Anchovy Spawning Stock Biomass of the Gulf of Cadiz in 2020 by the DEPM

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1. Introduction

The Daily Egg Production Method (DEPM) to estimate the Anchovy Spawning Stock Biomass (SSB) in the Gulf of Cádiz (ICES, Subdivision 9a South) is conducted by Spain (Centro Nacional Instituto Español de Oceanografía, CSIC) every three years, since 2005.

BOCADEVA 0720 is the sixth survey of the historical DEPM series for anchovy in the Gulf of Cádiz and was delivered on board R/V Ramón Margalef (CNIEO) from the 9th to the 17th of July 2020. The surveyed area extended from Strait of Gibraltar to Cape San Vicente (Spanish and Portuguese waters in the Gulf of Cadiz).

Plankton samples, along a grid of 21 transects perpendicular to the coast were obtained for the spawning area delimitation and density estimation of the daily egg production. The survey objectives also included the characterization of the oceanographic and meteorological conditions in the study area. The samples to estimate adult parameters (sex ratio, female mean weight, batch fecundity and spawning fraction) were obtained in the acoustic survey "ECOCADIZ 2020-07", carried out during the same period.

This working document provides a description of the survey, laboratory analysis and estimation procedures used to obtain the Gulf of Cadiz Anchovy SSB by DEPM for 2020 in the South-Atlantic Iberian Stock.

2. Methodology

Table 1 summarized a description of the methodology used to obtain egg (BOCADEVA 0720) and adult (ECOCADIZ 202007) samples.

Table 1. BOCADEVA 0720 & ECOCADIZ 2020-07. General sampling information.

Eggs	**Anchovy DEPM survey BOCADEVA 0720**
Survey area	(36º13'-36º50'N –6º07'-–8º55'W)
R/V	R/V Ramón Margalef
Date	9th to 17th July
Transects (Sampling grid)	21 (8x3)
Pairovet stations (150 □m)	162
Sampling maximum depth (m)	100
Hydrographic sensor	CTD SBE 911
Flowmeter	Yes
CUFES stations	152
CUFES (335 □m)	3 n miles (sample unit)
Environmental data	Temperature and Salinity
Adults	**Acoustic survey ECOCADIZ 2020-07**
Survey area	(36º11'-36º47'N –6º12'-–8º54'W)
R/V	R/V Miguel Oliver
Date	31th July to 13th August 2020
Gears	Pelagic trawl (63.5/51 Tuneado gear)
Trawls	30: 29 valid: - 25 for echo-traces identification; - 4 to collect hydrated females
Trawls time	From 06:58 to 21:19 hrs GMT (At night; 4 hauls targeted hydrated females)
Biological sampling:	On fresh material, on board of the R/V
Sample size	At least 60 individuals randomly picked; up to 120 (adding batches of 10 randomly picked anchovies) if a minimum of 30 mature females were not found for spawning fraction estimation. A minimum of 150 hydrated females for batch fecundity estimation.
Fixation	4% Phosphate buffered Formaldehyde
Preservation	4% Phosphate buffered Formaldehyde

2.1. Surveying

2.1.1. Plankton sampling

The strategy of egg sampling was identical to that used in previous BOCADEVA surveys. An adaptive sampling was carried out in the East - West direction using a PairoVET net in fixed stations as main sampler and a continuous recording with CUFES (*Continuous Underwater Fish Egg Sampler*) as secondary sampler (Figure 1).

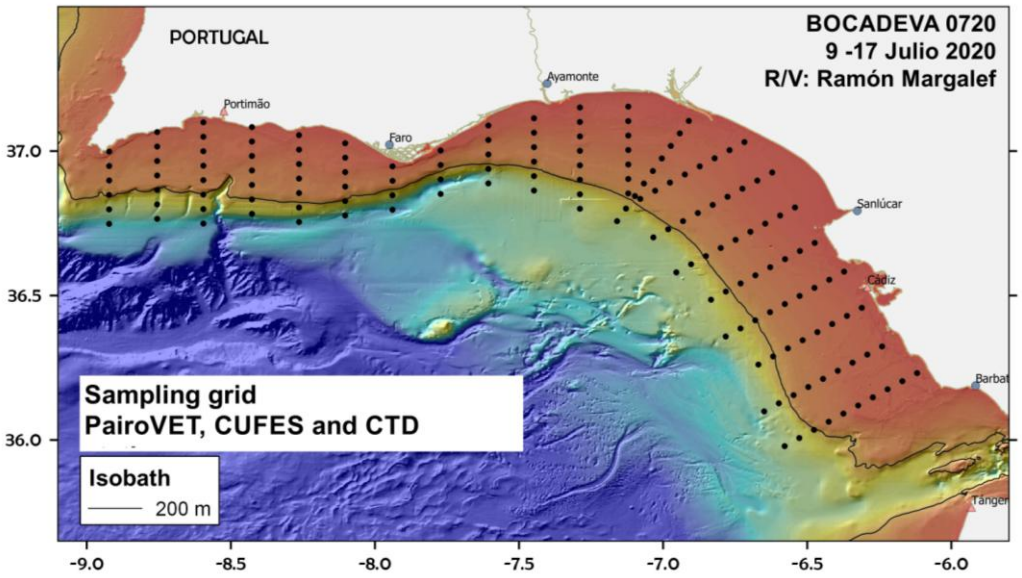


Figure 1. BOCADEVA 0720. Sampling grid. Planned plankton and CTD stations.

- *Vertical sampling (PairoVET)*

The sampling grid was established on the continental shelf following a systematic sampling scheme, with transects perpendicular to the coast and spaced 8 nm. Egg samples were always taken every 3 nm in the inner shelf (ICES, 2003). The inshore limit of transects was determined by bottom depth (as close to the shore as possible), while the offshore extension was decided adaptively depending on the results of the most recent CUFES sample.

Vertical hauls were carried out with a PairoVET sampler equipped with nets of 150µm mesh size. Hauls were carried out up to a maximum depth of 100m or of 5m above the bottom in shallower depths, speed of about 1 m/s. Sampling depth were recorded using an underwater positioning systems HiPAP 500 Kongsber fitted to the net. Flowmeters were used to calculate the volume of filtered water during each haul.

Due to COVID-19 constraints, the scientific crew on board was reduced and consequently, the PairoVET samples were not processed on board as usually. Samples were preserved in a 4 % buffered formaldehyde solution.

- *Continuous sampling (CUFES)*

The CUFES sampling (Checkley et al., 1997) is fixed at 5m depth. The volume of filtered water (600 l/min, approximately) was also integrated each 3 nm. The CUFES collector was arranged with a 335 µm net.

In order to decide the offshore extension of the transects, the CUFES samples collected over the 200 meters depth, were inspected on board to determine the presence/absence of the anchovy eggs. CUFES samples were all preserved in a 4 % buffered formaldehyde solution.

2.1.2. Adult fish surveying

Adult Anchovy samples for DEPM were obtained during the ECOCADIZ 2020-07 survey from pelagic trawl hauls (See Ramos et al., 2020).

Except for searching Anchovy females with hydrated gonads, fishing stations were mostly conducted during daylight hours and carried out over isobath, once echotraces supposedly belonging to Anchovy were detected by echo-sounder.

For the estimation of spawning fraction (S), a minimum of 30 mature, non-hydrated females per sample is sought, so a minimum of 60 random anchovies are sampled, adding batches of 10 random individuals to the sampling until the goal is achieved or a maximum of 120 anchovies are sampled. Sex-ratio (R), along with other parameters used in the DEPM is also obtained from this random sampling.

When hydrated females (HF) appeared, an additional sampling was done in order to obtain a minimum of 150 HF for the whole area prospected. Gonads from both hydrated and non-hydrated females were preserved in 4% buffered formaldehyde solution.

2.1.3. Hydrography

A CTD SBE 911 was used in each station to take temperature, salinity, fluorescence and oxygen of the water column. A continuous sampling of sea surface temperature and salinity don't was possible carry out, due the termosalinograf of the vessel was broken.

2.2. Laboratory analysis

2.2.1. Plankton samples

At the laboratory, PairoVET samples are sorted. Anchovy eggs and larvae from the two nets of the PairoVET samples are identified and counted, as well as other commercial species. The anchovy eggs are classified into 11 stages of development classification according to the key proposed by Moser and Ahlstrom (1985).

CUFES samples have been sorted and anchovy eggs are counted and classified in three development stages: No-Embryo (I-III), Early Embryo (IV-VI) and Late Embryo (VII-XI).

2.2.2. Adult fish samples

The preserved ovaries on board are then processed for histology at the laboratory; they are embedded in resin and the histological sections are stained with haematoxylin and eosin. The slides are examined and scored for their maturity stage (most advanced oocyte batch) and post ovulatory follicles (POFs) presence and age assignment to daily cohorts (Hunter and Macewicz 1985). Prior to fecundity estimation, hydrated ovaries are also processed histologically to check for POF presence and thus avoid underestimating fecundity. The individual batch fecundity is then measured, by means of the gravimetric method applied to the hydrated oocytes, on one to three whole mount sub-samples per ovary, weighing on average 50-150 mg (Hunter et al. 1985).

2.3. Data analysis

2.3.1. Egg Production (z, P_0 and P_{tot}) estimation and area calculation

All calculations for area delimitation, egg ageing and model fitting for egg production (P_0) estimation were carried out using the R packages *geofun, spatstat, eggsplore and shachar* available at *ichthyoanalysis* (http://sourceforge.net/projects/ichthyoanalysis).

The surveyed area (*A*) was calculated as the sum of the area represented by each station. The spawning area (*A+*) was delimited with the outer zero Anchovy egg stations, and was calculated as the sum of the area represented by those stations.

The model of egg development with temperature was derived from the incubation experiment carried out in Cádiz in July 2007 (Bernal *et al*., 2012). A multinomial model was applied (Ibaibarriaga *et al.,* 2007, Bernal *et al.,* 2008) considering only the interaction Age*Temp (other interactions were not significant).

$$N_{i,t} \sim \text{Mult}\,(N, p_{i,t})$$

$$p_{i,t} = f\,(\text{Age}, \text{Temp})$$

Egg ageing was performed by a multinomial Bayesian approach described by Bernal *et al*. (2008) and using *in situ* SST; a normal probability distribution was used with peak spawning assumed to be at 22:00h with 2h standard deviation. This method uses the multinomial development model and the assumption of probabilistic synchronicity (assuming a normal distribution).

$$p(age \mid stage, temp, time) \propto p(stage \mid age, temp)\; p(age \mid time)$$

ageing — development model — synchronicity

Daily egg production (P_0) and mortality (z) rates were estimated by fitting an exponential mortality model to the egg abundance by cohorts and corresponding mean age. The model was fitted using a generalized linear model (GLM) with negative binomial distribution. The ageing process and the GLM fitting were iterative until the value of z converged.

[depm.control (spawn.mu=22; how.complete=0.95; spawn.sig=2), initial z = 0.01].

$$P_{age} = P_0\, e^{-z\,age}$$

$$\log\left(\frac{N_{age}}{area}\right) = \log(P_0) - z\,age \;\rightarrow\; \log(N_{age}) = \log(area) + \log(P_0) - z\,age$$

Finally, the total egg production was calculated as: $P_{tot} = P_0\, A+$

2.3.2. Adult parameters

The adult parameters estimated for each fishing haul considered only the mature fraction of the population (determined by the fish macroscopic maturity data).

Before the estimation of the mean female weight per haul (W), the individual total weight of the hydrated females was corrected by a linear regression between the total weight of non-hydrated females and their corresponding gonad-free weight (Wnov).

The sex ratio (R) in weight per haul was obtained as the quotient between the total weight of females and the total weight of males and females.

The expected individual batch fecundity for all mature females (hydrated and non-hydrated) was estimated by modelling the individual batch fecundity observed (Fobs) in the sampled hydrated females and their gonad-free weight (Wnov) by a GLM.

The spawning fraction (*S*) is currently being determinate by histological analysis of the post-ovulatory follicles, POFs (Hunter and Macewicz, 1985). The fraction of females spawning per day (S) is determines, 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).

The mean and variance of the adult parameters for all the samples collected was then obtained using the methodology from Picquelle and Stauffer (1985; *i.e.*, weighted means and variances). All estimations and statistical analysis were performed using the R software.

2.3.3. Spawning Stock Biomass

The Spawning Stock Biomass (SSB) was computed according to:

$$SSB = \frac{P_{total} * W}{F * S * R}$$

Due to the COVID19 pandemic, and the consequent remote work, the histological processing of the gonad samples for the reading of POFs and estimation of the S has not been possible to finish it yet. So, the mean value of spawning fraction (S) of the historical series has been preliminary used to estimate the SSB for 2020.

3. Results

3.1. Sampling

3.1.1. Distribution and abundance of Anchovy eggs by PairoVET

The surveyed area (16223 km²) extends from Cabo de Trafalgar (Spain) to Cabo de San Vicente (Portugal). This area includes the continental shelf of the Gulf of Cadiz. The survey was carried out from East to West, starting in the radial 1- station 1, located close the Strait of Gibraltar (Figure 2).

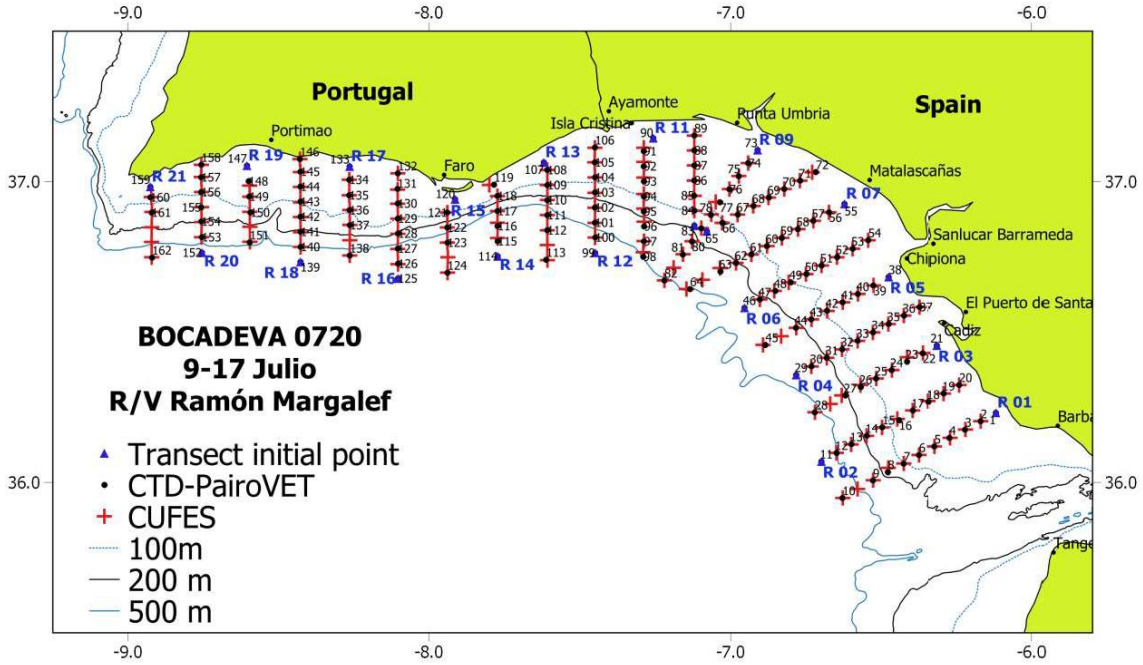


Figure 2. BOCADEVA 0720 survey. PairoVET, CUFES and CTD stations.

The icthyoplankton sampling almost covered the whole 24 hours' day-time period. A total of 162 PairoVET stations were carried out. In 86 stations (53%) there was presence of Anchovy eggs (positive stations). A total of 2916 Anchovy eggs were caught, and a maximum density (in number/m²) of 6161.6 was obtained (Table 2).

Table 2. BOCADEVA 0720. Anchovy eggs (number and density) by PairoVET.

By PairoVET	Anchovy eggs
N stations	162
N positive stations	86
N total eggs	2916
N máximum eggs	530
Total density (eggs/m²)	33874
Maximum density (eggs/m²)	6161

Temperature ranged between 17.16 and 24.70 degree (mean 21.35 degree), similar that 2017. Anchovy eggs presented a patched distribution along the area (Figure 3). The highest abundances were found in a station located close to Guadalquivir (6161 eggs/m^2) and 5 stations along the area registered densities higher than 1000 eggs/m^2. The station where were registered the maximum abundance is 107 m depth, in front of the Guadalquivir River, and the temperature 21.82 ºC.

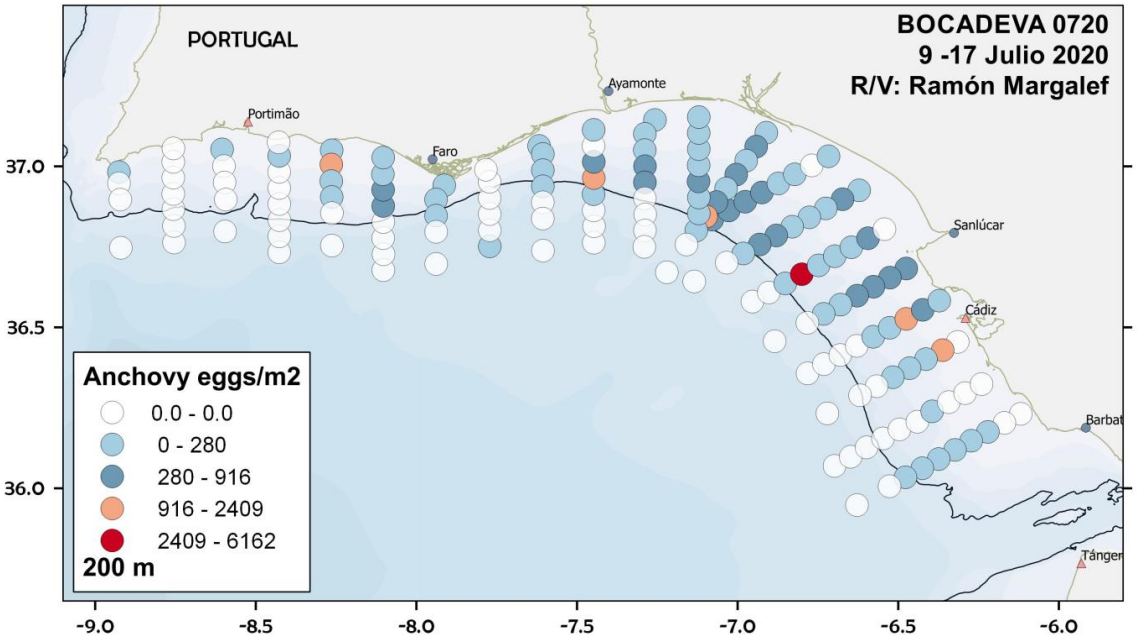
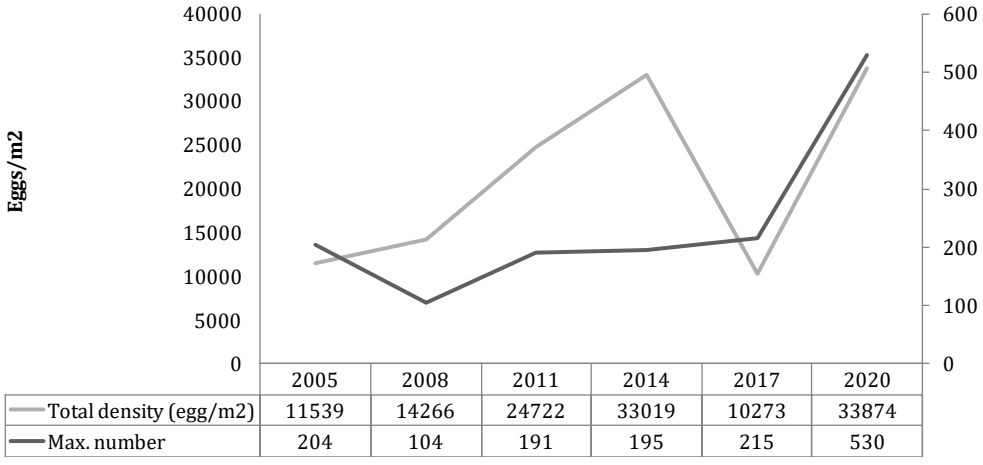


Figure 3. BOCADEVA 0720. Abundance distribution of Anchovy eggs density (eggs/m^2) by PairoVET.

The highest estimates of maximum egg density by station and total egg density as sampled by PairoVET were recorded during the 2020 survey (Figure 4).



	2005	2008	2011	2014	2017	2020
Total density (egg/m2)	11539	14266	24722	33019	10273	33874
Max. number	204	104	191	195	215	530

Figure 4. BOCADEVA 0720. Total Anchovy egg density and maximum number by PairoVet station

98% of Anchovy eggs were classified into 11 stages according to the degree of embryonic development (Figure 5). The most abundant were stage II (38.5%).

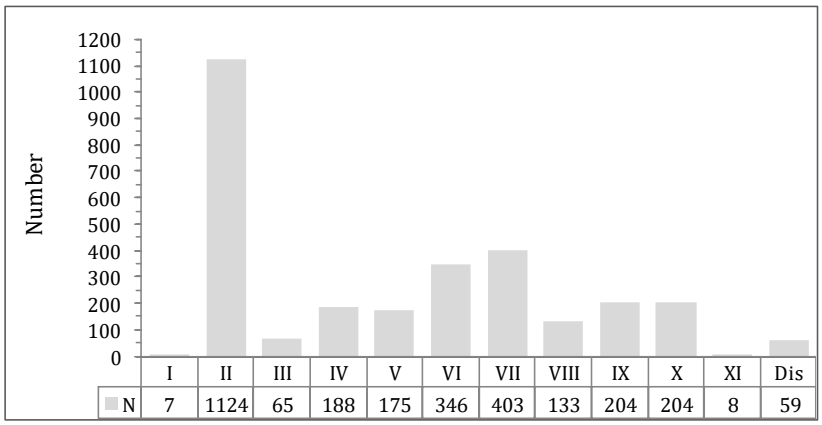


Figure 5. Number of Anchovy eggs by developmental stage.

Eggs in Stage I were caught mainly at 23:25 GMT, coincident to the peak spawning for the species in the area (22:00 GMT ± 2h) and the egg development model (Bernal et al., 2012).

A total of 152 stations by CUFES were carried out, 69% positive. A total of 36476 eggs of anchovy were caught, the third highest value in the historical series (2005-2020).

3.1.2. Adults. Results of the pelagic hauls

The samples to estimate adult parameters (sex ratio, female mean weight, batch fecundity and spawning fraction) were obtained in the acoustic survey "ECOCADIZ 2020-07" (see Ramos et al., 2020).

A total 21 fishing hauls which caught anchovies were performed during the acoustic survey, complemented by 4 samples conducted by night aimed at the collection of anchovy females with hydrated egg (Figure 6).

On the whole, almost 1691 anchovys were sampled, more than 686 ovaries were collected and 1677 otoliths were removed for age determination. A total of 180 hydrated females were caught for batch fecundity estimation.

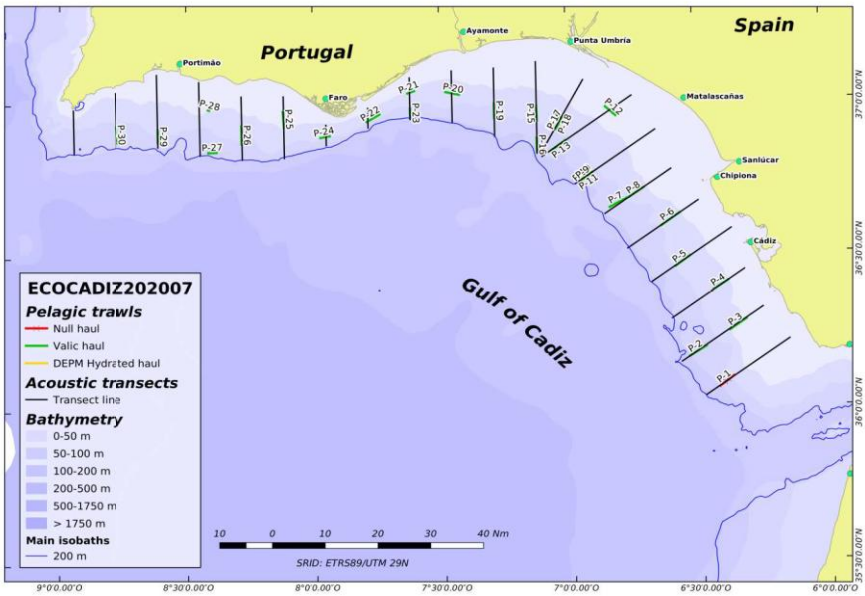


Figure 6. Spatial distribution of fishing hauls in ECOCADIZ 2020-07 survey.

3.2. Data analysis

3.2.1. Eggs parameters

The cumulative plot of the total density and temperature by range of temperature is show in Figure 7. The temperature at 5 m depth has been used for the estimates. Daily egg production (P_0) and mortality (z) rates were estimated by fitting an exponential mortality model to the egg abundance by cohorts and corresponding mean age (Figure 8). The model was fitted using a generalized linear model (GLM) with negative binomial distribution (Table 3 , Figure 9). The ageing process and the GLM fitting were iterative until the value of z converged. [depm.control (spawn.mu=22; how.complete=0.95; spawn.sig=2), initial z = 0.01].

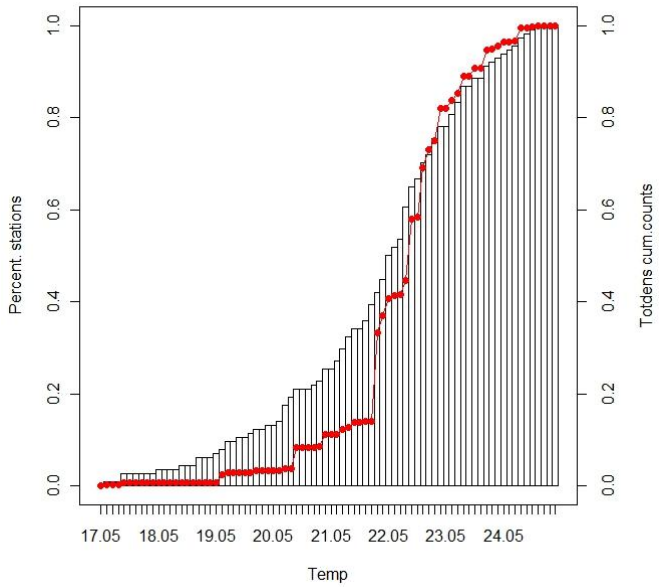


Figure 7. Anchovy DEPM Gulf of Cadiz 2020. Cumulative plot of total density and temperature by range of temperature (inter=0.1)

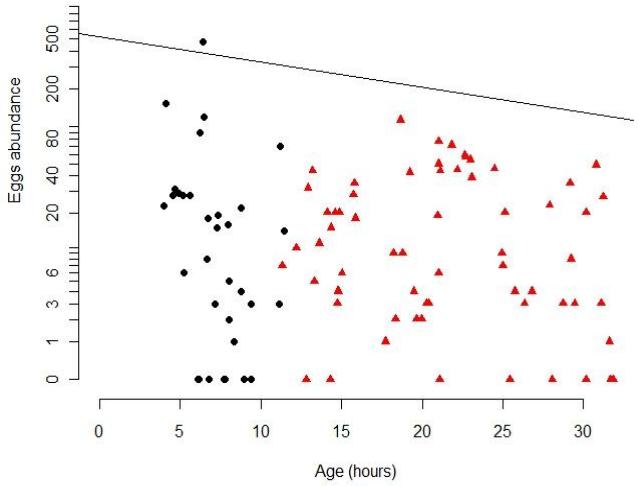


Figure 8. Anchovy DEPM Gulf of Cadiz 2020. Exponential mortality model by cohort.

Eggs mortality rate was negative, but not significant, and a high CV was observed. The results show that 67% of the eggs dying per day. The CV associated to total egg production 0.38. A post-stratified estimation was made, but the obtained results were not better.

Table 3. Anchovy DEPM Gulf of Cadiz 2020. Egg production and mortality rate. Selected Generalized Lineal model (GLM).

```
glm.nb(formula = cohort ~ offset(log(Efarea)) + age, data = aged.data,
    weights = Rel.area, init.theta = 0.438879629406381, link = log)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.4320  -1.0075  -0.4033   0.1832   2.7494

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  6.26032    0.38716  16.170   <2e-16 ***
age         -0.04639    0.02086  -2.224   0.0261 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Negative Binomial(0.4389) family taken to be 1)

    Null deviance: 102.476  on 96  degrees of freedom
Residual deviance:  97.134  on 95  degrees of freedom
AIC: 640.14

Number of Fisher Scoring iterations: 1


              Theta:  0.4389
          Std. Err.:  0.0668

 2 x log-likelihood:  -634.1440
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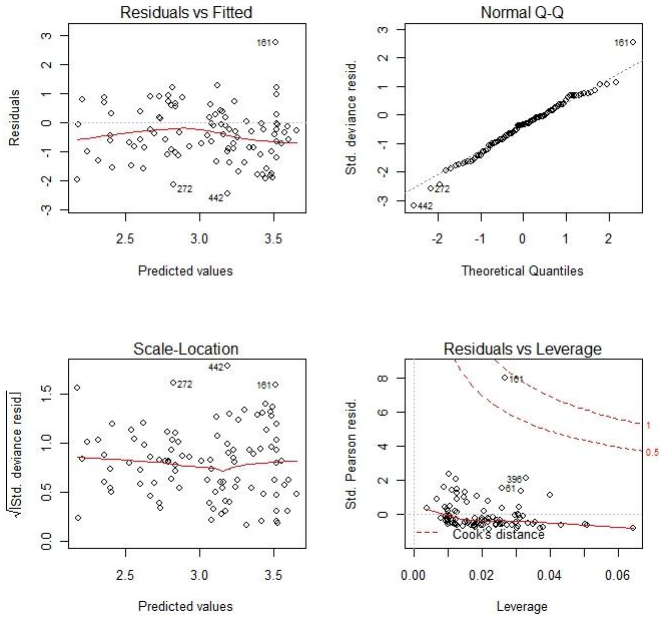

Figure 9. Residual inspection plots for the Generalized Linear Model fitted to Anchovy egg production data (Gulf of Cadiz 2020).

3.2.2. Adult parameters by haul

The total weight of hydrated females was corrected for the increase of weight due to the hydration process by a linear regression model between individual data of gonad-free-weight (Wnov) and its corresponding total weight (Wt) from non-hydrated females (Table 4, Figure 10). The expected female weight (Wexp) for all mature females was also estimated using this linear regression model.

Table 4. Anchovy DEPM Gulf of Cadiz 2020. Results of the linear regression model for the relationship between non-hydrated females total weight (Wt) and ovary-free weight (Wnov).

```
lm(formula = Wt ~ Wnov, data = adults.dat[which.weight, ])

Residuals:
       Min         1Q     Median         3Q        Max
-1.3420683 -0.1295539  0.0004695  0.1142538  1.9115503

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.218788   0.023099  -9.472   <2e-16 ***
Wnov         1.060947   0.001259 842.365   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.2957 on 703 degrees of freedom
Multiple R-squared: 0.999,      Adjusted R-squared: 0.999
F-statistic: 7.096e+05 on 1 and 703 DF,  p-value: < 2.2e-16
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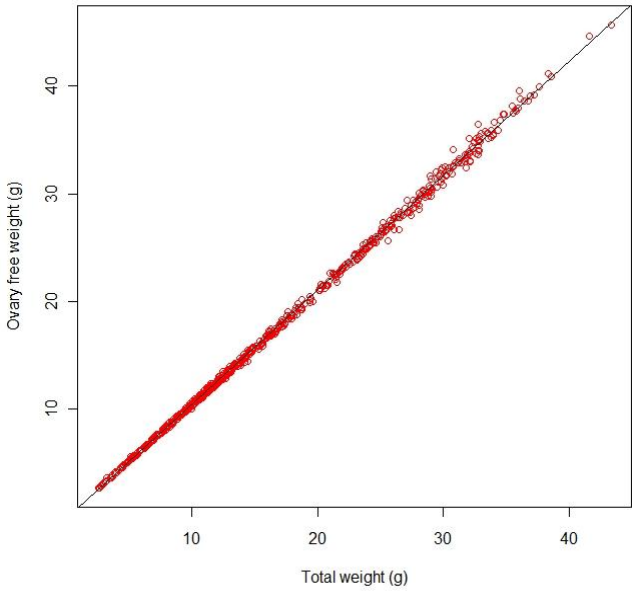

Figure 10. Anchovy DEPM Gulf of Cadiz 2020. Plot of the linear regression model for the relationship between non-hydrated females total weight (Wt) and ovary-free weight (Wnov).

The expected batch fecundity for all mature females (Fexp) was estimated by modelling the observed individual batch fecundity (Fobs) in hydrated females in function of their gonad-free-weights (Wnov) by a GLM model (Figure 11). Results of this model and the residual inspection plots are shows in Table 5 and Figure 12.

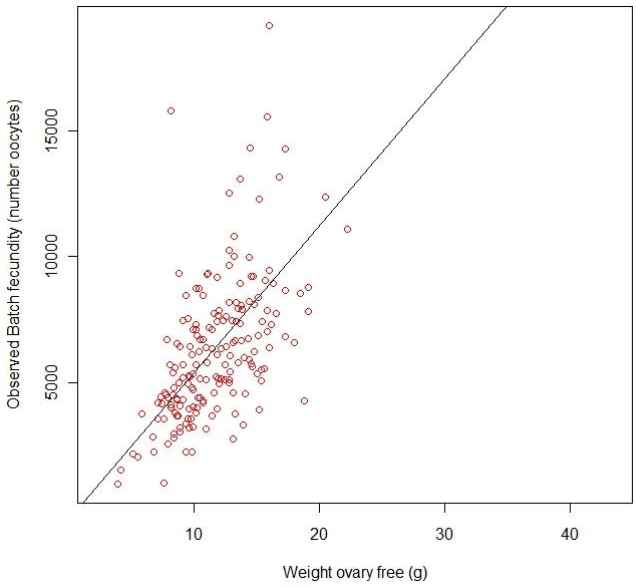

Figure 11. Anchovy DEPM Gulf of Cadiz 2020. Generalized linear model for the relationship between observed individual batch fecundity (Fobs) and ovary-free weight (Wnov).

Table 5. Anchovy DEPM Gulf of Cadiz 2020. Batch fecundity. Selected Generalized lineal model (GLM).

```
glm.nb(formula = Fobs ~ Wnov, data = adults.dat, na.action = "na.omit",
    link = identity, init.theta = 8.1304800390389)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-3.1789  -0.7723  -0.1586   0.4279   4.7313

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -466.80     440.43   -1.06    0.289
Wnov          584.34      44.13   13.24   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Negative Binomial(8.1305) family taken to be 1)

    Null deviance: 296.95  on 179  degrees of freedom
Residual deviance: 183.69  on 178  degrees of freedom
  (1506 observations deleted due to missingness)
AIC: 3260.2

Number of Fisher Scoring iterations: 1

              Theta:  8.130
          Std. Err.:  0.841

 2 x log-likelihood:  -3254.211
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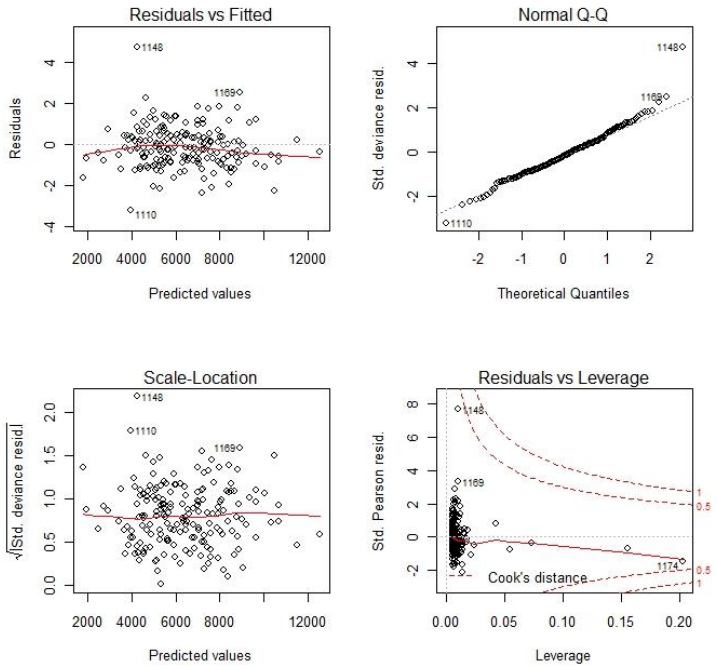

Figure 12. Anchovy DEPM Gulf of Cadiz 2020. Residual inspection plots for the Generalized Linear Model fitted to the Anchovy batch fecundity data.

3.2.3. Preliminary SSB 20202

The total spawning area (*A+*) was: 10058 km^2 (62% of the total area). In order to obtain a preliminary estimate of the SSB for 2020, the S mean value of the historical series has been used.

The results and their associated CV for the egg and adult parameters, and the preliminary SSB are summarized in the Table 6. A total Spawning Stock Biomass of 81466 tons (CV 0.43) has been estimated, the higher value of the historical series (Table7).

Table 6. Anchovy DEPM Gulf of Cadiz 2020. Summary of the results for egg and adult parameters and the preliminary SSB estimates (CVs in brackets).

Parameters	**Gulf of Cádiz 2020**
Eggs	
P_0 (eggs/m^2/day)	523.4 (0.38)
Z (day^{-1})	-1.112 (0.44)
P_{tot} (eggs/day) (x10^{12})	5.264 (0.38)
Spawning area (Km^2)	10058
Adults	
Female Weight (g)	16.63 (0.12)
Batch Fecundity	8212 (0.13)
Sex Ratio	0.54 (0.009)
Spawning Fraction*	0.241*
SSB	
Spawning Stock Biomass 1 (tons) (CV)	81466 (0.43)

*mean value of the historical series

Table 7. Anchovy SSB in the Gulf of Cadiz by DEPM. Historical series.

Year	2005	2008	2011	2014	2017	2020
Eggs						
P_0 (eggs/m^2/day) (CV)	50.8(0.80) / 224.5(0.69)	184(0.44) / 348(0.35)	276 (0.32)	313.5 (0.34)	145,8 (0,55)	523,4 (0.38)
Z (day^{-1}) (CV)	-0.039(0.75)	-1,43(0,29)	-0.29 (1.14)	-0.33 (1.19)	-0,16	-1.11 (0.44)
P_{total} (eggs/day) ($x10^{12}$) (CV)	0.07(0.76) / 1.06(0.65)	0.31(0.44) / 1.80(0.35)	1.87 (0.36)	1.95 (0.34)	0,74 (0,55)	5.26 (0.38)
Surveyed area (km2)	11982	13029	13107	14595	15556	16223
Positive area (Km^2)	6139	6863	6770	6214	5080	10058
Adults						
Female Weight (g) (CV)	25.2(0.03) / 16.7(0.04)	23.67 (0.06)	15.2 (0.11)	18.22 (0.08)	16.14 (0.17)	16.63 (0.13)
Batch Fecundity(CV)	13820(0.05) / 11160(0.05)	13.778 (0.07)	7486 (0.12)	7502 (0.08)	7507 (0.17)	8212 (0.14)
Sex Ratio (CV)	0.53(0.01) / 0.54(0.01)	0.528 (0.005)	0.531 (0.007)	0.54 (0.008)	0.53 (0.009)	0.54 (0.009)
Spawning Fraction (CV)	0.26(0.07) / 0.21(0.07)	0.218 (0.065)	0.276 (0.036)	0.276	0.234 (0.06)	0.241(*)
SSB						
Spawning Biomass –tons (CV)	14673	31527(0.32)	32757 (0.40)	31569 (0.30)	12392 (0.61)	81466 (0.43)

* Mean value of the historical series

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REFERENCES

Bernal, M., M.P. Jiménez and J. Duarte, 2011. Anchovy egg development in the Gulf of Cádiz and its comparison with development rates in the Bay of Biscay. *Fisheries Research*.

Bernal, M., Ibaibarriaga, L., Lago de Lanzos, A., Lonergan, M.E., Hernandez, C., Franco, C., Rasines, I., Valdes,
L. and Borchers, D.L., 2008. Using multinomial models to analyse data from Iberian sardine egg incubation experiments: a comparison with traditional techniques. *ICES J. Mar. Sci*., 65:51–59.

Checkley, D. M., Ortner, P. B., Settle, L. R., and Cummings, S. R. 1997. A continuous, underway fish egg sampler. Fisheries Oceanography, 6: 58-73

Díaz, P., MP. Jiménez and M.M. Angélico, 2015. Anchovy egg mortality in the Gulf of Cadiz and its application to egg production estimation. 39th Larval Fish Conference. Viena (Austria), del 13 al 17 de Julio de 2015

Díaz, P., P. Jiménez and MM Angélico, 2015. Implementing an external egg mortality model to estimate egg production for anchovy in the Gulf of Cadiz. ICES Working Group on Acoustic and Egg Surveys for Sardine and Anchovy in ICES Areas VIII and IX (WGACEEG). Lowestoft (UK), 16- 20 November 2015

Hunter, J.R., B.J. Macewicz, 1985. Measurement of spawning frequency in multiple spawning fishes. *In* : Lasker (ed.) An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: application to the northern Anchovy, *Engraulis mordax*. *NOAA Tech. Rep. NMFS* 36: 7-16 pp.

Hunter, J.R., N.C.H. Lo, R.J.H. Leong, 1985. Batch fecundity in multiple spawning fishes. *In*: Lasker (ed.) An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: application to the northern Anchovy, *Engraulis mordax*. *NOAA Tech. Rep. NMFS* 36: 66-77 pp.

Ibaibarriaga, L., Bernal, M., Motos, L., Uriarte, A., Borchers, D.L., Lonergan, M.E., and Wood, S.N. 2007. Characterization of stage-classified biological processes using multinomial models: a case study of Anchovy (*Engraulis encrasicolus*) eggs in the Bay of Biscay. Canadian Journal of Fisheries and Aquatic Sciences, 64:539–553.

ICES, 2003. Report of the Study Group on the Estimation of Spawning Stock Biomass of Sardine and Anchovy (SGSBSA),

ICES 2006. Report of the Working Group on Acoustic and Eggs Surveys for Sardine and Anchovy in ICES Areas VIII and IX (WGACEGG). ICES Living Resources Committee. ICES CM 2006/LCR:18. Ref.ACFM.

Jiménez, M.P., M. Millán, M. Bernal, G. Costas, 2005. Estimación de la biomasa de reproductores del stock de *Engraulis encrasicolus* del Golfo de Cádiz por el Método de Producción Diaria de Huevos (MPDH). Informe de incidencias y resultados preliminares. Documento interno IEO. 62 pp.

Moser H.G, E.H Ahlstrom, 1985 Staging Anchovy EggsIn: R. Lasker (editor), An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application t o theNorthern Anchovy, Engraulis mordax. NOAA Tech. Rep. NMFS 36. US. Dep. Commer., Wash., D.C., 99 p.

Picquelle, S., Stauffer, G., 1985. Parameter estimation for an egg production method of northern Anchovy biomass assessment. In: Lasker (ed.) An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: application to the northern Anchovy, *Engraulis mordax*. NNAA Tech. Rep. NMFS 36: 43-50 pp.

Ramos, F., Tornero, J. and Farias, C., 2020. 1Acoustic assessment and distribution of anchovy, sardine and chub mackerel in ICES Subdivision 9a South during the ECOCADIZ 2020-07 Spanish survey (August 2020) with notes on the distribution of other pelagic species.