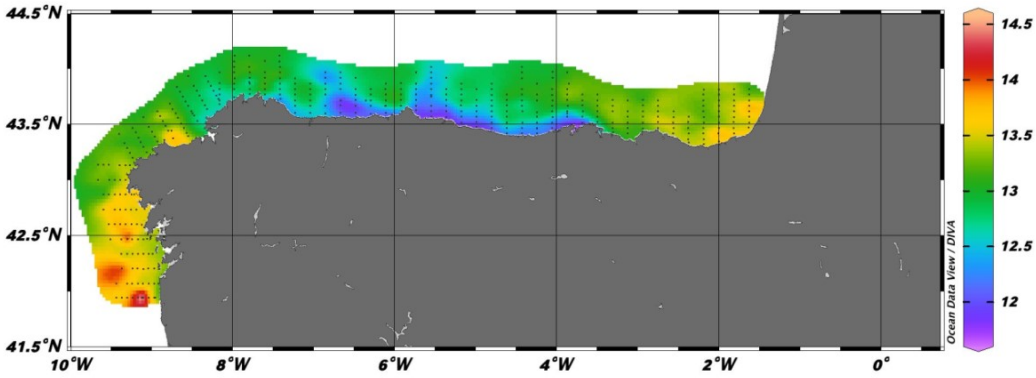


Figure 7. SAREVA 0321 survey. Distribution of Anchovy (top map), Mackerel (middle map) and Horse mackerel (bottom map) eggs abundance (eggs/m^2) obtained with PairoVET.



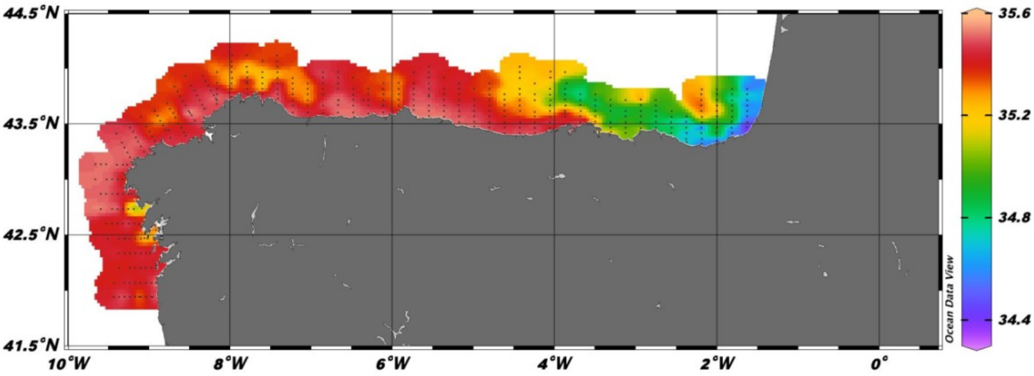


Figure 8. SAREVA 0321 survey. Sea temperature (above) in ºC and salinity in P.S.U. (down) registered during SAREVA 0321.

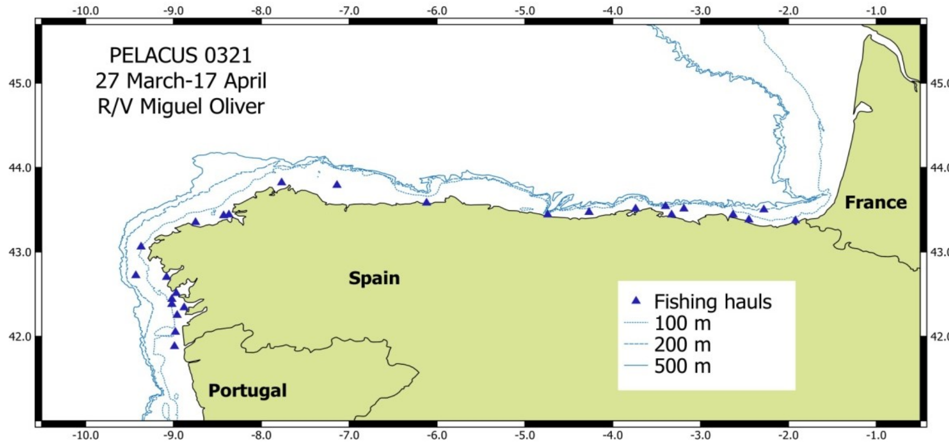


Figure 9. SAREVA 0321 survey. Spatial distribution of fishing hauls with sardine presence.

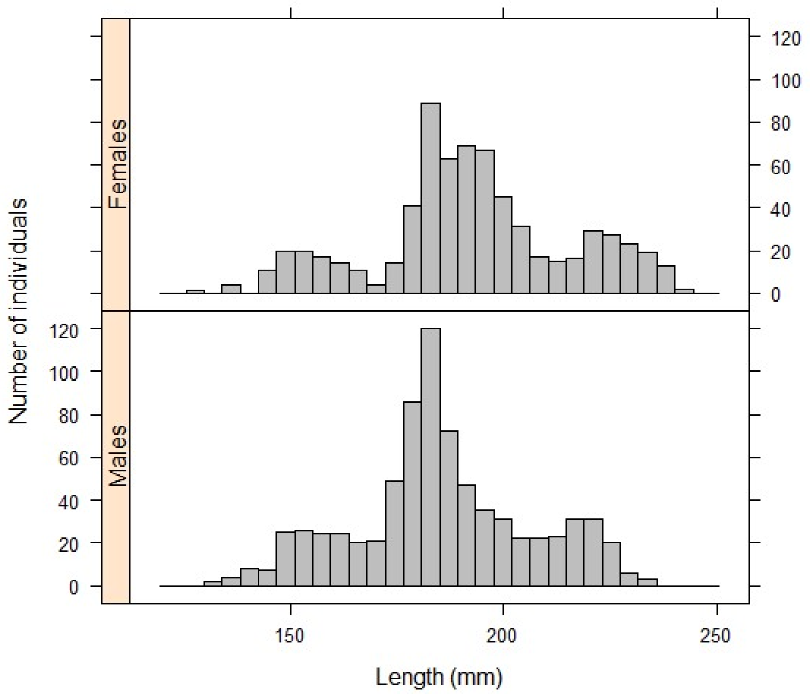


Figure 10. Sardine length (mm) distributions per sex. Only values from mature fish sampled randomly (absolute values) are shown.

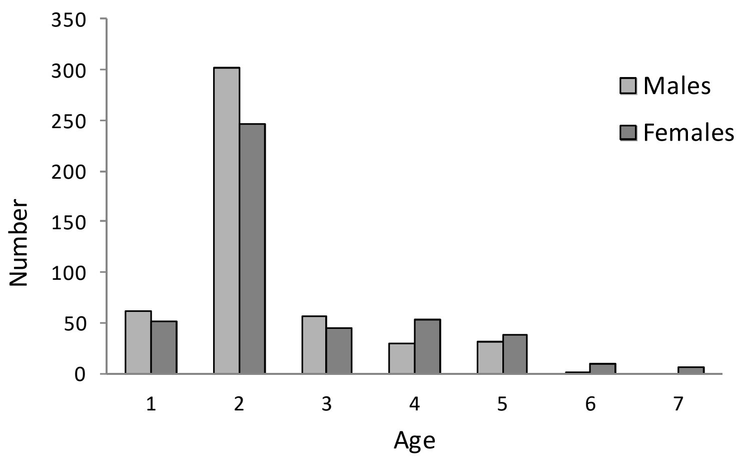


Figure 11. Sardine age composition (years) per sex. Only values from mature fish sampled randomly (absolute values) are shown.

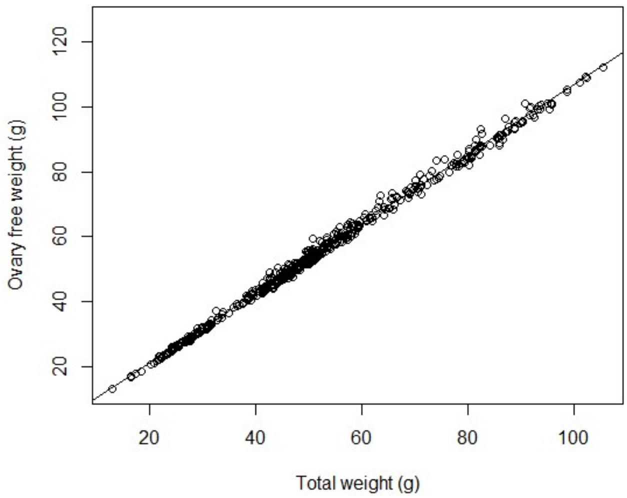
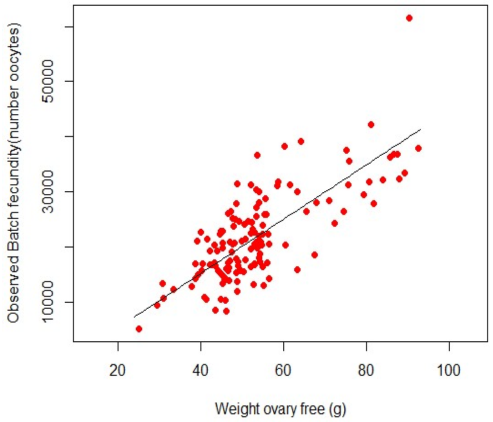


Figure 12. Plot of the linear regression model for the relationship between non-hydrated females total weight (g) and ovary-free weight (g).




```
glm.nb(formula = Fobs ~ Wnov, data = adults.dat, na.action = "na.omit",
   link = identity, init.theta = 16.15806578)
Deviance Residuals:
   Min       1Q   Median      3Q      Max
-2.7498  -0.7710  -0.1529  0.6376  2.2660
Coefficients:
          Estimate Std. Error z value Pr(>|z|)
(Intercept) -4532.51    1791.15  -2.531  0.0114 *
Wnov         493.20      37.24  13.244  <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Dispersion parameter for Negative Binomial(16.1581) family taken to be 1)
   Null deviance: 307.86  on 136  degrees of freedom
Residual deviance: 138.42  on 135  degrees of freedom
  (1568 observations deleted due to missingness)
AIC: 2734.5
Number of Fisher Scoring iterations: 1
           Theta:  16.16
       Std. Err.:  1.93
 2 x log-likelihood:  -2728.525
```

Figure 13. Preliminary observed batch fecundity vs. gonad free weight of the 137 hydrated females, the regression line of the corresponding model for the area (9a N + 8c) (left panel) and results of the GLM obtained (right panel).

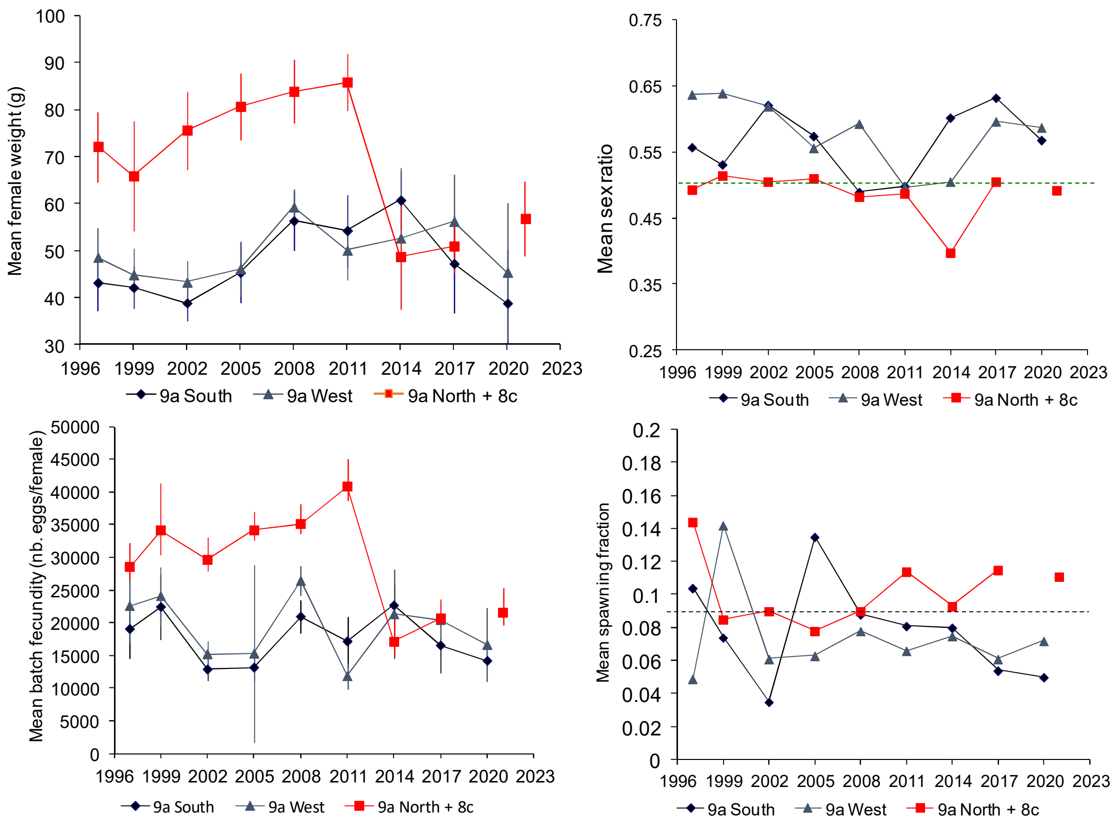


Figure 14. Evolution over time (1997-2021) of mean female weight (W) (in grams), Sex ratio (R), Batch fecundity (F) (number eggs/female) and Spawning fraction (S) estimates for the three strata (9a South - black, 9a West – blue, and 9a North + 8c - red); vertical lines indicate approximate 95% confidence intervals (i.e., ± 2 standard-deviations).

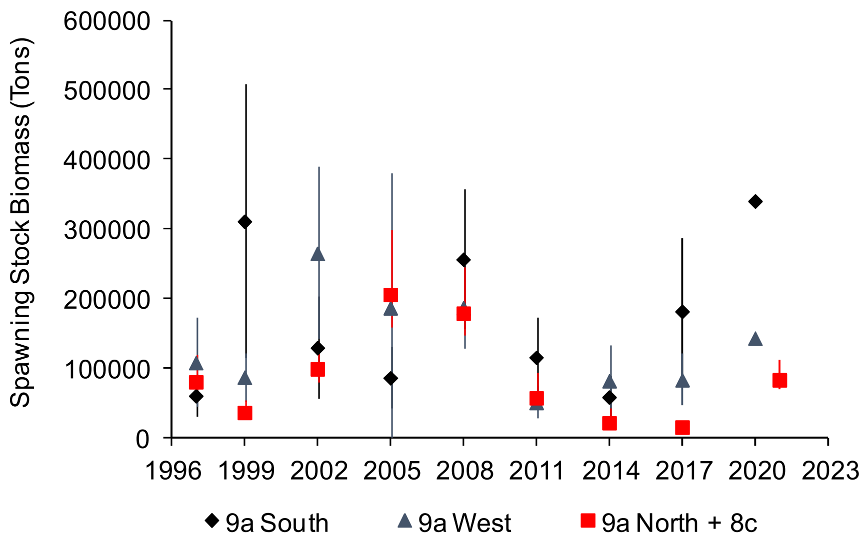


Figure 15. Spawning Stock Biomass (Tons) by geographical stratum for the historical series 1997-2021; black – 9a South, blue - 9a West, red – 9a North + 8c. Dots and lines indicate the estimates of SSB and their confidence intervals.