WD. Calculation of mackerel adult parameters for the application of the DEPM in the western spawning area .

by
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1. Introduction

Following the decision in the WKMSPA (ICES, 2012) of calculate mackerel biomass using both, the Annual Egg Production Method (AEPM) and the Daily Egg Production Method (DEPM), during the 2019 Mackerel and Horse Mackerel Egg Survey DEPM method was implemented simultaneously with the AEPM as had been done in 2013 and 2016. Thus, this is the third in the five-point series to compare AEPM and DEPM methods. The calculation of the Total (annual) Fecundity following the DEPM is based in the batch fecundity and the number of spawning events per year.

Sampling at sea, histological processing, image analyses and aging of postovulatory follicles were carried out by the laboratories of the countries involved in the 2019 Egg Survey following the WGMEGS Survey Manual (ICES, 2019a) & Fecundity manual (ICES, 2019b), yielding biological data of all the sampled fish, oocytes diameter measurements and age of post ovulatory follicles (POFs) of the sampled ovaries as raw data that were used in this work to calculate the DEPM adult parameters.

The daily method requires an intensive sampling for DEPM adult parameters calculation as it's very difficult to achieve the planned number of samples because of sea conditions and other unforeseen events. Ovary samples taken for batch fecundity and spawning fraction calculation are chosen according to macroscopic maturity criteria and have to be screened to test this suitability for each analysis. The number of samples collected for batch fecundity is subsequently reduced after histological screening looking for valid samples showing a defined batch.

This document describes the methodology used for the calculation of the adult parameters, that is, the average female weight, the sex ratio, the batch fecundity, and the spawning fraction from the raw data calculated in the different institutes and the decrease of the number of samples looking for those that are valid for each of the analyses. The results of the DEPM carried out in the 2016 egg surveys are also shown.

2. Material and methods

2.1 Adult sampling

From the sampling schedule described in the survey manual (ICES, 2019,a), only mackerel was caught in 67 hauls of those planned. The optimum number of fish to sample per haulis 100 individuals,randomly selected, to take total size, weight, sex and asess maturity. Those data are used for sex ratio (R) and spawning fraction (S) estimations. Many of the 67 hauls reached the 100 fish sampled, being the Cantabrian Sea, the Gulf of Biscay and the South of Irelandthe areas whith the best sampling coverage (Figure 1b). Sampling was more intense during the spawning season (periods 3 and 4) as can be seen in the map of the Figure 1. A total of 4324 mackerel were sampled. The 59% of the sampled fish were captured during the peak of spawning (periods 3 and 4) and latitudes from 41º to 50 °N (Table 1).

Table 1. Number of total fish (male and females) sampled by period and latitude. The proportion of the total fish is also shown.

	Period 2		**Period 3**		**Period 4**		**Period 5**		**Period 6**		**Period 7**		
Latitude °N	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	**Total**
36-40	81	(1.9)											81
41-45	109	(2.5)	1176	(27.2)	505	(11.7)	66	(1.5)					1856
46-50			733	(17.0)	100	(2.3)	418	(9.7)	49	(1.1)			1300
51-55			13	(0.3)	18	(0.4)			200	(4.6)	137	(3.2)	368
56-60			63	(1.5)	100	(2.3)	155	(3.6)	203	(4.7)			458
61-65							48	(1.1)	150	(3.5)			198
Total n	**190**		**1922**		**723**		**687**		**602**		**137**		**4324**

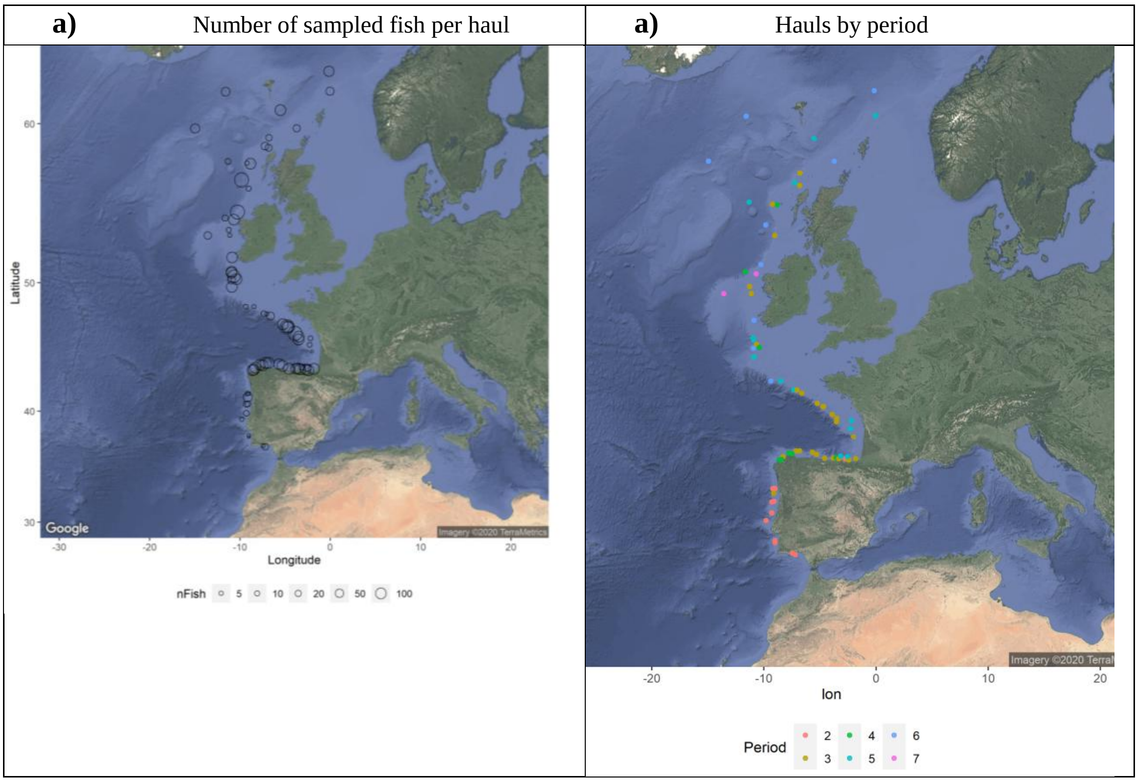

Figure 1. a) Number of sampled fish per haul. The optimum sample size is 100 indivuals per haul (WGMEGS protocol). Sampling was succesful at the Cantabrian Sea, the Gulf of Biscay and the South of Ireland. **b)** Distribution ohaul by time period.

Fish size (total length) ranged from 211 to 459 mm with a modal size in 350 mm, being females more frequent at bigger sizes (Figure 2). Mackerel sizes do not vary with latitude, unless from 36 to 40°N where only small individuals were sampled (Figure 3).

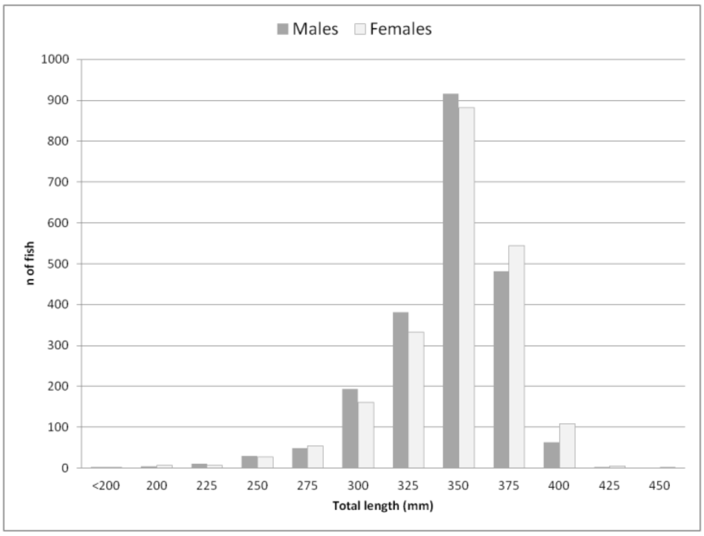

Figure 2. Total length frequency histogram by sex.

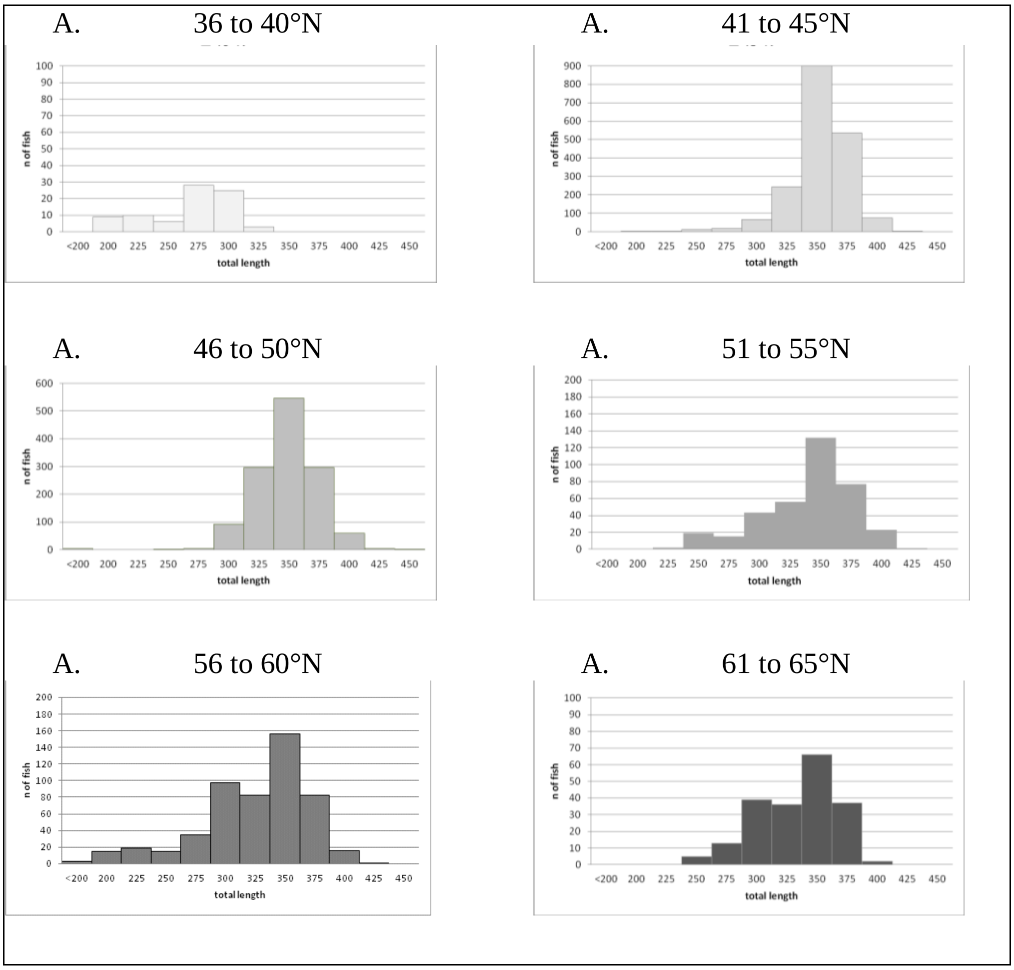

Figure 3. Total length frequency histograms by latitude.

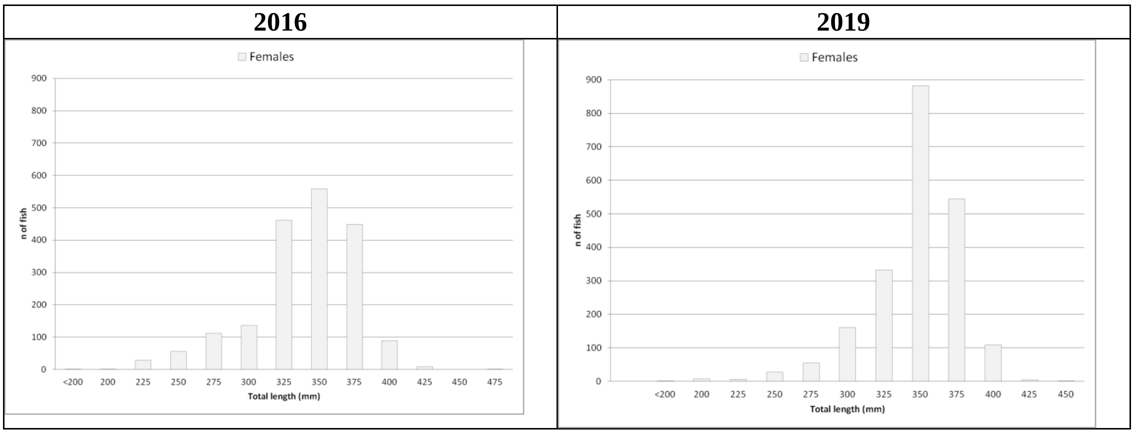

Figure 4. Mackerel female length frequency distributions in 2016 and 2019.

2.2 **Sampling of ovaries for batch fecundity and spawning fraction estimations.**

In each of the hauls, ovary samples were taken from 30 females in the 100 sampled fish. The distribution by time (period) and latitude of the ovary samples are shown in Table 2. Most of the samples were taken in the periods 3 and 4, coinciding with the peak of spawning so it's more likely that valid samples will be obtained. From each of the 30 females the ovary is weighted and different samples are taken: 4 micropipette samples, a portion of the ovary and a complete lobe following the protocols in the Fecundity manual (ICES, 2019b). Those ovary samples are taking for batch fecundity and spawning fraction estimations but not all samples are valid. Thus, all ovary samples are procesed using histology to screen the ovary under microscope and select samples valid for batch fecundity and for spawning fraction. This year, a total of 1383 ovaries were histologically procesed and the histological slides were examined under the microscope yielding 198 samples valid for batch fecundity (Figure 4) and 791 selected for spawning fraction (Figure 5).

Table 2. Number of screened ovary samples using histology.

	Period						
Latitude	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
61-65				25	44		**69**
56-60			30	42	51		**123**
51-55			2		43	47	**92**
46-50		302	40	60	23		**425**
41-45	36	408	166	40			**650**
36-40	24						**24**
Total	**60**	**710**	**238**	**167**	**161**	**47**	**1383**

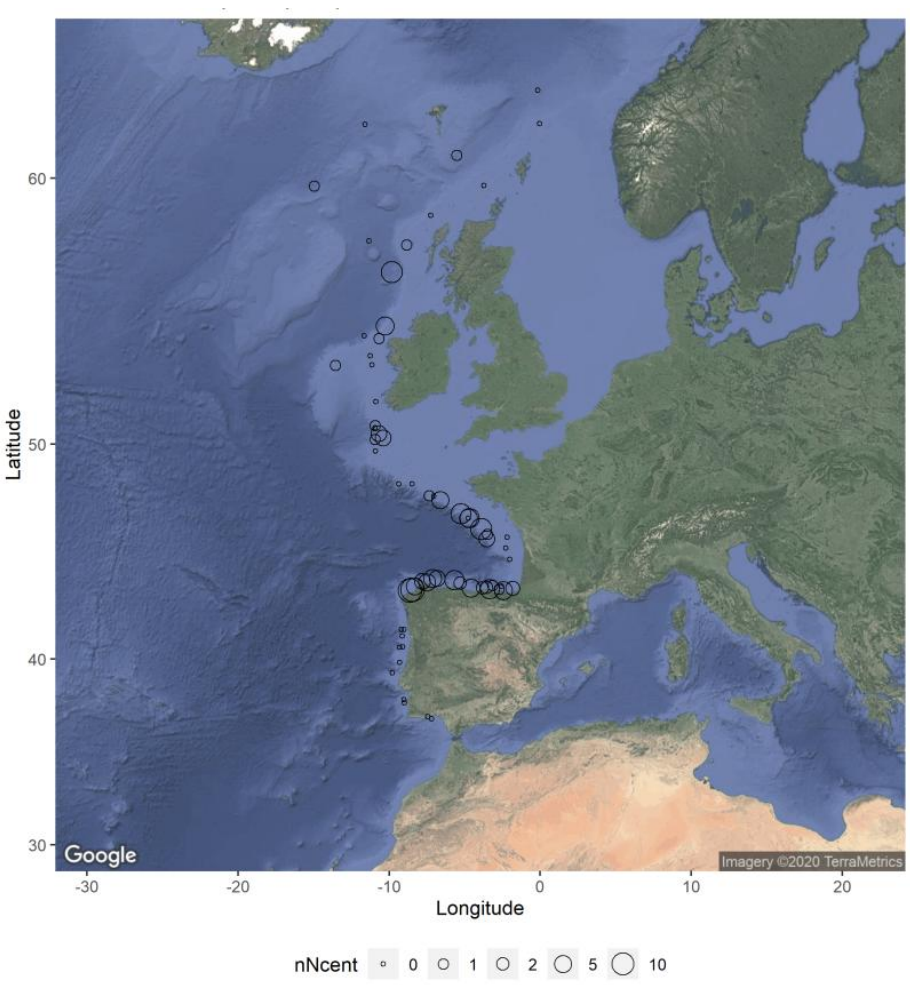

Figure 4. Distibution of the samples used for batch fecundity estimation.

2.3 **Batch fecundity (F)**.

Batch fecundity (F) is the number of eggs the female lays in a single spawning batch. Before being spawned, the oocytes increase in size by hydration and separates from the other oocytes in the ovary in a batch.

Batch fecundity was estimated from 198 ovaries containing hydrated oocytes. In the different laboratories, oocytes diameters were measured in a 0.1 g whole mount ovary sample by aid of image analysis (ICES 2019b).

For the calculation of the batch fecundity in each of the females, the oocytes were grouping in 50 µm size clusters (Figure 5). A batch was considered valid if hydrated oocytes were separated from the standing stock of oocytes by a gap of at least 50 µm. When a valid batch exist (i.e. those surrounded by squares in Figure 5), the number of oocytes is recorded.

The relative batch fecundity (number of hydrated oocytes per gram of gonad-free weight) was then calculated. The relationship between batch fecundity and gonad-free weight was fit to a negative binomial model to estimate the batch fecundity by haul (Fh). These analyses were done using an R script (see section 2.5).

diameter	Samples C082	C036	C086	A010
<250				
250-299	49	206	61	33
300-349	41	71	96	31
350-399	66	75	148	84
400-449	83	295	230	49
450-499	21	174	98	47
500-549	30	39	54	39
550-599	30	19	48	61
600-649	23	20	65	62
650-699	12	55	55	88
700-749	3	48	3	9
750-799	1	78	1	
800-849		27	2	
850-899		3		
900-949		1	12	
950-999			17	1
1000-1049			9	26
1050-1099			1	26
1100-1149				3
1150-1199				

Figure 5. Example of oocyte diameter frequency distribution in 4 ovaries. The squares mark the batch. Oocytes are grouped in 50 µm clusters. A gap of 50 µm is the minimum separation to consider a differenciated batch.

2.4 **Spawning fraction (S)**

792 histological ovary sections (Table 3) were examined to be assigned post ovulatory follicles (POFs) ages according the criteria described in the fecundity manual (ICES 2019b). Most of the examined ovary sections (83%) were taken at the periods 3 and 4, that is, the peak of the spawning and at 40 to 50 degrees north latitude. The spawning fraction (S) was estimated as the proportion of active females from the total of mature ones in each haul. The number of active females was estimated from the addition of females presenting day 1 and day 2 POFs (recent spawning) divided by 2. Finally, the total S was the average of the S by haul.

Table 3. Number of histological slides examined for POF aging.

POF age	**Period**						
Latitude	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
61-65				6	11		17
56-60			15	10	12		37
51-55			2		10	12	24
46-50		208	25	15	6		254
41-45	9	289	147	10			455
36-40	5						5
Total	**14**	**497**	**189**	**41**	**39**	**12**	**792**

2.5 **R script for adult parameters estimation**

The adult parameters were estimated using an R script used for sardine DEPM in the WGACEGG that was adapted for mackerel.

A. The script has the following sections:

   1. Correction of expected weight to avoid weight bias produced by hydration.
   2. Estimation of sex ratio in weight (R) by haul
   3. Estimation of average weight of mature females (W) by haul
   4. Estimation of batch fecundity (F) by haul
   5. Estimation of spawning fraction (S) by haul
   6. Graphs for post-stratification
   7. Estimation of average parameters (by stratum) and variances
   8. Summary of estimates and variances

   Sections 1-3 and 5 used random samples.

   Section 4 is performed by joining the hydrated females of the random sampling with the hydrated females of the directed sampling.

B. Variables to be calculate:

   - Wh   average weight of mature females (W) at every haul
   - Rh   sex ratio at every haul
   - Fh   Batch fecundity at every haul
   - Sh Female Spawning fraction at every haul
   - W   average weight of mature females in the population
   - R sex ratio in the population
   - F Batch fecundity in the population= average number of eggs per mature female per batch (g).
   - S Female spawning fraction in the population= Female spawning fraction that spawns per day.

C. Conditions for the different parameters

   - It's necessary to identify from which maturity stage a fish is considered mature.
   - A minimum number of adult fish by haul to estimate the parameters.

3. Results and discussion

Table 4 shows the valid sampling used in DEPM adult parameters calculations in 2016 and 2019. Twelve more hauls were made in 2019 than in 2016, although the number of sampled fish was slightly lower. The number of mature females in 2019 was 13% higher than in 2016 and the number of valid fish for batch fecundity was a 22% higher than in 2016. On the other hand, the number of POFs aged for the calculation of the spawning fraction increased by 11%.

Table 4. Summary table of valid sampling for 2019 survey by period. Summary information for 2016 is also shown.

Period	Hauls	Total Fish	Mature Females	Spawning fraction valid samples	Batch Fecundity valid samples
2019					
2	11	190	106	14	1
3	27	1985	958	497	91
4	8	723	314	189	40
5	14	687	332	41	4
6	9	602	336	39	4
7	2	137	51	12	2
Total	**71**	**4324**	**2097**	**792**	**142**
2016					
2	6	597	316	39	5
3	11	1003	432	182	46
4	16	1270	562	229	51
5	14	775	299	94	14
6	8	622	188	63	12
7	4	78	23	9	0
Total	**59**	**4345**	**1820**	**616**	**128**

The results of the DEPM adult parameters for the whole population are shown in table 5. The average weight per female is 355.7 grams, 8% higher than in 2016 which was 326.8grams. The sex ratio was very close to 50% both years.

Table 5 . 2019 and 2016 DEPM mackerel adult parameters estimated for the population and their coefficients of variation (cv).

	2019		**2016**	
Adult patameters	estimate	cv	estimate	cv
Average Female Weight (g)	355.66	0.0218	326.77	0.0305
Sex ratio (nº of females/total)	0.520	0.0100	0.515	0.0052
Batch Fecundity (n° eggs/batch per mature female)	12257	0.0106	8820	0.0413
Spawning fraction (n° of spawning females per mature female)	0.198	0.0904	0.163	0.1238

3.1 Batch fecundity

In 2019 DEPM analysis, the relationship between batch fecundity and gonad-free female weight was poor (Figure 6). Batch fecundity by haul (Fh), ranged from 9342 to 14410 hydrated oocytes, with a media for all the population of 12257 hydrated oocytes. This value is a 28% higher than in 2016, when population batch fecundity was 8820 hydrated oocytes (Table 5).

Batch fecundity variability is high from year to year and even within each year. This variability can be attributed to different feeding conditions. In 2019 the average female weight was 355.66 g an 8% higher than in 2016 that was of 326.77 (Table 5), what indicates bigger females or females with better condition in 2019 than in 2016. Larger females spawn more eggs per batch and in 2019 there were more females in the larger size classes than in 2016 (Figure 4).

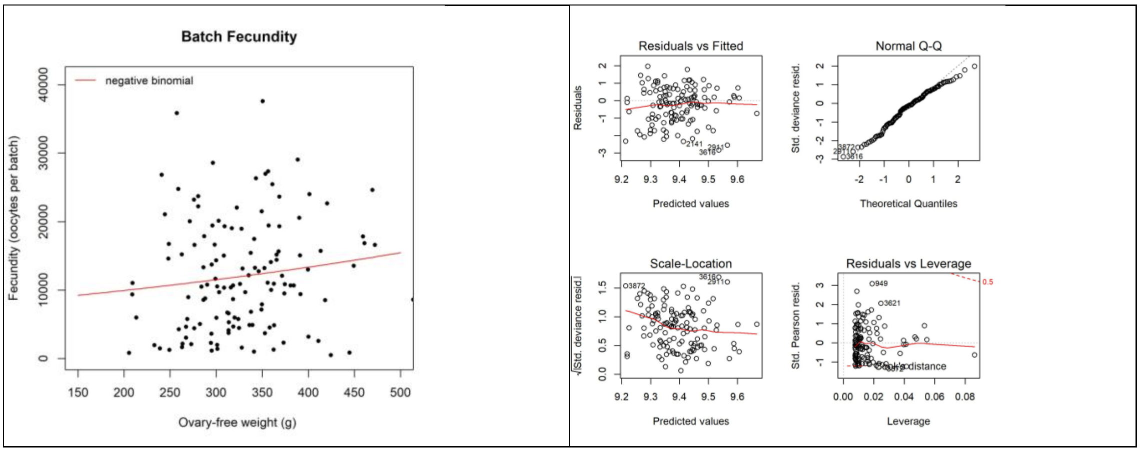

Figure 6. Description of the negative binomial model fitness in batch fecundity data

3.2 Spawning fraction

In 2019 from each 100 mature females, 19.8 were spawning. This value is higher than in 2016, when the 16,3 % of females were spawning (Table 5).

3.3 DEPM adult parameters variation by latitude

Seasonal and spatial differences in adult parameters have often been reported in Scombrids, requiring some spatial approaches to obtain unbiased estimates (ICES, 2012). During their reproductive migration, western mackerel move northwards from the south of Portugal to Iceland. In this broad range of latitude the environmental conditions change and it's likely that also do the fecundity parameters of adults, as the batch fecundity and the frequency of spawning vary throughout the spawning period.

In order to study the latitudinal heterogeneity, the adult parameters have been calculated separately for areas south and north of the 44.5° N (Table 6).

Table 6. 2019 and 2016 DEPM adult parameters estimated values and coefficients of variation (cv) south and north of 44.5 N.

	2016		2019	
North of 44.5 N	**estimate**	**cv**	**estimate**	**cv**
Average Female Weight (g)	328.53	0.0339	361.55	0.032
Sex ratio (nº of females/total)	0.517	0.0075	0.527	0.0157
Batch Fecundity (nº eggs/batch per mature female)	8833	0.1054	12528	0.0697
Spawning fraction (number of spawning females per mature female)	0.151	0.1688	0.17	0.1623
South of 44.5 N	**estimate**	**cv**	**estimate**	**cv**
Average Female Weight (g)	323.39	0.0628	347.22	0.0264
Sex ratio (nº of females/total)	0.512	0.0061	0.509	0.0064
Batch Fecundity (nº eggs/batch per mature female)	8544	0.121	12014	0.0691
Spawning fraction (number of spawning females per mature female)	0.186	0.1812	0.23	0.0859

Average female weight and batch fecundity were higher in the northern area than in the southern area. On the other hand, there were more females spawning in the south (19% of mature females) than in the north (15%). The impact that these differences may have on the calculation of spawning biomass should be studied.

4. Bibliography

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5. Acknowledgements

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