# Daily Egg Production Estimates for the Atlantic Iberian Sardine in 2020,

### (ICES areas 9a and 8c)

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### 1. Background

The spawning stock biomass (SSB) of the Atlantic Iberian sardine (stock pil.27.8c9a, ICES divisions 8c and 9a) has been estimated using the Daily Egg Production Method (DEPM) since the late 90s. The surveys and analyses are internationally coordinated in the framework of ICES-WGACEGG where IEO (Instituto Español de Oceanografía, Spain) and IPMA (Instituto Português do Mar e da Atmosfera, Portugal) are represented. Every three years the two institutes conduct a coordinated survey covering the Iberian shores. The Portuguese campaign surveys the waters from the entrance of the Strait of Gibraltar to the border of Portugal and Galicia (ICES area 9a), while the Spanish survey monitors the northern area of the stock from the border, at river Minho, to the south of the Armorican shelf in French waters (ICES areas 9a North and 8c).

The DEPM surveys comprise ichthyoplankton, fish and hydrographic sampling. Plankton samples are collected, along a grid of parallel transects perpendicular to the coast, for spawning area estimation and daily egg production calculation. Concurrently, fishing hauls are carried out for estimation of daily fecundity (sex ratio, female weight, batch fecundity and spawning fraction) for the mature sardines in the population.

In 2020, IPMA's survey took place, onboard RV Vizconde de Eza (ES) between the 3<sup>rd</sup> and the 28<sup>th</sup> of February, during the peak spawning period for sardine in the region. The utilization of the Spanish vessel entailed a reduction in the survey period, usually 35 days, so that the Portuguese campaign could be accommodated in the RV operations programme. By the time the Spanish survey was set to start, at the end of March, the COVID19 pandemic had reached alarming proportions and the imposed lockdown, consequently the programmed campaign was cancelled to observe the health security directives in place. For this reason, in 2020, the area of the Atlantic-Iberian sardine stock was not fully surveyed.

This working document provides a description of the Portuguese survey, including the laboratory analyses and estimation procedures used to obtain the egg and adults parameters for the 2020 DEPM.

Due to the lack of data for the northern stratum (IEO sampling area) the spawning stock biomass (SSB) estimate for the whole stock was achieved raising the SSB calculated to the southern and western areas by an estimated factor, 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; details of these analyses are presented in a separate WD (Diaz et al. 2020).

### 2. Methodology

### 2.1 Surveying

In 2020, the Portuguese survey (PT-DEPM20-PIL) took place in the scheduled period, during the peak spawning season for sardine in the southern and western Atlantic-Iberian shores. It was carried out on board RV Vizconde de Eza from the 3<sup>rd</sup> to the 28<sup>th</sup> of February. The survey duration was reduced to 26 days to conform to the RV availability.

The Spanish DEPM survey (SAREVA 0320) was cancelled due to work restrictions during the first phase of the COVID-19 pandemic in Europe.

### Plankton sampling

The Portuguese DEPM ichthyoplankton sampling follows the standard agreed protocol (table 1). Vertical plankton hauls are 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 is dependent on the bottom depth (as close to the shore as possible), while the offshore extension is 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 includes two rings (Ø 25cm) fitted with 150  $\mu$ m mesh size nets (1.5 m long) and a CTDF probe. Sampling covers the water column from bottom, or 150m depth (beyond the 150 isobath), to the surface. PairoVET samples are 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 nm beyond the inner shelf, depending on the egg results from the CUFES sampler. The CUFES system is used as an auxiliary egg sampling gear, helping in defining vertical hauls density and the offshore extension of the transects. The outer limit of a transect is reached when two consecutive CUFES samples are negative (zero sardine eggs) beyond the 200 m depth.

All plankton samples are preserved in a solution of buffered 4% formaldehyde in distilled water. Both nets (table 1) are used for egg density estimation all the analyses are carried out (for the CUFES and the PairoVET samples) in the laboratory after the survey as it is not possible to have onboard enough elements for plankton sampling and analyses and adult fish sampling.

### *Temperature, salinity and fluorescence.*

The water column structure (temperature, salinity and fluorescence) is surveyed by CTD(F) profiling during the PairoVET hauls. IPMA used in 2020 two CTDF probes, a Valeport microCTD for the first 10 days and a SBE19 for the remainder. During the Portuguese survey the surface water layer was sampled continuously with the CT(F) probes (SBE45) connected to water pumped by the CUFES system.

### Adult fish surveying

Onboard RV Vizconde de Eza in 2020, the fishing hauls were performed with a pelagic net<sup>(1)</sup> following sardine schools detection by the scientific echo-sounder. The number of samples and its spatial distribution is organized to ensure good and homogeneous coverage of the survey area. However, with this year's reduction of the survey period, the available time for trawling was also considerably shortened, consequently decreasing the number of fish hauls accomplished during the survey (table 2 and figure 2). All was also endeavoured to complement the samples collected by the RV during the Portuguese survey with fish obtained from the fishing fleet (purse-seiners) at three ports (Matosinhos, Peniche, Portimão), acquired within 1-3 weeks of the surveying by the RV in each area. Nevertheless, as the sardine fishery was closed at this time of the year, and despite official fishing authorizations delivered by the Portuguese authorities for the purpose of this survey, many purse-seiners were not operating, and significantly fewer additional samples from the commercial vessels were obtained, compared to previous years (table 2). Finally, as a supplementary effort to meet with the DEPM sampling objectives, additional fish samples (n = 5) were also collected during the acoustic survey

PELAGO20 that took place shortly after the DEPM campaign, covering the same geographical area, but the sampling of these fish eventually distanced themselves of about 3-4 weeks from the DEPM ichthyoplankton surveying in the same areas and the microscopical analysis of these fish ovaries sampled showed a contrasting spawning activity in comparison to the ones observed during the DEPM survey in the same areas (see section 3.3.), and it was decided not including them in the estimations.

Onboard the RV, and for each haul, a minimum of 60 sardines are randomly selected and biologically sampled (table 1). In some occasions sampling is 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, gutted weight, sex, maturity state) is recorded for all fish, the ovaries are preserved for histology (with a 4% buffered formaldehyde solution) and to obtain gonad weight (measured in laboratory for IPMA), and the otoliths removed for age determination. The biological sampling and ovaries fixation are always carried out in fresh material, with the exception of some commercial samples for which the ovaries are removed from the fish body and preserved immediately after the fish are landed, while the remaining body of the fish is frozen for posterior biological sampling in laboratory.

<sup>(1)</sup> A 63.5/51 pelagic fishing gear with a sweep attached to the net's footrope composed of small rubber dishes (20 cm diameter), with a vertical opening of about 14-16 m and 30 in horizontal plane, and a 20mm codend.

### 2.2 Laboratorial analyses

#### Plankton samples

All sardine eggs are sorted from PairoVET and CUFES samples at IPMA's laboratory. The eggs from the vertical hauls (2 nets) are all counted and staged according to the 11 stages of development classification (adapted from Gamulin and Hure, 1955). The eggs from the CUFES samples are all counted and staged but due to the poor condition of the eggs because of damage caused by the pumping system, a second staging scale with 5 stages is now in use. As a consequence of the lockdown imposed for several months the laboratory work for IPMAs CUFES samples is not yet completed and will be presented at a later stage.

### Adult fish samples

The preserved ovaries are weighed in laboratory at IPMA and the weights obtained corrected by a conversion factor (between fresh and formaldehyde fixed material) established previously. These ovaries are then processed for histology for spawning fraction estimation: they are embedded in paraffin, the histological sections are 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 are also processed histologically to check for POF presence and thus avoid underestimating fecundity (Pérez et al. 1992b). The individual batch fecundity is 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 100-150 mg (Hunter et al. 1985).

### 2.3 Data analyses

All calculations for area delimitation, egg ageing and model fitting for egg production (P<sub>0</sub>) estimation are carried out using the R packages (*geofun, eggsplore and shachar*) available within the open source project *ichthyoanalysis* (<u>http://sourceforge.net/projects/ichthyoanalysis</u>). Some routines of the R packages used were updated since the 2008 versions.

#### Survey area and spawning area delimitation

Area delimitation is attained through an automated procedure included in the spatstat library. However, during the process of defining the area represented by each sampling station limits are set in order to prevent very extreme values. The range 25-175 km<sup>2</sup> 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). Nonetheless, the extreme values usually occur on the borders of the survey area and therefore do not affect the estimation of the positive area (A+, spawning area).

### Strata definition

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.

In 2020 data is available only for strata 1 and 2 (Portuguese survey).

### Mortality and Egg Production

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 stratrum using the average mortality estimates previously obtained. The exponential model:  $E[P] = PO e^{-Z age}$  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.

The methodology currently being used for mortality and egg production estimations can be described in more detail as follows:

- Step 1 Estimation of age and cohort abundance
- Step 2 Model design/fitting for Mortality estimation
- Step 3 Calculation of PO with the mortality estimates obtained in step 2

### Step 4 - Calculation of total egg production

### Step 1 - Estimation of age and cohort abundance

- Data from plankton hauls from several surveys with abundances by stage and in situ temperature are used (several surveys from the 90s to present)

- Egg stage and age are related to temperature with a multinomial model. 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) (previously a normal PDF was considered); peak spawning time (21h) 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 (realistic) spawning period and therefore few eggs are excluded from the analyses (how.complete=0.95).

### Step 2 - Model design/fitting for mortality estimation

- The mortality curve is fitted to the estimates of abundance-by-cohort.

- A model for mortality is designed with the historic data set and tested. 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:

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

E [Na] = expected number of eggs in a cohort of mean age a

D0 = the rate of egg production

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.

- 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 fitting 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 + 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 (three more years: 2011, 2014, 2017, 2020 (stratum 1 and 2) have been added).

The application of the above model allows the estimation of coherent mortality values for each stratum for all surveys of the series. Each update of the model with new data will also update (slightly) the series mortality estimates. Such small changes in the series values should only be considered during the course of a benchmark evaluation.

### Step 3 - Calculation of PO with the mortality estimates obtained in step 2

- To obtain P0 an egg production model that can accommodate mortality estimates external to the estimation procedure (as obtained in step 2) 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 the areas represented by each station.

The application of the above model allows the estimation of PO for each stratum for all the surveys in the historic series. Each update of the model with new data will also update (slightly) the series PO estimates. Such small changes in the series values should only be considered during the course of a benchmark assessment.

### Step 4 - Calculation of total egg production

Finally, total egg production (P<sub>tot</sub>) is calculated, per stratum for each survey, by multiplying the daily egg production (P0) estimates by the corresponding spawning area (A+) values.

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

### 2.3.2. Fish data

The adult parameters estimated for each fishing haul considers only the mature fraction of the population (determined by the fish macroscopic maturity data, the latter corrected by the microscopical analysis, if needed, for the females with gonads preserved for histology) and is based on the biological data collected from both surveys and commercial samples.

### Mean female weight (W) and Sex ratio (R)

Before the estimation of the mean female weight per haul (W), the individual total weight (Wt) of the hydrated females is 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 is obtained as the quotient between the total weight of females on the total weight of males and females.

### Batch fecundity (F)

The expected individual batch fecundity (Fexp) for all mature females (hydrated and non-hydrated) is estimated by modelling the individual batch fecundity observed (Fobs) in the sampled hydrated females and their gonad-free weight (Wnov) by a GLM (with an identity link function and a negative binomial distribution).

### Spawning fraction (S)

The fraction of females spawning per day (S) is 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 is corrected by the average number of females with Day-1 or Day-2 POF, and the hydrated females are not included) (Pérez et al. 1992a, Ganias et al. 2007). For the 2020 samples, and considering the results of the microscopical analysis obtained, other (pre- and post-spawning) markers than solely Day-1 and Day-2 POFs were also included in the average (see section 3.3.)

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. Final adult parameters include estimates for each of the strata (this year only for Southern and Western areas).

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

### 3. Results

### 3.1 Temperature and salinity distributions

During the Portuguese survey in 2020, the sea surface temperature (SSS) and salinity (SSS) patterns observed were the typical for the winter period in the region (figure 3) As usual SST was higher in the south (14.5-17.0°C) and lower in the west coast (13.5-16.0°C), and decreasing from south to north. In the northwestern shelf, to the north of Cape Mondego, a very large area was occupied by water with comparatively lower temperature (13.5-14.5°C). However, this low temperature plume was not characterised by low salinity. In fact, the rivers outflow signatures were quite weak in February 2020 as a consequence of the rather dry period that preceded the campaign, the Douro river being the only exception to this pattern. The cold SST in the north was probably the result of atmospheric cooling observed towards the end of the survey after some days of continuous northerly winds.

### 3.2 Eggs

In total, 490 PairoVET and 606 CUFES samples were collected in 2020, along the 58 transects of the Portuguese survey. The CUFES samples from IPMAs campaign are still being processed (figure 1, table 2). A total of 8286 sardine eggs were collected by the PairoVET sampler, 5032 in the southern coast and 3254 in the western shelf (figure 4). As commonly occurs eggs of stages VI and II were the more abundant in the samples (likely because of their longer duration); the high proportion of recently spawned eggs (stage I and especially stage II) in a few samples in the southern stratum indicates spots of spawning activity. Overall, sardine eggs were observed in 30% of the samples, however the percentage of positive stations was higher in the south (41%) than in the west (24%). During the 2020 survey an increase in the number of eggs collected and also in the number of samples with sardine eggs was observed compared to the previous DEPM survey, but it is worth remembering that in 2017, due to logistics constraints, the survey took place late in the spawning season.

Nonetheless, the egg distribution general patterns in 2020 and 2017, were not very different with the same larger patches appearing in the south and in the northwest. However, a clear increase in egg density was observed in the south and in the southwest regions. In the central western shore, between Cape Carvoeiro and Aveiro hardly any eggs were collected (possibly related to the structure of the sardine population in the area, with a large proportion of young individuals, and also maybe influenced by the low water temperature at the time of the survey). The patches with higher egg density in 2020, were observed in the southern region, in the central Cadiz region and in the Algarve from Cape Sta Maria to the west and in the western coast in the southwest, mainly from Sines to Setúbal and in the far north between Douro and Lima (figure 1).

In total 48249 km<sup>2</sup> were surveyed (18689 in the south and 29560 in the west), of which 16971 km<sup>2</sup> were considered the spawning area (7844 in the south and 9127 in the west). The spawning stratum decreased in 2020, comparing to 2017, in the west, but was similar for the southern region in the two years (figure 5, table 3).

Since the 2016 WGACEGG revision (undertaken in preparation for the 2017 sardine benchmark workshop) the procedure adopted to estimate mortality and egg production follows the approach published by Bernal et al 2011a e 2011b and described in the methods section. Mortality and egg production are estimated for each of the three strata (two in 2020). The results from the model update are shown in table 4 and the parameters estimated for the 2020 survey appear in table 3.

The mortality estimates obtained (considering survey temperatures and the data from the whole historic series) were within the range observed for the DEPM series (revised in 2016) but slightly

higher in the south and little lower in the west compared to the previous estimates in 2017 (table 3, annex 3).

The Daily Egg Production (eggs/m<sup>2</sup>/day) was considerably higher in the south and in the west, in 2020, than in 2017 (S: 450.85, W: 243.95; figure 6, table 3, annex 3). Consequently, the Total Daily Egg Production (eggs/day) was also considerably higher in 2020 than in 2017 (figure 7, table 3, annex3). The Total Daily Egg Production (POTot) estimated for the southern and western strata of the Atlantic Iberian stock (areas 9a, south and west) during the 2020 survey was  $5.77 \times 10^{12}$  ( $3.54 \times 10^{12}$  corresponding to the south and  $2.23 \times 10^{12}$  to the west) (figure 7). These results represent an increase of POTot of 62% in the south and of 100% in the west. In fact, the increase in POTot was the highest registered for the past three DEPM surveys (the highest since 2011).

### 3.3 Adults

As referred previously, both the shortening of this year's survey duration and the sardine fishery closure at the time of the survey constrained the possibility of obtaining the number of samples that were desirably expected in comparison to previous years, and this despite all efforts made. However, contrarily to what had been observed during the surveys undertaken since 2008, in 2020 sardines appeared more available to trawling, most of the survey hauls having resulted positive for the species (91%). A total of 15 fishing hauls which caught sardines were performed during the DEPM survey, complemented by 5 samples obtained from the Portuguese commercial fleet and 5 samples provided by the PELAGO20 survey (table 2 and figure 2). On the whole, 1556 sardines were sampled, nearly 800 ovaries were collected and preserved (among which 85 from hydrated females, a similar number to the ones obtained in 2017 for the 9a South and the 9a West strata), and ca. 1450 otoliths were removed for age determination.

The length frequency distribution of the fish sampled was plurimodal for both West and South coasts, with two modes (13 and 20 cm) for the former and three modes (11.5, 17.5 and 19.5 cm) for the latter (figure 8), with the smallest fish (<11.5 cm) only sampled off the South coast. The fish from the 5 samples obtained during the PELAGO20 survey 3-4 weeks later, off the West coast, presented a very similar length distribution compared to the DEPM survey in the same area, with the exception of the larger fish from the 2<sup>nd</sup> mode that were lacking from these 5 samples (it should be noted that these 5 samples are just a subset of the fish trawled in the West during the PELAGO20 survey, and should thus not be considered here as representative of the sardine population present during the Acoustic survey).

The sardine age distribution for the South stratum was narrower than for the West: in the South, sardines were aged up to 5 years-old, in comparison to sardines sampled off the West up to 10 years-old (figure 8). In the South, 3/4 of the fish were aged 0 to 2 years, with a mode in the 2-years old cohort that could possibly be related to the regular significant recruitments occurring in the Cadiz Spanish waters since 2018 (cf. results from the PELAGO and IBERAS surveys series). In the West, two modes were observed in the fish sampled, 1 and 4 years-old, the first one corresponding to the high recruitment observed in the NW of Portugal in 2019. The difference observed in the length distribution between the DEPM and PELAGO20 sardines was obviously similarly observed for the age composition, both surveys presenting the 1-year old mode, but the PELAGO20 sardines almost completely lacking the 4-years old cohort observed in the DEPM survey.

Most sardine females sampled were mature in both strata (98% and 94% for the South and West coast, respectively), the great majority being actively reproducing (~80%), while ca. 16% in a developing stage (these being almost exclusively sardines sizing  $\leq$  16 cm, and thus likely first-time spawners) (results not shown).

Due to the circumstances already explained previously, the analyses for the estimation of the adult parameters in 2020 were carried out only for strata 9a South and 9a West (strata 1 and 2), referring to the data collected during the Portuguese DEPM survey. Data were analysed as follows:

(a) The same linear regression between the non-hydrated females Wt and their corresponding Wnov was used for the whole surveyed area (Wt =  $1.046 \times Wnov - 0.369$ , R2 = 0.999).

(b) The observed batch fecundity (Fobs) data were modelled using both the ovary-free female total weight (Wnov) and the stratum as covariates, the GLM model obtained is shown in figure 9; though the slopes corresponding to the two strata are very similar, the model was statistically significantly different from the GLM without the stratum as covariate.

(c) The spawning fraction (S) estimates obtained per haul showed a relatively high dispersion (from 0 to 13.3%), with ca. half of the hauls with an S = 0, especially in the West coast (among which the 5 hauls obtained from the PELAGO20) (figure 10), resulting in low mean spawning fractions for the whole survey in both areas.

In some of these hauls with a null or very low S estimate, the females presented POFs older than 2 days (indicating that spawning events would have taken place more than 2 days before) and/or oocytes at final maturation stages (with migratory nucleus MN or hydrated) (indicating that these females could possibly have spawned within the following 24h) (figure 2). The null S estimates obtained for these hauls (because of the lack of females with recent POFs in the sample, cf. the methodology to estimate S, section 2.3.2) may be in part "unrealistic" in biological terms, i.e., these estimates may have failed to correspond to the "real" spawning activity in these areas, and to the potential contribution of these females to the production of the eggs sampled in the plankton. The causes for these observations are unknown, a hypothesis being that the females in these areas might have skipped the spawning of one batch of oocytes during 2-3 consecutive days for any physiological/environmental reasons.

For the reasons exposed here before, the group suggested that additional spawning markers than solely the Day-1 and Day-2 POFs could be included in the calculation of S, provided that all these spawning markers correspond to a single spawning event (batch) and that there is no bias on their sampling. This alternative methodology, before proving being sufficiently accurate and robust, will require several sensitivity and statistical analyses using the DEPM historical series data, analyses that the group recommended to be carried out during next year. Still, after having tested different S estimators with various combinations of spawning markers, taking into account the current knowledge on the spawning behaviour, the ovarian dynamics and the POFs degeneration pattern for sardine in Iberian waters, the group agreed that for the 2020 survey, S be estimated based on both MN oocyte stages and Day-1 and Day-2 POFs, according to the equation:

[(1/3 \* (MN + Day-1 + Day-2)) + MN + Day-1 + Day-2 + Day >2 + No POFs],

where MN = number of mature females with MN oocytes; Day-1, Day-2 and Day>2 = number of mature females with POFs aged 1, 2 or more than 2 days, respectively; No POFs = number of mature females with no POFs In their ovaries.

Females with hydrated oocytes were not included in the S estimator, as Ganias and Nunes (2011) suggested that sardine imminent spawners form ephemeral spawning aggregations at depth, with a consequently possible bias in the proportion of these hydrated females in the samples. Likewise, it is not guaranteed that the POFs classified as Day>2 would correspond to a unique daily cohort, i.e. to a single spawning event.

As previously explained in both this section and section 2.1, the samples obtained during the PELAGO20 were not included in the calculations.

Based on the above analyses, the adult estimates derived from the 2020 DEPM survey for 9a West and 9a South strata, summarized in table 3, showed a general decrease. Female mean weight (W) decreased in both South and West coasts (22% and 36%, respectively) to values similar to those

estimated in 2002 and 2005 for these areas, being among the lowest of the time series, and possibly related to the sardine high recruitments in 2000, 2004 and 2019 that preceded these 3 surveys (figure 11, annex 3). Subsequently, mean batch fecundity (F) also decreased in all the surveyed area (17% in the South and 35% in the West), whereas relative batch fecundity remained very similar to the ones estimated in 2017 (~365 eggs/gram female for both strata; results not shown). Regarding spawning fraction (S), and based on the alternative methodology described above, the estimate obtained in the South was very similar to the one calculated in 2017 (though lower), and the one for the West coast increased slightly, both values being within the range of the historical series (figure 11, annex 3).

Consequently, mean daily fecundity (DF) for sardine during the 2020 survey slightly decreased in the South (13%; 10.4 eggs/day.gram female) and increased in the West (17%; 15.5 eggs/day.gram female) (figure 12).

### 3.4 SSB estimate

Due to the cancelation of SAREVA 0320 DEPM survey because of the COVID-19 pandemia, Spawning Stock Biomass (SSB) estimates derived from the DEPM were only possible for the 9a South and 9a West strata.

However, several methodological approaches to compensate for the lack of the SAREVA survey covering the 9a North+8c stratum were explored (described in detail in the WD Díaz et al, 2020), the results obtained having suggested that the SSB index for total Iberian Peninsula (including the 3 strata) could be obtained by raising the SSB estimated from the Portuguese survey by a factor of 1.3.

The final SSB estimates obtained from the application of the DEPM in 2020 increased substantially in comparison to the previous survey, having almost doubled in the whole surveyed area (an increase of 87% and 71%, respectively, for the South and for the West: 341.2x10<sup>3</sup> and 144.0x10<sup>3</sup> tons) (figure 13, annex 3). The SSB in the South is the second highest of the time series, whereas the value in the West is at the level of the biomass indexes obtained in the first decade of the 2000's. Jointly with the SSB calculated for the North stratum according to the method of Diaz et al, 2020, the SSB index estimated for the total Iberian Peninsula accounts for 630.7x10<sup>3</sup> tons, which more than doubled in relation to 2017 (an increase of 123%; annex 3).

### 4. Remarks

- Area 9a+8c for DEPM PIL not fully surveyed in 2020, SAREVA survey cancelled due to COVID19
- IPMA survey: PT-DEPM20-PIL conducted in the scheduled period (3-28 Feb), full area coverage (Cape Trafalgar, in Cadiz, to border Portugal-Galicia), but duration reduced in relation to planned. In 2017 IPMAs survey was late in the season and did not cover whole area adequately.
- Hydrographic conditions typical for the winter period, SST South ~ 14.5-17.0°C; West ~ 13.5-16.0°C; extensive area of lower SST (13.5-14.5 °C) in the NW; weak plumes of lower salinity
- Icthyoplankton samples collected in the whole study area according to pre-defined grid; fishing hauls in lower numbers than usual due to time constraints and adverse weather conditions in the NW region
- Similar pattern of egg distribution in 2020 and 2017 but higher egg abundance; egg density higher in the south, eggs more evenly distributed; NW patchier distribution

- Increase in spawning area: S, SW; decrease in NW (S: 42% of area surveyed with eggs; W 31% of area surveyed with eggs)
- Mortality higher in S and lower in W compared to 2017 (possibly temperature related)
- Daily Egg prod (eggs/m<sup>2</sup>/day) considerably higher in S and W in 2020 than in 2017 (peak spawning!)
- Total Daily Egg Prod (eggs/day) considerably higher in S and W in 2020 than in 2017 and past 3 surveys (62% increase in S and 100% in W); 2020 results comparable to estimates of 2005 in the West and close to 2008 values in the South (reflecting increase in PIL abundance!)
- Lower number of fishing hauls due to the reduced duration of the survey, but higher availability of sardine
- Fish sampled with a plurimodal length/age distribution in both South and West; small fish (from 2019 recruitment) present in both areas
- Spawning fraction (S) estimation adopting an alternative methodology in 2020
- Decrease in all adult parameters, and in both areas, but relative batch fecundity almost identical to 2017.
- SSB almost doubled in both South and West areas (341.2x10<sup>3</sup> and 144.0x10<sup>3</sup> tons, respectively); final SSB estimate for the total Iberian Peninsula (following a methodological approach to compensate for the lack of the SAREVA survey covering the North stratum) more than doubled in relation to 2017 (630.7x10<sup>3</sup> tons).

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

DEPM Surveys	Portugal
DEFINI SULVEYS	(IPMA)
Survey	PT-DEPM2020-PIL
Survey area	(9a S, 9a W) South-West
SURVEY EGGS	
Sampling grid	8 (transect) x 3(station)
PairoVET Eggs staged (n egg)	
(stages Gamulin and Hure, 1955)	All (2 net)
Sampling maximum depth (m)	150
Temperature for egg ageing	3-5 m
Peak spawning hour	(PDF Lognormal, 21 ± 2 * 3)
Egg ageing	Bayesian (Bernal et al, 2008)
Strata	No strata/Stratum (South,West)
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	3nm (sample unit)
CUFES Eggs counted	All
CUFES Eggs staged	Subsampled of a minimun of 100
Hydrographic sensor	CTDF (FSI)
Flowmeter	Y
Clinometer	Y
Environmental data	Fluorescence, 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)
Sample size	60 indiv randomly ; extra if needed (30 females min for histology) and if hydrated females found
Sampling for age	Otoliths from the same females sampled for histology, and from 30 random males
Fixation	Buffered formaldehyde 4% (distilled water)
Preservation	Buffered formaldehyde 4% (distilled water)
Histology:	
- Embedding material	Paraffin
- Stain	Haematoxilin-Eosin
S estimation	Day 1 and Day 2 POFs (according to Pérez et al. 1992a and Ganias et al. 2007) (in 2020, exception of inclusion of MN oocytes stage)
R estimation	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

## Table 2. General Sampling for DEPM 2020

Institute	IPMA	IPMA			
Survey area	9a South	9a West			
SURVEY EGGS					
R/V	Vizconde de Eza	Vizconde de Eza			
Date	05-14/02	14-28/02			
Transects	21	37			
PairoVET stations	190	300			
Positive stations	78	72			
Tot. Eggs	5032	3254			
Max eggs/m2	11720	4330			
Temp (ºC) min/mean/max	14.9/16.3/17.7	13.5/14.7/16.3			
Max age	55.8	62.5			
CUFES stations	250	356			
Positive CUFES stations	lab processing underaway	lab processing underaway			
Tot. Eggs CUFES	lab processing underaway	lab processing underaway			
Max eggs/m3	lab processing underaway	lab processing underaway			
Hydrographic stations	190	300			
SURVEY ADULTS					
Number Hauls R/V	9	8			
Number Hauls C/V	0	5			
Number R/V (+) trawls	8	7			
Date	05-17/02	19-28/02			
Depth range (m)	27-66	29-60			
Time range (hh:mm)	09:00 - 17:07	01:00 - 13:40			
Total sardine sampled	476	1080			
Length range (cm)	10.5-22.5	11.5-24.5			
Weight range (g)	8-83	10-110			
Females for histology	265	531			
Hydrated females	64	21			
Otoliths	459	996			

**Table 3.** DEPM parameters derived from 2020 sardine DEPM surveys with their CV (%) in brackets by institution and stratum (in 2020, only 9a South and 9a West estimates were based on the survey; cf. note below). Surveyed and positive areas (km<sup>2</sup>), Mortality Z (hour<sup>-1</sup>), Daily egg production P0 (eggs/m<sup>2</sup>/day), Total egg production P0 tot (eggs/day) (x10<sup>12</sup>), 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).

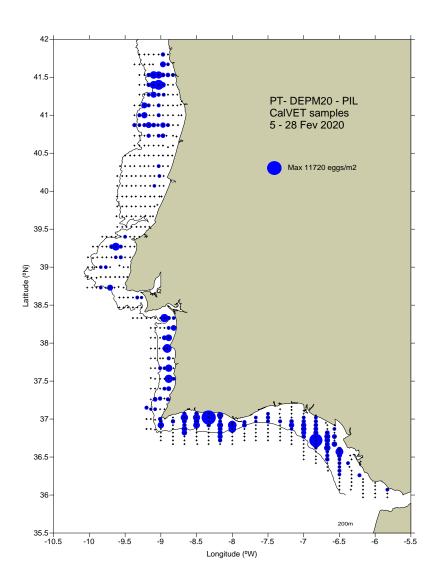
Institute	IPMA	IPMA	TOTAL	Total (Iberian		
Area	9a South	9a West	(9a S + 9a W)	Península) (*)		
Survey area (Km <sup>2</sup> )	18689	29560	48249			
Positive area (Km <sup>2</sup> )	7844	9127	16971			
Z (hour <sup>-1</sup> )(CV%)	-0.030 (7.0)	-0.023(5.7)				
P0 (eggs/m2/day)(CV%)	450.85 (23.7)	243.95 (20.4)				
P0 tot (eggs/day) (x10 <sup>12</sup> ) (CV%)	3.54 (23.7)	2.23 (20.4)	5.77 (17)			
Female Weight (g) (CV%)	38.80 (14.7)	45.40 (16.2)				
Batch Fecundity (CV%)	14176 (15.3)	16637 (17.0)				
Sex Ratio (CV%)	0.568 (8.3)	0.587 (7.3)				
Spawning Fraction (CV%)	0.050 (22.0)	0.072 (23.8)				
Daily Fecundity (eggs/day.g female)	10.38	15.49				
Spawning Biomass (tons) (CV%)	341164 (39.4)	143984 (39.8)	485148 (30.2)	630692 (*)		

(\*) Eggs and adult parameters for the ICES subdivision 9a North and division 8c 9a are not available in 2020 due to the cancelation of SAREVA 0320 DEPM survey because of the COVID-19 pandemic. The total Iberian Peninsula SSB was estimated raising the Portuguese SSB index (9a South and 9a West) by 1.3 (see separate WD, Díaz et al, 2020, for the analyses that resulted in this index).

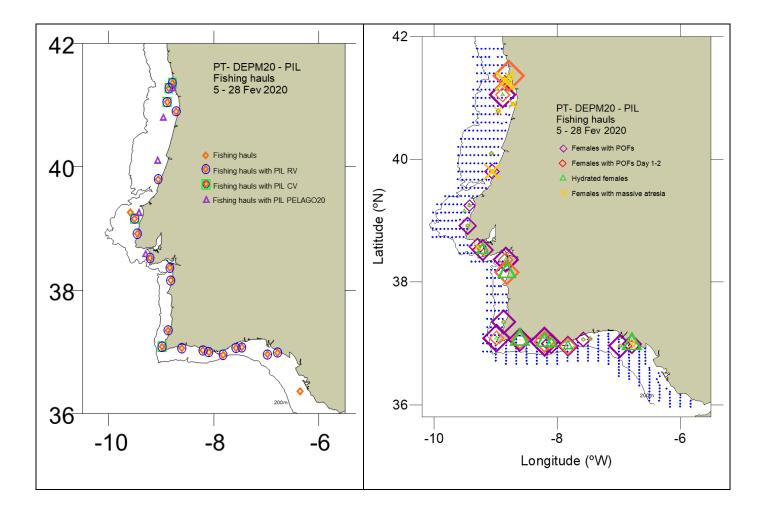
**Table 4.** Fitted parameters of the final mortality model updated with 2011, 2014, 2017 and 2020data.

	Estimate	Std. Error	z value	Pr(> z )	
Sstrata1	0.658	0.996	0.661	0.508856	
Sstrata2	6.875	0.799	8.601	< 2e-16	***
Sstrata3	-1.235	0.807	-1.529	0.126152	
Tstrata	4.647	0.909	5.112	3.19E-07	***
Тетр	0.468	0.058	8.025	1.01E-15	***
age	0.054	0.014	3.738	0.000186	***
Sstrata:Tstrata	-0.337	0.154	-2.185	0.028863	*
Sstrata:Temp	-0.129	0.072	-1.789	0.073611	
Sstrata:Temp	-0.556	0.060	-9.226	< 2e-16	***
Tstrata:Temp	-0.354	0.065	-5.425	5.80E-08	* * *
Temp:age	-0.005	0.001	-5.287	1.25E-07	***

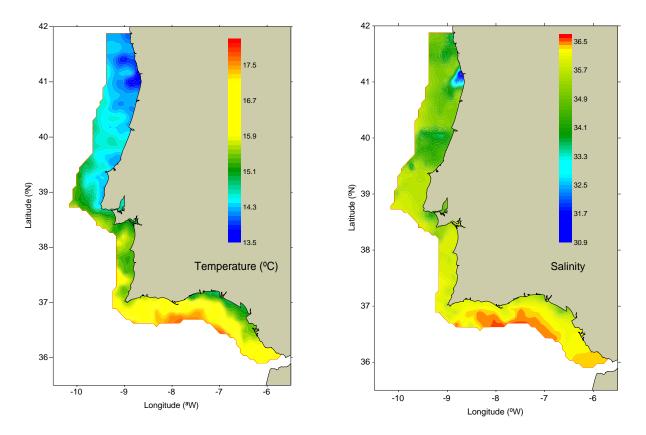
The z-value indicates the value of the z-statistics used to test the significance, and Pr(|z|) the probability of the null hypothesis (H0: parameter does not differ from zero).



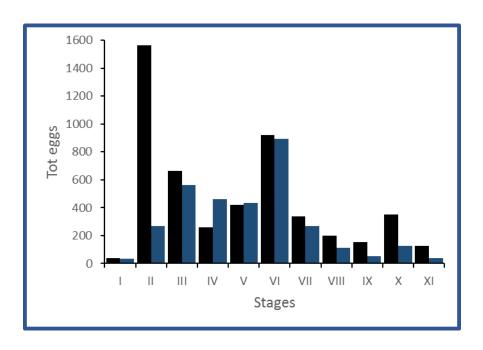
**Figure 1.** Sardine egg density distribution (Egg/m<sup>2</sup>) obtained from PairoVET sampling during IPMA's survey (PT-DEPM20-PIL) (+, egg absence).



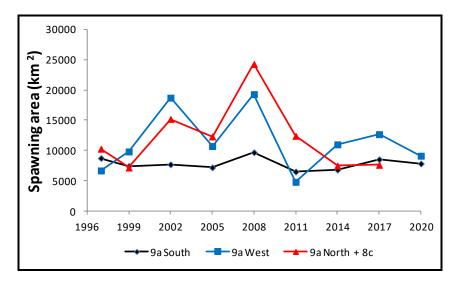
**Figure 2.** Spatial distribution of the fishing hauls. Left panel: Hauls with sardine presence from the DEPM 20 research vessel (blue circle surrounding the orange diamond), from the commercial fleet (green square surrounding the orange diamond), and from the PELAGO20 survey (purple triangles). Right panel: for each haul, the proportion of females with POFs (purple diamond), with POFs of Day-1 and Day-2 (red diamond), with hydrated oocytes (green triangle) and with massive atresia (>50% of the most advanced batch of oocytes atretic; yellow cross); the size of the symbols is equivalent to the proportions referred above. Hauls from the PELAGO20 were ultimately not used in the estimations (see text).



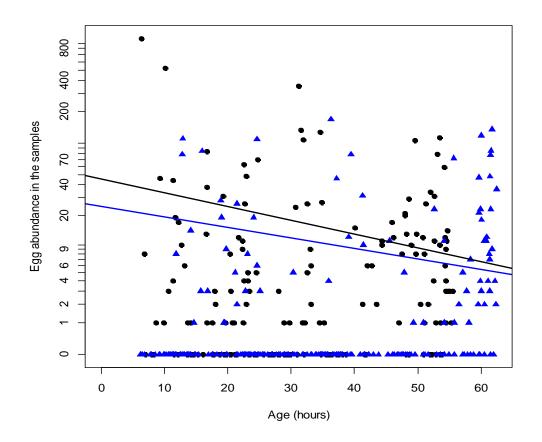
**Figure 3.** Distribution of sea surface temperature (left) and, salinity (right) during IPMA's survey (PT-DEPM20-PIL) (see table 2 for surveying dates).



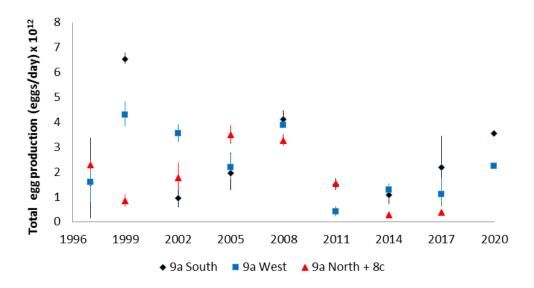
**Figure 4.** Number of sardine eggs (total eggs) per development stage by stratum from the PairoVET sampler. South (9a S) in black, West (9a W) in blue.



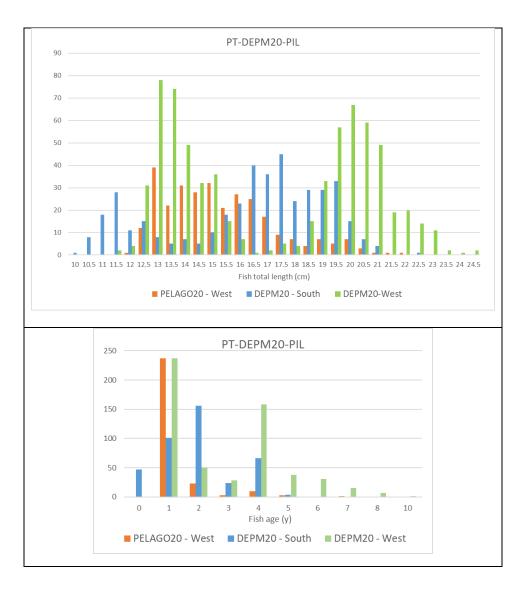
**Figure 5**. Spawning area (km<sup>2</sup>) by spatial stratum (black = south, blue = west, red = north) for the historic series 1988-2020.



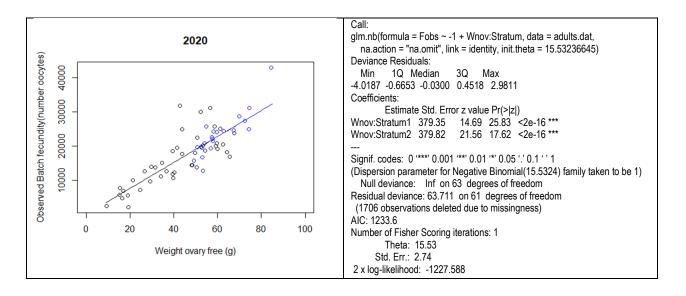
**Figure 6**. Abundance by age of eggs in the two spatial strata (black = south, blue = west) and its corresponding fitted mortality curve.



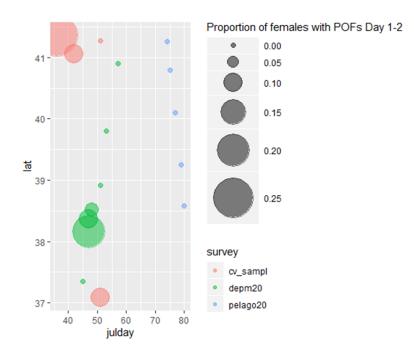
**Figure 7**. Total egg production (eggs/day\*10<sup>12</sup>) by spatial strata (black = south, blue = west, red = north) for the historic series 1988-2020. Dots and lines indicate the estimates of egg production and their confidence intervals.



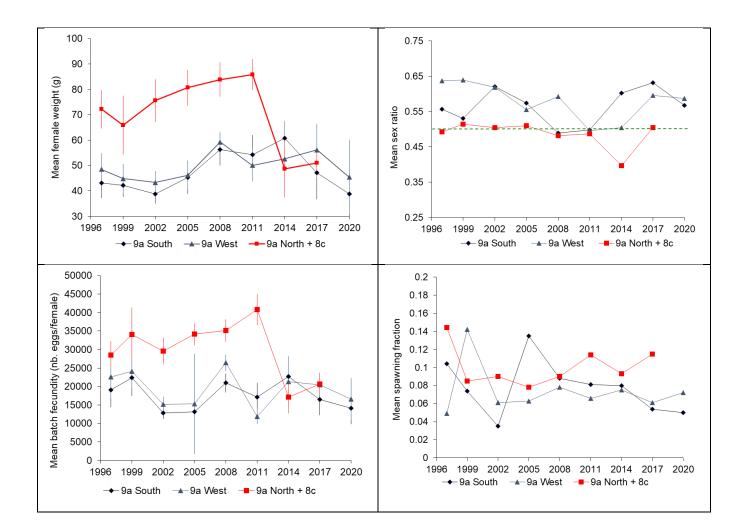
**Figure 8**. Sardine length (cm) (upper panel) and age (lower panel) composition of the females sardine sampled, per stratum (only were considered the fish randomly sampled).



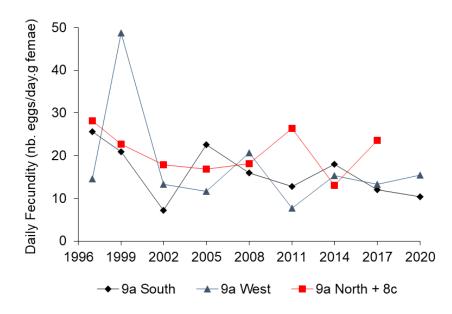
**Figure 9**. Observed batch fecundity vs. gonad free weight of the hydrated females, the regression lines of the corresponding model (left panel), and the results of the GLM obtained (right panel) for the South (black) and West (blue) strata.



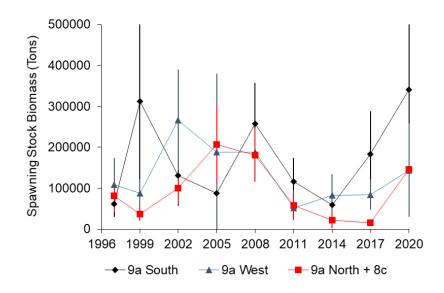
**Figure 10.** Proportion of females with POFs of Day-1 and Day-2 for each haul from the 9a West stratum, plotted against the date the fish were trawled (in Julian days) and the geographical location of the haul (in latitude coordinates), considering the three origins of the samples (DEPM survey, in green; PELAGO survey, in blue; commercial fleet, in pink); the size of the bubbles is linearly proportional to the plotted values.



**Figure 11.** Evolution over time (1997-2020) 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 and 8c - red); vertical lines indicate approximate 95% confidence intervals (i.e.,  $\pm 2$  standard-deviations).



**Figure 12**. Daily fecundity (nb. eggs/day.g female) by geographical stratum, for the historical series 1997-2020; black – 9a South, blue - 9a West, red – 9a North + 8c.



**Figure 13**. Spawning Stock Biomass (Tons) by geographical stratum for the historical series 1997-2020; black – 9a South, blue - 9a West, red – 9a North + 8c. Dots and lines indicate the estimates of SSB and their confidence intervals. Due to the cancelation of SAREVA 0320 DEPM survey because of the COVID-19 pandemic, a "real" SSB estimated for the 9a North+8c stratum is not available, the estimate graphed here for this area results from the analyses described in WD, Díaz et al, 2020.

### ANNEXES:

**ANNEX 1.** Summary of plankton sampling for sardine DEPM surveys (9a South and West surveyed by IPIMAR/IPMA and 9a North and 8c surveyed by IEO). In 1990, only 9a North and 8c areas were sampled. In 2020, due to the pandemic situation, only 9a South and West were surveyed.

			Research	Transects and	PairoVET	Eggs	Max	Temp (°C)	Survey	Positive	CUFES	Eggs	Max eggs/m3
Year	Strata	Dates	¥1	Grid nm (transects x	Stations (% with		eggs/m2	-				CUFES	CUFES
			Vessel	stations)	eggs)	PairoVET	PairoVET	Min-Max	area (km2)	area (km2)	Stations	CUFES	CUFES
	9a South	28/03-30/03 01-08/03-21-	Noruega	15 (7x7)	55(25.5)	344	1680	14.5-17.2	9037	2144			
1988	9a West	28/03		42 (7x7)	249(35.7)	944	1360	12.8-16.1	39073	14889			
	9a North+8c	31/03-05/05	Cornide de Saavedra	68 (6x6-3)	516(51.7)	3922	2758.3	10.6-15.5	55492	26644			
	Iberian Peninsula			125	820(45.1)	5210	2758.3	10.6-17.2	103602	43676			
	9a South												
1990	9a West												
	9a North+8c	18/04-10/05	Investigador		475(36.6)	1494	2063.4	12.8-18.5	64185	30555			
	Iberian Peninsula												
	9a South	18/03-25/03	Noruega	29 (7x7)	135(43.0)	868	5593.8	16-19.3	19951	8745			
1997	9a West	01/03-16/03		39 (7x7)	238(16.0)	586	2012.3	14-16.9	37757	6696			
	9a North+8c	05/03-29/03	Cornide de Saavedra	44 (15 GAL,7.5 CANT x3)	515(16.7)	1465	5381	13.2-15.9	55870	10275			
	Iberian Peninsula			112	888(20.5)	2919	5593.8	13.2-19.3	113577	25716			
	9a South	10/01-19/01	Noruega	77 (6x6)	147(36.7)	3184	13431	14-17.1	20633	7451			
1999	9a West	19/01-03/02	Cornide de Saavedra	(6x6)	272(23.2)	1926	6060	12.6-16.3	36919	9829			
1999	9a North+8c	17/03-03/04		50 (15 GAL,7.5 CANT x3)	290(25.9)	900	1196.6	12.2-13.8	30316	7174			
	Iberian Peninsula				707(27.2)	6010	13431	12.2-17.1	87868	24454			
	9a South	27/01-02/02		53 (8x3-6)	152(32.2)	530	1733.4	14.5-16.9	16504	7702	168	2955	29.4
	9a West	08/01-27/01	Noruega	(8x3-6)	332(41.9)	2077	8328.2	12.1-16.8	34442	18711	375	8774	131
2002	9a North+8c	20/03-16/04	Cornide de Saavedra	36 (8x3-6)	220(58.6)	1939	1896.1	10.9-17.5	25476	15202	441	9669	40.6
	Iberian Peninsula				704(45)	4546	8328.2	10.9-17.5	76422	41615	984	21398	131
	9a South	13/02-22/02	Capricórnio	(8x3-6)	159(41.5)	1733	4825.6	13.1-15.4	17321	7201	186	4991	30.4
	9a West	29/01-12/02		(8x3-6)	249(32.9)	1942	8020	11.6-14.8	26808	10723	312	4278	55.6
2005	9a North+8c	13/04-01/05	Cornide de Saavedra	56 (8x3)	371(32.3)	3216	3231	12.4-16	38476	12307	323	9748	85
	Iberian Peninsula				779(34.4)	6891	8020	11.6-16	82605	30231	821	19017	85
	9a South	20/01-27/01		22 (8x3)	174(56.3)	5727	9842.5	14.8-17.1	18164	9692	181	10710	124.9
	9a West	28/01-15/02	Noruega	36 (8x3)	288(51.7)	7895	8142.4	13.3-16.7	30318	19296	315	19632	140
2008	9a North+8c	02/04-27/04	Cornide de Saavedra	56 (8x3)	426(54.2)	3788	8354.2	11.9-15.2	42381	24264	416	17225	162.7
	Iberian Peninsula	02/04-27/04	connuc uc suaveura	114	888(53.8)	17410	9842.5	11.9-17.1	90863	53252	912	47567	162.7
	9a South	10/02-20/02		21 (8x3)	170(31.8)	2208	4950	14.6-16.9	17578	6523	184	4607	81.7
	9a West	20/02-08/03	Noruega			833	2970	13.5-16.1	32098	4817	308	4007	6
2011			Comite to Come 1	36 (8x3)	309(12.9)	833						479	6 97.3
	9a North+8c	25/03-10/04	Cornide de Saavedra	56 (8x3)	337(38.6)		1537	12.5-14.6	33832	12405	291		
	Iberian Peninsula			113	816(27.5)	4835	4950	12.5-16.9	83508	23745	783	24914	97.3
	9a South	15-26/04	Noruega	20 (8x3)	134(46.3)	2019	5500	14.5-19.1	14558.7	6825	146	2695	78.3
2014	9a West	15-21/3; 4-15/4		38 (8x3)	265(38.1)	2164	1550	12.8-18.5	27357.3	11001	313	12709	61.7
	9a North+8c	29/3-9/4;16-21/4	Visconde de Eza	54 (8x3)	394(16.8)	313	704	12.3-14.9	38914.4	7495	339	2186	25.2
	Iberian Peninsula			112	793(28.9)	4496	7754	12.3-19.1	80830.5	25320	798	17590	78.3
	9a South 9a West	11-19/03	Noruega	23 (8x3)	173(46)	2958	5580	14.6-18.3	19104	8548	251	448	8.3
2017	9a West 9a North+8c	25/4-24/05 29/03-09/04	Visconde de Eza	28 (8x3 or 16x3) 53 (8x3)	177(25) 378(18)	922 343	1510	13.8-19.7	26768	12682	254	3408 1584	112.3
	Iberian Peninsula	29/05-09/04	+isconde de EZa	33 (0,3)	728(29)	4223	5580	12.6-14.2	82812	28857	838	5440	17.3
	9a South	05-17/02		21 (8x3)	190 (41)	5032	11720	14.9-17.7	18689	7844	250		ng underaway
2020	9a West	19-28/02	Visconde de Eza	37 (8x3)	300 (24)	3254	4330	13.5-16.3	29560	9127	356	-	ng underaway
2020	9a North+8c												
	9a South + 9a West			58	490 (31)	8286	11720	13.5-17.7	48249	16971	606		

**ANNEX 2.** Summary of adult sampling in Iberian Peninsula (9a South, 9a West and 9a North + 8c) sardine DEPM surveys. In 2020, due to the pandemic situation, only 9a South and West were sampled.

Year	Strata	Fishing hauls (% positive)	Total sardine sampled	Males	Females	Females for histology	Hydrated females	Mature females (%)
	9a South							
1000	9a West							
1988	9a North+8c							
	Iberian Peninsula							
	9a South							
	9a West							
1990	9a North+8c							
	Iberian Peninsula							
	9a South	12(83.3)	537	232	305	131	24	304(99.7)
	9a West	28(57.1)	804	298	506	142	6	506(100)
1997	9a North+8c		402	142	260	255	113	259(99.6)
	Iberian Peninsula	9(77.8) 49(67.3)	1743	672	1071	528	113	1069(99.8)
1999	9a South	12(100)	1208	536	672	151	19	624(92.9)
	9a West	28(100)	2732	1125	1580	283	86	1479(93.6)
	9a North+8c	19(57.9)	997	532	463	100	19	422(91.1)
	Iberian Peninsula	59(86.4)	4937	2193	2715	534	124	2525(93)
	9a South	31(96.8)	2416	934	1478	499	47	1462(98.9)
2002	9a West	43(93.0)	2811	1104	1472	576	66	1217(82.7)
	9a North+8c	28(100)	2058	1019	1039	470	69	1038(99.9)
	Iberian Peninsula	102(96.1)	7285	3057	3989	1545	182	3717(93.2)
	9a South	24(91.7)	1652	759	891	510	52	851(95.5)
2005	9a West	42(97.6)	2915	1323	1533	983	1	1366(89.1)
2005	9a North+8c	76(46.1)	1625	721	897	562	115	755(84.2)
	Iberian Peninsula	142(69)	6192	2803	3321	2055	168	2972(89.5)
	9a South	27(92.6)	1745	838	906	643	103	842(92.9)
	9a West	58(87.9)	3195	1352	1839	1371	76	1554(84.5)
2008	9a North+8c	41(87.8)	2392	1157	1235	594	183	1235(100)
	Iberian Peninsula	126(88.9)	7332	3347	3980	2608	362	3631(91.2)
	9a South	18(88.9)	975	480	495	397	11	495(100)
	9a West	40(80)	2069	1028	1037	827	25	954(92)
2011	9a North+8c	53(18.9)	718	334	384	230	31	380(99)
	Iberian Peninsula	111(52.3)	3762	1842	1916	1454	67	1829(95.5)
	9a South	17(94.1)	938		582	444		582(100)
	9a West			356			70	
2014	9a North+8c	47(70.2)	1635	969	666	705	21	646(97.0)
	Iberian Peninsula	57(26.3) 121(52.9)	755 3328	443 1768	624 1872	262 1411	119 210	624(100) 1540(98.7)
	9a South							
	9a West	19(74) 44(45)	987 1173	266 434	457 443	359 482	56 23	457(100) 443(96)
2017	9a North+8c	65(28)	1534	746	788	482	178	786(100)
	Iberian Peninsula	128(41)	3694	1446	1688	1323	257	1688(99)
	9a South	9 (88.9)	476	192	284	265	64	284 (97.9)
2020	9a West	18 (94.4)	1080	486	594	531	21	594 (94.3)
2020	9a North+8c							
	9a South + 9a West	27 (92.6)	1556	678	878	796	85	878 (95.4)

Veen	Etroto	Morta	ality	Pt	ot	W		R		F		S		SS	В
Year	S trata	Estim	C.V	Estim	C.V.	Estim	C.V.								
	9a South	-0.026	0.08	0.98	0.26										
1988	9a West	-0.019	0.07	1.93	0.10										
1900	9a North+8c	-0.018	0.09	4.15	0.07										
	Total Iberian Peninsula			7.06	0.06										
	9a South	-0.032	0.11	1.57	0.16	43.1	0.07	0.557	0.05	19062	0.12	0.104	0.13	61337	0.25
1997	9a West	-0.028	0.09	1.58	0.18	48.5	0.07	0.637	0.04	22569	0.13	0.049	0.18	108870	0.30
1997	9a North+8c	-0.022	0.07	2.28	0.14	72.2	0.05	0.493	0.14	28544	0.07	0.144	0.1	81180	0.24
	Total Iberian Peninsula			5.43	0.09									251387	0.16
	9a South	-0.029	0.09	6.53	0.19	42.1	0.05	0.531	0.03	22436	0.11	0.074	0.22	311982	0.32
1999	9a West	-0.022	0.06	4.28	0.15	44.9	0.06	0.639	0.05	24086	0.09	0.142	0.05	87832	0.20
1999	9a North+8c	-0.014	0.16	0.84	0.16	65.9	0.09	0.514	0.04	34137	0.1	0.09	0.09	37104	0.23
	Total Iberian Peninsula			11.66	0.12									436919	0.23
	9a South	-0.029	0.09	0.94	0.18	38.8	0.05	0.621	0.05	12881	0.06	0.035	0.19	130406	0.28
2002	9a West	-0.025	0.07	3.53	0.11	43.3	0.05	0.619	0.03	15212	0.07	0.061	0.18	265984	0.23
2002	9a North+8c	-0.018	0.08	1.78	0.11	75.6	0.05	0.505	0.08	29623	0.06	0.09	0.11	99989	0.20
	Total Iberian Peninsula			6.25	0.07									496379	0.15
	9a South	-0.021	0.07	1.96	0.16	45.4	0.07	0.574	0.11	13169	0.08	0.135	0.13	87103	0.26
2005	9a West	-0.018	0.08	2.18	0.12	46.2	0.06	0.556	0.06	15304	0.44	0.063	0.21	187676	0.51
2003	9a North+8c	-0.018	0.08	3.48	0.12	80.7	0.04	0.51	0.07	34147	0.04	0.078	0.17	206668	0.23
	Total Iberian Peninsula			7.63	0.08									481447	0.23
	9a South	-0.03	0.1	4.12	0.14	56.3	0.06	0.489	0.07	20956	0.06	0.088	0.08	257403	0.19
2008	9a West	-0.024	0.07	3.87	0.10	59.3	0.03	0.593	0.03	26424	0.04	0.078	0.1	187640	0.15
2000	9a North+8c	-0.018	0.09	3.27	0.09	83.9	0.04	0.482	0.06	35139	0.04	0.09	0.13	179983	0.18
	Total Iberian Peninsula			11.27	0.07									625026	0.11
	9a South	-0.028	0.09	1.49	0.16	54.3	0.07	0.498	0.09	17157	0.11	0.081	0.09	116566	0.24
2011	9a West	-0.021	0.07	0.40	0.18	50.1	0.06	0.496	0.04	11838	0.09	0.066	0.08	51502	0.23
2011	9a North+8c	-0.017	0.09	1.54	0.11	85.9	0.03	0.487	0.12	40844	0.05	0.114	0.26	58304	0.31
	Total Iberian Peninsula			3.43	0.09									226372	0.16
	9a South	-0.027	0.09	1.07	0.16	60.72	0.05	0.602	0.08	22673	0.07	0.080	0.15	59500	0.25
2014	9a West	-0.023	0.07	1.27	0.12	52.63	0.14	0.505	0.06	21322	0.16	0.075	0.19	82767	0.31
2014	9a North+8c	-0.016	0.12	0.29	0.14	48.70	0.11	0.397	0.15	17118	0.12	0.093	0.34	22346	0.43
	Total Iberian Peninsula			2.63	0.09		-				-			164613	0.19
	9a South	-0.026	7.3	2.18	0.14	47.22	0.11	0.632	0.06	16546	0.13	0.054	0.18	182486	0.29
2017	9a West	-0.026	6.9	1.11	0.16	56.2	0.09	0.596	0.06	20444	0.07	0.061	0.08	84099	0.22
	9a North+8c	-0.017	9.4	0.38	0.15	50.95	0.06	0.505	0.06	20698	0.07	0.115	0.16	16129	0.25
	Total Iberian Peninsula			3.68	0.1									282714	0.20
	9a South	-0.03	7	3.54	0.24	38.8	0.15	0.568	0.08	14176	0.15	0.05	0.22	341164	0.39
	9a West	-0.023	5.7	2.23	0.2	45.4	0.16	0.587	0.07	16637	0.17	0.072	0.24	143984	0.40
2020	9a North+8c														
	Total 9a South - 9a West			5.76	0.17									485148	0.30
	Total Iberian Peninsula													630692	0.30

**ANNEX 3:** Sardine DEPM surveys for the Atlanto-Iberian stock. Summary of the results for eggs, adults and SSB estimates.

Note: Eggs and adult parameters for the ICES subdivision 9a North and division 8c 9a are not available in 2020 due to the cancelation of SAREVA 0320 DEPM survey because of the COVID-19 pandemic. The total Iberian Peninsula SSB was estimated raising the Portuguese SSB index (9a South and 9a West) by 1.3 (see separate WD, Díaz et al, 2020, for the analyses that resulted in this index).