New species for EU aquaculture

Deliverable Report

Deliverable No:	D12. 2	**Delivery Month:**	59
Deliverable Title	Recommendations for wreckfish broodstock feeds		
WP No:	12	**WP Lead beneficiary:**	P19. CMRM
WP Title:	Nutrition- wreckfish		
Task No:	12.2	**Task Lead beneficiary:**	P8. IEO
Task Title:	Influence of broodstock feeding regimes for fecundity and spawn quality		
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Objective: Determine the influence of broodstock feeds on fecundity and spawn quality.

Description:

The deliverable presents:

- A preliminary study about biochemical composition of some tissues of wild wreckfish and a comparison with the biochemical composition of tissues of intensive reared wreckfish
- The biochemical composition of different broodstock feeds with special attention to the fatty acid contents
- The effect of different feeding regimes based on fresh and commercial dry feeds on oocytes fatty acid composition.
- The effect of different feeding regimes based on fresh and commercial dry feeds on eggs fatty acid composition.
- The effect of feeding regime on fecundity and egg and sperm quality.

Some recommendations for wreckfish broodstock feeds are mentioned.

1.-INTRODUCTION

The wreckfish *Polyprion americanus* is considered a good candidate for the marine aquaculture. This species is characterized by its strong appearance and sharp saw-shaped opercles, and has a wide geographic distribution and a long life. Its fast growth, good adaptation to captivity (Machias et al., 2003; Papandroulakis et al., 2004; Rodriguez Villanueva et al., 2014) good flesh quality (Linares et al., 2015), high market value and consumer acceptance internationally make the wreckfish a good candidate for aquaculture. An adequate broodstock nutrition is essential to obtain success in fish intensive culture. In marine fish, dietary lipids and in particular polyunsaturated fatty acids (PUFAs) play a critical role in the successful production of high quality gametes and eggs (Izquierdo et al., 2001; Sargent et al., 2002).

The role of PUFAs in broodstock nutrition, which are involved in several metabolic pathways as energy production, membrane structure and function, eicosaenoids production and control of lipid homeostais has been documented (Watanabe, 1982; Tocher, 2003). Marine fish have restricted ability or are unable to synthetize n-3 and n-6 Long Chain -PUFAs from their precursors, alpha linolenic acid (18:3n-3) and linoleic acid (18:2n-6) respectively (Tocher, 2003; Izquierdo and Koven, 2011; Oliva-Teles, 2012). Therefore, 22:6n-3(DHA), 20:5n-3(EPA) and 20:4n-6(ARA) are considered essential fatty acids for marine fish and must be included in the diets.

Research about wreckfish nutrition is very scarce and only some information is available from studies from feeding habits of wild population (Brick Peres & Haimovi, 2003), feeding rates (Papandroulakis et al., 2004), biochemical composition of tissues from wild fish (Linares et al., 2015) or from results obtained in other relative species (Anderson et al., 2012). More recently a study about proximate composition, fatty acid profile and cholesterol content of wild Mediterranean wreckfish was performed (Roncarati et al., 2014).

Nutrition studies performed in DIVERSIFY were focused mainly on broodstock feeds for enhancing fecundity and spawn quality and the development of adequate live prey enrichments for wreckfish larvae. These are the first steps for the development of proper nutrition and culture of this serranid species.

Due to the scarce information about wreckfish nutrition and with the objective of knowing the nutritional requirements, a study of the composition of different tissues (muscle, liver and gonad) from wreckfish wild fish was done as preliminary studies to get some basic information of this species.

The information about the composition of tissues from wild fish and eggs was very useful to advance on the knowledge of wreckfish nutritional requirements, identify potential nutritional deficiencies and formulate suitable diets for wreckfish broodstock. It is known that diet is the most important factor influencing fish fatty acid composition (Cowey and Sargent, 1972). A specific dry food for wreckfish broodstock was formulated and manufacturated and the effect of this diet was compared with other fresh diets. Analyses of oocytes and eggs from reared females fed with the different diets supplied were performed.

The aim of this study was to determine the influence of broodstock feeds on fecundity and spawn quality. Some recommendations for wreckfish broodstock feeds are done.

2.-MATERIALS AND METHODS

Preliminary studies about wild fish

Sampling procedure

Samples from wild fish

Samplings of wild wreckfish were obtained from February 2014 to April 2015. A total of 91 fish were sampled from the Azores (North Atlantic, Portugal). Total length (cm), perimeter (cm) and total and eviscerated weight (kg) were recorded for each fish. Perivisceral fat was collected and its percentage of total weight was determined. Gonads, liver, stomach and intestine were also collected from each fish and weighed to determine the respective indexes: the gonadosomatic index (GSI=gonad weight/body weight) x 100) and hepatosomatic index (HSI=liver weight/body weight) x 100) and viscerosomatic index (VSI= visceras weight/ body weight) x 100).

The stage of the reproductive development was checked. Sample collection of muscle, liver and gonads were carried out for biochemical analysis to know the nutritional status of wild fish. 24 samples of muscle and 58 of liver and inmature gonads were processed in the CIMA (CMRM), stored at -80ºC and freeze dried to perform biochemical analysis. Histological analysis were performed and the sex was identified: males, females and undetermined individuals. The biochemical analysis were carried out of

all the gonads together and separated by sex. Additionally, some samples of developed gonads of wild wreckfish were obtained and analysed.

Samples from reared fish (broodstock)

Seven samples of muscle and liver and 10 samples of gonads from reared fish from different broodstocks (IEO, IGAFA, MC2 and Acuario O Grove) were collected, stored at -80ºC and freeze dried to be analysed in the CIMA (CMRM) to compare the results with the ones obtained in wild fish.

Wreckfish broodstocks. Feeding protocole

Description of different broodstocks and type of food used.

The different diets supplied to wreckfish broodstock from the beginning of the project are shown in **Table 1**. Analyses of proteins, total lipids and fatty acids of all the diets were performed. The nutrition studies were carried out with two wreckfish broodstocks (IEO and IGAFA) using four different types of food (**Table 1**): semi-moist diet and dry food 1 supplied in the first experiment and semi-moist diet, dry food 2 and a mixture of hake/squid (half and half) supplied in the second experiment. The P32. MC2 (AF) broodstock was not used for the feeding experiments since it is placed in an Aquarium (Aquarium Finisterrae, A Coruña) and the feeding control became more difficult.

Table 1. Type of food used for wreckfish broodstocks

	2014	2015	2016	2017
Stock IEO Tank S1 n-5	Semi-moist diet	Semi-moist diet	Semi-moist diet	Semi-moist diet
Stock IEO Tank S2 n-6	Semi-moist diet	Dry food 1	Dry food 2	Dry food 2
Stock IGAFA n=10	Vitalis Repro/ Vitalis Cal	Squid	Squid	Hake/Squid
Stock AF n=17	Semi-moist diet & Fish breeders-M	Semi-moist diet & Fish breeders-M	Semi-moist diet & Fish breeders-M	Semi-moist diet & Fish breeders-M

The semi-moist diet was a mixture of 14.8% white fish, 14.8% of fish oil, 18% mussels, 17.6% squid and 34.8% fishmeal. The ingredients of Dry food 1 are shown in **Table 2**. The first experiment was performed fed the wreckfish broodstock with semi-moist diet and dry food 1. It was carried out at the IEO facilities during the year 2014. The results obtained with dry food 1 and the first data of tissues composition of wild wreckfish, muscle, liver and specially gonads as well as the first data of wrekfish eggs composition and an extensive bibliographic revision make a modification of this feed necessary. At the end of 2014 a new dry food specific for this species was formulated by FCPCT and Sparos S.A. (Portugal), dry food 2. The ingredients of dry food 2 are shown in **Table 3** and consisting of 50% fish meal, 12.5% squid meal, 6% krill, etc. A new experiment was performed at the IEO facilities: semi-moist diet was supplied to the stock of S1 tank and dry food 2 was supplied to the stock of tank S2.

The IGAFA broodstock was fed during 2014 with Vitalis Repro/Vitalis Cal from Skretting and the food was changed at the end of this year, because of the fish had a big amount of fat. The first years (2015 and 2016) it was fed with squid and in 2017 by a mixture of hake and squid (half and half). The results of feeding with this diet were compared with semi-moist diet and dry food 2. Samples of oocytes and eggs were taken out from wreckfish females fed with the different diets: semi-moist diet, dry food 1, dry food 2 and hake/squid. All the samples were frozen at -80ºC and freeze dried to be analysed. Furthermore samples of all the diets were taken out for biochemical analysis (fatty acid profiles)

Biochemical analysis

The analysis (proximal) of the diets shown in this work were made by commercial companies (Skretting and Sparos). The analysis of tissues and eggs of wreckfish were carried out by the CIMA by the following methods: Proteins were determined by the Bradford method (1976) in a UV spectrophotometer Lambda 35 (Perkin Elmer, Beaconsfield, UK). The total lipids extraction was carried out following the Blight and Dyer method with chloroform:methanol (2:1) and gravimetric determination (Blight and Dyer (1959), modified by Fernandez-Reiriz et al. (1989). Fatty acids methyl esters (FAME) were obtained by transesterification and methylation according to Lepage and Roy (1986). FAME analyses were performed in a Clarus gas chromatograph (Perkin Elmer, Beaconsfield, UK) fitted with a flame ionization detector at 260 °C in triplicate. The separation was achieved using a capillary column SPTM-2330 fused silica (30 m length, 0.25 mm internal diameter and 0.2 µm film thickness). After holding at 140 °C for 5 min the temperature was raised at 1 °C/min to 177 °C, 0.50 °C/min to 180 °C and 2 °C/min to 210 °C and maintained for 7 min with the injector at 275 °C. Injection was made in a split ratio mode (ratio 10:1) and the quantification was done using the area of the internal standard 19:0 (nonadecanoic acid).

Table 2. Ingredients of Dry food 1

Ingredients	*Dry food 1 %*
Fishmeal 70 LT FF Skagen	**50.000**
CPSP 90	7.5000
Squid meal	**12.500**
Krill meal (Aker Biomarine)	**6.000**
Wheat Gluten	6.000
Wheat Meal	4.940
Tuna oil	2.000
Incromega DHA 500TG	2.000
VEVODAR	3.000
Vit & Min Premix PV01	2.000
Lutavit E50	0.060
Soy lecithin - Powder	2.000
Macroalgae mix	1.000
Antioxidant powder (Paramega)	0.200
Antioxidant liquid (Naturax)	0.200
SelPlex - Se yeast	0.020
Carophyll Pink 10% - astaxanthin	0.050
Nucleotides (Nucleoforce)	0.030
L – Taurine	0.500
Total	100.000

Table 3. Ingredients of Dry food 2

Ingredients	*Dry food 2 %*
Fishmeal 70 LT FF Skagen	**25.000**
CPSP 90	10.000
Squid meal	**34.200**
Krill meal (Aker Biomarine)	**7.500**
Wheat Gluten	7.000
Wheat Meal	7.250
Tuna oil	1.000
Algatrium 70% DHA	0.200
Incromega DHA 500TG	1.000
VEVODAR	1.300
Vit & Min Premix PV01	2.000
Lutavit E50	0.050
Soy lecithin - Powder	1.500
Macroalgae mix	1.000
Antioxidant powder (Paramega)	0.200
Antioxidant liquid (Naturax)	0.200
SelPlex - Se yeast	0.020
Carophyll Pink 10% - astaxanthin	0.050
Nucleotides (Nucleoforce)	0.030
L – Taurine	0.500
Total	100.000

3.-RESULTS

Preliminary studies

Biochemical composition of tissues from wild fish

Biochemical analysis, proximate composition and fatty acid profiles (**Table 4**) show that wild wreckfish have a big amount of proteins in muscle (84.4%DW) and low level of lipids (6.9%). A high variability among individuals was found in liver and gonad composition. The average values of protein and lipids in liver were 38.2 and 39.3% respectively and in gonads the protein content reached 46.9% and the lipids

were 26.3%. All the gonads were immatures, but as it is said above, histological analysis were done and sex were identified in some individuals. Analyses of males, females and undetermined were carried out separately (**Table 5**), showing significately higher amount of proteins in females gonads (56%DW) than in males 43.8% and undetermined with 38.5%. Lipid levels vary between 22% in females, 26% in undetermined and 30% in males.

Regarding fatty acid composition in muscle (**Table 4**), PUFA (polyunsaturated fatty acids), SAFAs (saturated fatty acids) and MUFA (monounsaturated fatty acids) fractions have average values of 39.1, 28.8 and 32.1% of total fatty acids respectively. DHA presents a very high content (26.4% TFA), EPA 4.5% and ARA the 3.1%. DHA+EPA represent more than 30% of TFA in the muscle of wild wreckfish.

In the liver, the average value of PUFA was 16.7% of total fatty acids (TFA), SAFAs content reached 26.5% TFA and MUFAs 55.6%, DHA level represents the 8.7% TFA, EPA 2.9% and ARA 1.5%. All the values have shown a high variability among individuals.

With respect fatty acid profile of immature gonads (**Table 5**), even though no significant differences were found in fatty acid content between males, females and undetermined gonads, the PUFA content is slightly higher in female gonads (31.5%TFA) than in male and undetermined gonads (27-29%). n-3 and n-6 PUFA represent 25.7% and 5.4% in female gonads and DHA, EPA and ARA reached values of 16.7, 4.8 and 4.4% TFA respectively. The EPA/ARA ratio is 1.4 in females and males inmature gonads and 1.7 in undetermined gonads.

Table 4. Biochemical composition. Proteins, lipids and fatty acids (means±std) of muscle, liver and gonads of wild wreckfish

	Muscle	*Liver*	*Inmature Gonads*
	Proximate analysis (% dry matter)		
Proteins	84.41±7.34	38.16±12.89	46.87±14.11
Lipids	6.92±3.39	39.34±15.03	26.31±14.40
	Fatty acids content (% TFA)		
14:0	2.08±0.45	1.91±0.65	2.81±0.81
16:0	19.37±0.83	17.79±3.95	17.87±1.90
17:0	1.04±0.22	1.09±0.38	1.21±0.25
18:0	5.89±0.65	5.37±1.16	6.35±1.38
Saturated (SAFAs)	28.83±1.28	26.48±5.21	28.76±2.78
16:1 n-9	0.47±0.06	1.12±0.37	0.67±0.15
16:1 n-7	5.10±1.23	8.58±3.46	6.11±1.25
18:1 n-9	16.68±3.50	30.79±7.00	21.29±3.35
18:1 n-7	4.01±1.04	8.25±1.87	5.14±0.95
20:1 n-9	2.16±0.85	3.32±1.16	3.46±2.60
Monoenoics (MUFAs)	32.09±5.43	55.64±10.72	42.40±8.86
18:2 n-6	0.97±0.11	0.96±0.45	0.96±0.17
20:4 n-6 (ARA)	3.11±0.79	1.48±0.92	3.70±2.29
20:5 n-3 (EPA)	4.55±0.70	2.90±1.54	4.55±1.49
22:6 n-3 (DHA)	26.38±3.33	8.69±5.37	15.56±4.84
Polyunsaturated (PUFAs)	39.08±4.41	16.66±8.88	28.84±8.51
Σn-3	34.51±3.75	14.01±7.69	23.80±6.83
Σn-6	4.08±0.81	2.44±1.29	4.66±2.29
n-3/n-6	8.50±1.18	5.48±1.75	5.44±2.00
DHA/EPA	5.69±1.23	2.92±0.96	3.35±0.81
EPA/ARA	1.54±0.37	2.02±0.75	1.49±0.64

HUFA.highly unsaturated fatty acids; ARA. arachidonic acid; DHA.docohexaenoic acid; EPA.eicosapentaenoic acid

Table 5. Biochemical composition of immature gonads of wild wreckfish

	Male Gonads	*Female Gonads*	*Undeterminate Gonads*
	Proximate analysis (% dry matter)		
Proteins	43.81±10.96b	55.95±13.93a	38.52±13.11b
Lipids	29.98±14.80	22.07±16.72	25.96±7.70
	Fatty acids content (% TFA)		
14:0	2.93±1.01	2.62±0.72	2.87±0.48
16:0	18.33±2.69	17.06±0.71	18.28±0.78
17:0	1.16±0.27	1.29±0.28	1.17±0.13
18:0	6.43±1.57	6.50±1.49	5.98±0.75
Saturated (SAFAs)	29.38±3.95	27.98±1.22	28.81±1.49
16:1 n-9	0.73±0.16	0.64±0.13	0.59±0.09
16:1 n-7	6.14±1.43	5.58±1.06	6.85±0.79
18:1 n-9	21.07±4.33	21.25±3.00	21.76±1.45
18:1 n-7	4.90±1.03	5.00±0.85	5.81±0.65
20:1 n-9	4.18±3.60	3.03±1.66	2.79±0.78
Monoenoics (MUFAs)	43.92±12.28	40.51±6.23	42.46±2.63
18:2 n-6	0.92±0.21	0.99±0.16	0.98±0.12
20:4 n-6 (ARA)	3.47±2.28	4.44±2.73	2.97±1.15
20:5 n-3 (EPA)	4.29±1.91	4.82±1.23	4.64±0.94
22:6 n-3 (DHA)	14.37±6.91	16.73±2.33	15.98±2.06
Polyunsaturated (PUFAs)	26.70±11.66	31.51±5.47	28.72±3.12
Σn-3	21.91±9.61	25.74±3.49	24.34±2.83
Σn-6	4.40±2.31	5.43±2.70	3.95±1.14
n-3/n-6	4.94±1.75	5.39±2.40	6.47±1.50
DHA/EPA	3.12±1.02	3.52±0.64	3.51±0.50
EPA/ARA	1.41±0.51	1.45±0.83	1.69±0.56

HUFA.highly unsaturated fatty acids; ARA. arachidonic acid; DHA.docohexaenoic acid; EPA.eicosapentaenoic acid

Furthermore some samples of mature gonads were taken out: four females from Vigo market and two males from Canary Islands market. Proximate composition and fatty acid composition are shown in **Table 6**.

Table 6. Biochemical composition of mature gonads (mean values ±std) of wild wreckfish

	Female gonads	Male gonads
Proximate analysis (% dry matter)		
Proteins	59.77±10.50	44.02±3.77
Lipids	20.73±4.78	13.21±3.08
Fatty acid content (%TFA)		
14:0	1.15±0.36	0.93±0.18
16:0	17.27±2.91	19.63±0.85
17:0	1.08±0.12	0.68±0.16
18:0	6.42±0.46	7.69±0.23
Saturated (SAFAs)	26.30±2.96	29.24±0.36
16:1 n-9	1.15±0.68	0.38±0.14
16:1 n-7	3.97±1.96	1.57±0.64
18:1 n-9	15.45±5.52	9.58±1.95
18:1 n-7	6.84±3.74	4.24±0.96
20:1 n-9	1.70±0.21	1.99±0.15
Monoenoics (MUFAs)	31.23±11.69	19.37±3.87
18:2 n-6	0.50±0.38	0.59±0.10
20:4 n-6 (ARA)	7.07±1.40	10.10±1.10
20:5 n-3 (EPA)	5.35±1.53	5.35±0.07
22:6 n-3 (DHA)	25.12±7.36	31.40±4.91
Polyunsaturated (PUFAs)	42.47±9.72	51.39±3.60
Σn-3	34.57±8.88	39.88±4.95
Σn-6	7.57±1.16	10.69±1.15
n-3/n-6	4.54±0.84	3.81±0.87
DHA/EPA	4.70±0.25	5.87±0.94
EPA/ARA	0.77±0.22	0.54±0.06

HUFA.highly unsaturated fatty acids; ARA. arachidonic acid; DHA.docohexaenoic acid; EPA.eicosapentaenoic acid

The results showed a high values of proteins (60% in females and 44% in males) and the lipid content represented (21% in females and 13% in males) in wild gonads, n-3 PUFA values reached 35% in wild females and 40% in males gonads having a big amount of DHA (25-31%), EPA represented 5% in both females and males and ARA content was the 7 and 10% of total fatty acids in females and males wild gonads respectively.

Biochemical composition of tissues from reared fish.

The results of biochemical composition of reared fish are shown at **Table 7**.

Table 7. Biochemical composition. Proteins, lipids and fatty acids (means±std) of muscle, liver and gonad of captive-reared wreckfish

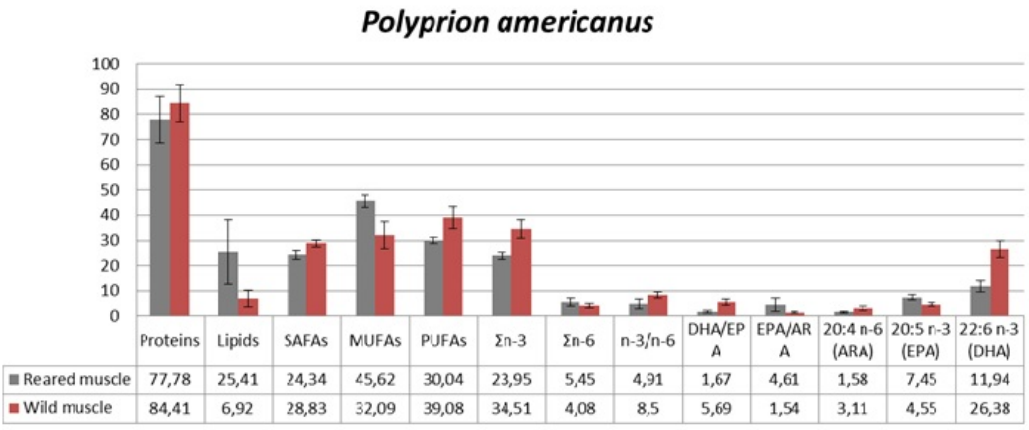
	Muscle	*Liver*	*Gonads*
	Proximate analysis (% dry matter)		
Proteins	77.78±9.30	43.97±16.02	58.11±8.84
Lipids	25.41±12.71	43.30±21.87	27.97±13.51
	Fatty acids content (% TFA)		
14:0	3.24±0.63	1.49±0.32	2.50±0.78
16:0	15.88±1.85	18.92±4.56	16.21±0.92
17:0	0.69±0.23	0.91±0.26	0.78±0.21
18:0	4.18±0.23	5.66±1.47	5.28±1.02
Saturated (SAFAs)	24.34±1.73	27.25±4.81	25.10±1.49
16:1 n-9	0.57±0.07	1.02±0.32	0.83±0.26
16:1 n-7	7.85±0.95	5.37±1.53	5.45±1.11
18:1 n-9	25.13±1.35	24.68±12.04	20.72±2.40
18:1 n-7	6.24±1.27	8.13±1.91	5.82±1.40
20:1 n-9	2.45±0.57	2.22±0.99	2.34±0.69
Monoenoics (MUFAs)	45.62±2.33	44.12±16.59	38.41±4.76
18:2 n-6	3.87±1.97	2.64±1.67	2.82±1.68
20:4 n-6 (ARA)	1.58±0.51	1.80±1.14	2.73±1.48
20:5 n-3 (EPA)	7.45±1.12	4.34±1.46	7.75±1.63
22:6 n-3 (DHA)	11.94±2.28	16.61±9.61	18.20±6.03
Polyunsaturated (PUFAs)	30.04±1.36	28.64±11.89	36.49±5.29
Σn-3	23.95±1.31	23.88±10.71	30.42±6.12
Σn-6	5.45±1.60	4.44±1.84	5.55±2.09
n-3/n-6	4.91±1.86	5.79±2.63	6.39±3.16
DHA/EPA	1.67±0.47	3.58±1.30	2.44±0.89
EPA/ARA	4.61±2.68	2.75±2.13	3.40±2.04

HUFA.highly unsaturated fatty acids; ARA. arachidonic acid; DHA.docohexaenoic acid; EPA.eicosapentaenoic acid

The first results showed that fish from intensive culture had more lipids in muscle and liver 25.4% (DW) and 43.3% respectively than those obtained in wild fish with 6.9% in muscle and 39.3% in liver. A high variability between reared individuals composition was found specially in liver.

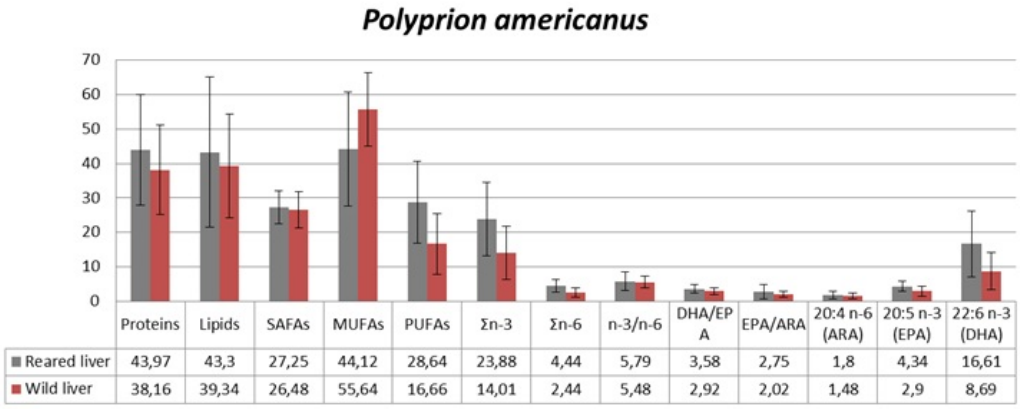
Protein content is higher in muscle of wild wreckfish (84.4%) than in captive-reared fish (77.8%). Some significant differences were also observed in the values of fatty acids (% total fatty acids). Values of PUFA and Σn-3 are higher in wild wreckfish, 39.1 and 34.5% TFA respectively than in reared fish, 30 and 23.9%. However, MUFA values are higher in reared fish (45.6%) than in wild fish (32.1%). DHA values represent 11.9% in cultured fish and 26.4% in wild fish. EPA content represents 7.4% in reared fish and 4.5% in wild fish and ARA 1.6% and 3.1% in reared and wild fish respectively. EPA/ARA ratio have values of 4.6 from reared fish and 1.5 from wild fish (**Fig. 1**).

High differences among individuals were found in liver of reared fish, as it was said above for wild fish. Proteins and lipids have values of 44 and 43.3% respectively, higher than in wild fish with values of 38.2 and 39.3% respectively. PUFAs, Σn-3 and Σn-6 are higher in liver from reared fish than from wild fish, while MUFAs content is lower in reared fish than in wild fish (**Fig. 2**). These tendencies are opposite of those found in muscle (**Fig. 2**).



	Proteins	Lipids	SAFAs	MUFAs	PUFAs	Σn-3	Σn-6	n-3/n-6	DHA/EPA	EPA/ARA	20:4 n-6 (ARA)	20:5 n-3 (EPA)	22:6 n-3 (DHA)
Reared muscle	77,78	25,41	24,34	45,62	30,04	23,95	5,45	4,91	1,67	4,61	1,58	7,45	11,94
Wild muscle	84,41	6,92	28,83	32,09	39,08	34,51	4,08	8,5	5,69	1,54	3,11	4,55	26,38

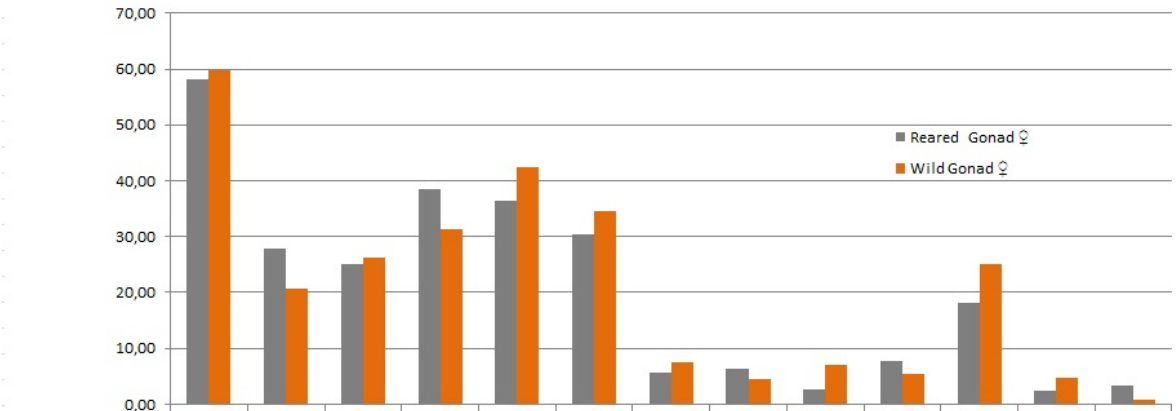
Figure 1. Protein, lipid and fatty acid composition of muscle from wild and captive-reared wreckfish



	Proteins	Lipids	SAFAs	MUFAs	PUFAs	Σn-3	Σn-6	n-3/n-6	DHA/EPA	EPA/ARA	20:4 n-6 (ARA)	20:5 n-3 (EPA)	22:6 n-3 (DHA)
Reared liver	43,97	43,3	27,25	44,12	28,64	23,88	4,44	5,79	3,58	2,75	1,8	4,34	16,61
Wild liver	38,16	39,34	26,48	55,64	16,66	14,01	2,44	5,48	2,92	2,02	1,48	2,9	8,69

Figure 2. Protein, lipid and fatty acid composition of liver from wild and captive-reared wreckfish

The comparison of the values of composition between females gonads from wild and captive-reared wreckfish is shown in the **Fig.3**, showing that the values of proteins from wild fish are higher (59.8%) than in reared fish (58.1%), while lipid content is higher in gonads from reared fish (28%) than in gonads of wild fish (20.7%). Regarding fatty acid content, PUFA content reached higher values (42.5%) in wild female gonads than in gonads from reared fish (36.5%) being n-3 PUFA a little higher in wild (34.6%) than in reared gonads (30.4%) and the same tendency was observed in n-6 PUFA content which represented 7.6% and 5.5 % in wild and reared gonads respectively. DHA level is higher in wild (25%) than in reared (18%), EPA is higher in reared (7.7%) than in wild gonads (5.3%) and ARA is higher (7.1%) in wild than in reared gonads (2.7%).



	Proteins	Lipids	SAFAs	MUFAs	PUFAs	Σn-3	Σn-6	n-3/n-6	20:4 n-6 (ARA)	20:5 n-3 (EPA)	22:6 n-3 (DHA)	DHA/EPA	EPA/ARA
Reared Gonad ♀	58,11	27,97	25,10	38,41	36,49	30,42	5,55	6,39	2,73	7,75	18,20	2,44	3,40
Wild Gonad ♀	59,77	20,73	26,30	31,23	42,47	34,57	7,57	4,54	7,07	5,35	25,12	4,7	0,77

Figure 3. Protein, lipid and fatty acid composition of female gonads from wild and captive-reared wreckfish

Feeding analysis of wreckfish broodstock

All the diets supplied to the IEO and IGAFA broodstocks were analysed. Proteins, lipids and fatty acids were performed.

Proteins and lipids

The values of proteins, total lipids and fatty acid profiles of the diets supplied to two different wreckfish broodstocks placed in IEO and IGAFA from 2014 to 2018 are shown in **Tables 8, 9 and 10**.

Table 8. Protein and lipid composition (% of dry weight) of broodstock wreckfish diets

Diets	Proteins	Lipids
Semi-moist diet*	64.66	17.35±2.45
Vitalis Repro*	52	16.00±0.50
Vitalis Cal*	54	18.00±0.55
Dry food 1*	60.3	16.40±0.33
Dry food 2*	68.2	12.50±0.53
Hake	82.5±4.65	5.54±0.46
Squid	57.54±1.66	10.79±0.38
Hake/Squid	62.93±4.23	7.89±4.23

*values supplied by commercial companies (Skretting and Sparos)

Semi-moist diet presents 64.7% of proteins and 17.3% of lipids. Dry food 2 and dry food 1 have 68.2 and 60.3% of proteins and 12.5 and 16.4% of lipids respectively. Hake has the highest level of proteins (82.5%) and the lowest amount of lipids (5.5%) of the broodstock feed, squid has 57.5% of proteins and

10.8% of lipids and hake/squid has 62.9% of proteins and 7.9% of lipids. With respect to dry food, which is currently used as feed of other fish broodstock, Vitalis Repro and Vitalis Cal have 52 and 54% of proteins and 16 and 18 % of lipids respectively (**Table 8**).

The fatty acid profile of Vitalis Repro and Vitalis Cal supplied to IGAFA broodstock during 2014 is shown in Table 9. As it is said above, the diet was changed in 2015, first to squid and in 2017 to a mixture of hake and squid (half and half) because of the big amount of fat found in fish, more than 10% of perivisceral fat and around the heart, air bladder, etc.

Table 9. Fatty acid profile of diets Vitalis Repro and Vitalis Cal supplied to IGAFA broodstock (2014)

	Vitalis Repro	*Vitalis Cal*
14:0	4.93±0.06	6.66±0.13
16:0	18.40±0.13	19.26±0.13
17:0	0.99±0.04	0.79±0.03
18:0	4.58±0.10	4.66±0.08
Saturated (SAFA´s)	29.44±0.29	31.86±0.34
16:1n-7	5.87±0.05	7.56±0.06
18:1n-9	17.15±0.13	12.46±0.06
18:1n-7	3.66±0.08	3.47±0.01
20:1n-9	2.54±0.01	1.22±0.01
22:1n-11	1.84±0.05	0.83±0.01
Monoenoics (MUFA´s)	33.64±0.10	27.27±0.03
18:2n-6	8.88±0.08	6.50±0.01
18:3n-3	1.74±0.02	1.18±0.01
18:4n-3	1.52±0.05	2.12±0.01
20:4n-6	1.16±0.04	0.95±0.03
20:5n-3	9.42±0.20	13.09±0.20
22:5n-3	1.40±0.02	1.57±0.03
22:6n-3	10.61±0.25	12.05±0.20
Polyunsaturated (PUFA´S)	36.92±0.36	40.87±0.36
Σn-3	25.37±0.40	30.67±0.38
Σn-6	10.04±0.12	7.45±0.02
n-3/n-6	2.53±0.07	4.11±0.05
DHA/EPA	1.13±0.02	0.92±0.00
EPA/ARA	8.11±0.41	13.78±0.57

The fatty acid composition of diets supplied to IEO and IGAFA broodstocks from 2015 to 2018 is shown in **Table 10**. The results obtained in the first experiment of wreckfish broodstock feeding have shown that the amount of fat was too high to feed wreckfish broodstock and a specific dry food for wreckfish broodstock (dry food 2) was formulated to be used at the IEO facilities (tank S2) since February 2016.

Dry food 2 has lower fat content (12.5% DW) than dry food 1 (16.4%). The dry food 2 fatty acid profile (Table 10) shows 46.9% of PUFA, 30.3% of n-3 and 16.2% of n-6. ARA content represents the 6.9% of TFA less than the one obtained in dry food 1 (9.1%) and the EPA/ARA ratio was 1.1% similar to the one obtained previously in tisues of wild fish (**Tables 4, 5 and 6**).

In the second experiment, three broodstock diets were used: semi-moist diet and dry food 2 supplied to the IEO broodstock and hake/squid supplied to the IGAFA broodstock. With regards hake/squid there was a higher amount of PUFA (54.8% TFA) than dry food 2 (46.9%) and semi-moist diet (38.5%), n-3PUFA values represent the 49.4% TFA in hake/squid and 30.3% and 29.4% in dry food 2 and semi-moist diets respectively, while n-6 PUFA content reaches a high value in dry food 2 (16.2%) followed by semi-moist diet (8.3%) and hake/squid (3.6%). The diet with the highest level of EPA is hake/squid (14.3%). Dry food 2 has the highest level of ARA (6.9%) while in semi-moist diet and hake/squid it represents 1.2% and 2.7% of total fatty acids. The EPA/ARA ratio is 5.2 in hake/squid and 1.2 in dry food 2 which is similar to the one previously obtained in tissues of wild fish.

Table 10. Fatty acid profile of diets supplied to IEO and IGAFA broodstock (2015-2018)

	Semimoist diet	*Dry food 1*	*Dry food 2*	*Hake*	*Squid*	*Hake/Squid*
14:0	4.81±0.59	3.92±0.10	4.07±0.05	5.65 ± 0.99	3.42 ± 0.13	2.48 ± 0.11
16:0	19.51±0.93	14.88±0.10	17.61±0.04	21.40 ± 0.43	23.28 ± 0.48	21.32 ± 0.40
17:0	1.01±0.18	0.78±0.05	0.88±0.01	0.96 ± 0.10	0.81 ± 0.01	0.69 ± 0.00
18:0	4.05±.0.30	4.47±0.04	4.14±0.15	4.03 ± 0.24	5.39 ± 0.13	5.03 ± 0.06
Saturated (SAFA´s)	29.96±.1.19	24.44±0.22	27.1±0.21	32.14±1.08	33.45 ± 0.37	29.92 ± 0.43
16:1n-7	4.79±0.51	3.05±0.02	3.45±0.03	3.31 ± 0.50	2.30 ± 0.31	2.45 ± 0.23
18:1n-9	12.21±1.46	14.48±0.09	12.33±0.11	8.34 ± 0.54	3.56 ± 0.22	6.02 ± 0.46
18:1n-7	3.82±0.35	2.17±0.04	3.44±0.01	4.28 ± 0.24	2.20 ± 0.12	2.71 ± 0.13
20:1n-9	3.55±0.64	3.79±0.03	2.68±0.02	0.47 ± 0.05	2.99 ± 0.08	1.98 ± 0.06
22:1n-11	3.53±1.03	4.86±0.22	1.79±0.05	0.01 ± 0.02	0.24 ± 0.04	0.06 ± 0.01
Monoenoics (MUFA´s)	31.55±0.97	30.83±0.15	26.01±0.11	19.98 ± 1.94	13.94 ± 0.48	15.25 ± 0.78
18:2n-6	7.03±0.64	7.55±0.01	9.35±0.09	1.51 ± 0.10	0.35 ± 0.04	0.85 ± 0.07
18:3n-3	1.16±0.14	1.23±0.02	1.4±0.04	0.83 ± 0.15	0.20 ± 0.03	0.46 ± 0.05
18:4n-3	1.66±0.26	1.49±0.11	1.39±0.05	1.78 ± 0.36	0.55 ± 0.11	0.88 ± 0.09
20:4n-6	1.25±0.28	9.06±0.06	6.9±0.12	0.97 ± 0.06	4.11 ± 0.29	2.73 ± 0.07
20:5n-3	8.84±0.48	6.61±0.02	8.19±0.11	10.32 ± 0.14	16.94 ± 0.31	14.27 ± 0.23
22:5n-3	1.29±0.24	0.99±0.15	1.01±0.14	0.83 ± 0.03	0.98 ± 0.07	0.97± 0.06
22:6n-3	15.91±1.16	16.67±0.32	17.94±0.05	31.02 ± 1.88	28.35 ± 0.90	32.55 ± 1.10
Polyunsaturated (PUFA´S)	38.49±1.65	44.72±0.37	46.89±0.1	47.88 ± 1.47	52.61 ± 0.40	54.83 ± 0.97
Σn-3	29.40±1.57	27.61±0.43	30.31±0.14	45.10 ± 1.63	47.40 ± 0.62	49.44 ± 1.00
Σn-6	8.28±0.56	16.62±0.07	16.25±0.06	2.48 ± 0.13	4.45 ± 0.26	3.58 ± 0.01
n-3/n-6	3.56±0.23	1.66±0.03	1.87±0.01	18.20 ± 1.53	10.67 ± 0.72	13.81 ± 0.31
DHA/EPA	1.43±0.66	2.52±0.04	2.19±0.02	3.00 ± 0.15	1.67 ± 0.08	2.28 ± 0.09
EPA/ARA	1.86±0.73	0.73±0.01	1.19±0.04	10.67±0.80	4.14±0.29	5.22±0.17

Fatty acid values ≥1% of total. Values expressed in mean ±SD.

Fatty acid profiles of oocytes from females fed with different types of food

Semi-moist diet and Dry food 1

The first experiment was carried out during 2015 and some samples of oocytes were taken out from females fed with semi-moist diet (oocytes S1) and dry food 1 (oocytes S2). Fatty acid profile of both diets (**Table 10**) showed that dry food 1 had a higher PUFA content (44.7% of TFA) than semi-moist

diet (38.5%) and this is due to the amount of n-6 PUFA in dry food 1 (16.6%) which is higher than in semi-moist diet (8.3%). The main difference between both diets was found in ARA content with values of 9.1% in dry food 1 and 1.2% in semi-moist diet.

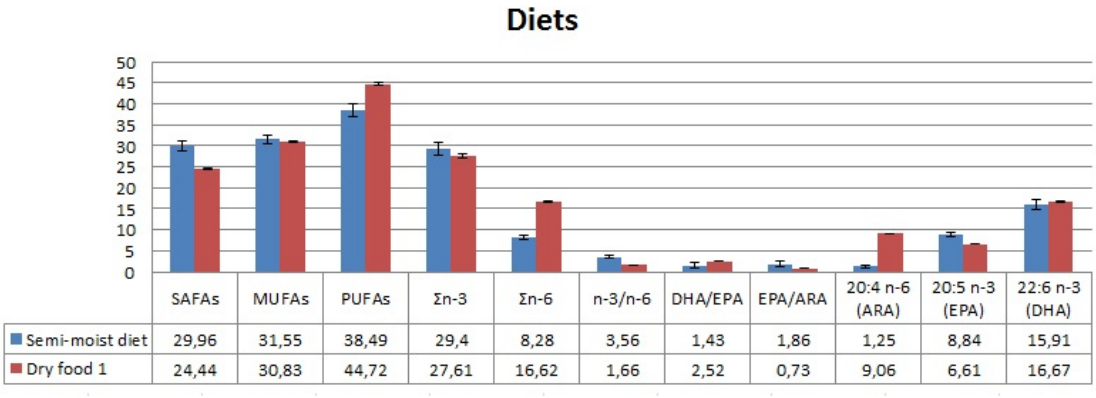
The fatty acid profiles of oocytes from females fed with semi-moist diet and dry food 1(**Table 11**) show that saturates content varies between 21-23%, MUFA had values of 32% and PUFA between 44.5-47% TFA. n-3 PUFA represents the 39% TFA in both oocytes and the main difference between the oocytes are in n-6 PUFA content which represents 5.6% TFA and 8% in oocytes from females fed with semi-moist diet and dry food 1 as the ARA content that has values of 1.8% and 3.7% TFA respectively in both diets.

Table 11. Fatty acid profile of oocytes from females fed with semi-moist diet and dry food 1

	Oocytes S1	*Oocytes S2*
14:0	1.47±0.07	1.42±0.02
16:0	15.35±0.65a	13.75±0.55b
17:0	1.02±0.20	0.91±0.19
18:0	4.92±0.28	4.70±0.19
Saturated (SAFA´s)	23.12±0.86a	21.09±0.76b
16:1n-9	1.34±0.14a	0.91±0.03b
16:1n-7	4.11±0.13a	3.45±0.17b
18:1n-9	17.54±0.97	17.45±1.01
18:1n-7	4.94±0.46	4.64±0.51
20:1n-9	1.74±0.05b	2.27±0.18a
Monoenoics (MUFA´s)	32.41±0.86	31.69±0.46
18:2n-6	3.87±0.18	4.14±0.48
20:4n-6	1.76±0.16b	3.72±0.22a
20:5n-3	7.70±0.32a	6.61±0.31b
22:5n-3	2.63±0.18	2.70±0.15
22:6n-3	26.33±1.07	27.92±1.06
Polyunsaturated (PUFA´S)	44.47±1.62b	47.23±1.14a
Σn-3	38.62±1.57	39.18±1.16
Σn-6	5.63±0.20b	7.96±0.59a
n-3/n-6	6.86±0.32a	5.01±0.44b
DHA/EPA	3.42±0.08b	4.23±0.27a
EPA/ARA	4.40±0.44a	1.78±0.11b

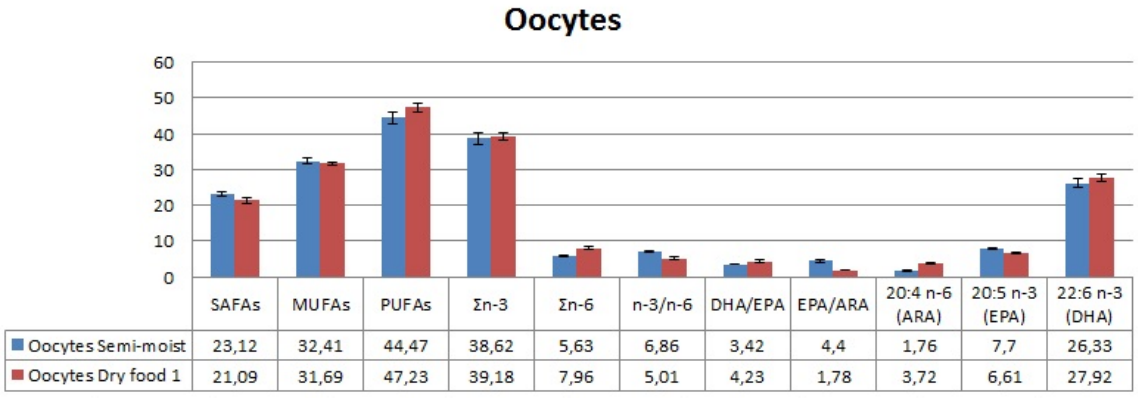
Fatty acid values ≥1% of total. Values expressed in mean ±SD. Different letters within a line denote significant differences (P<0.05)

These first results show a big relationship between the fatty acid profiles of diets supplied to broodstocks and oocytes. (**Fig. 4a, b**)



	SAFAs	MUFAs	PUFAs	Σn-3	Σn-6	n-3/n-6	DHA/EPA	EPA/ARA	20:4 n-6 (ARA)	20:5 n-3 (EPA)	22:6 n-3 (DHA)
Semi-moist diet	29,96	31,55	38,49	29,4	8,28	3,56	1,43	1,86	1,25	8,84	15,91
Dry food 1	24,44	30,83	44,72	27,61	16,62	1,66	2,52	0,73	9,06	6,61	16,67

Figure 4a. Fatty acid composition of semi-moist diet and dry food 1



	SAFAs	MUFAs	PUFAs	Σn-3	Σn-6	n-3/n-6	DHA/EPA	EPA/ARA	20:4 n-6 (ARA)	20:5 n-3 (EPA)	22:6 n-3 (DHA)
Oocytes Semi-moist	23,12	32,41	44,47	38,62	5,63	6,86	3,42	4,4	1,76	7,7	26,33
Oocytes Dry food 1	21,09	31,69	47,23	39,18	7,96	5,01	4,23	1,78	3,72	6,61	27,92

Figure 4b. Fatty acid composition of oocytes from females fed with semi-moist diet and dry food 1

Semi-moist diet, dry food 2 and hake/squid

Following the experiments done before with semi-moist diet and dry food 1, the effect of three different feeding broodstock regimes, semi-moist diet, dry food 2 and hake/squid (half and half) on fatty acid composition of oocytes and eggs from females fed with these diets was checked.
Samples of oocytes (Ø>700µ) were obtained by gonadal biopsies of females fed with the three diets which were described above.

Results of oocytes fatty acid composition from IGAFA broodstock females fed with a mixture of hake and squid compared with oocytes composition from IEO broodstock females fed with semimoist diet (S1) and dry food 2 (S2) are shown in **Table 12**.

Table 12. Fatty acid profile of oocytes (Ø >700 µ) from females fed with different diets (%TFA)

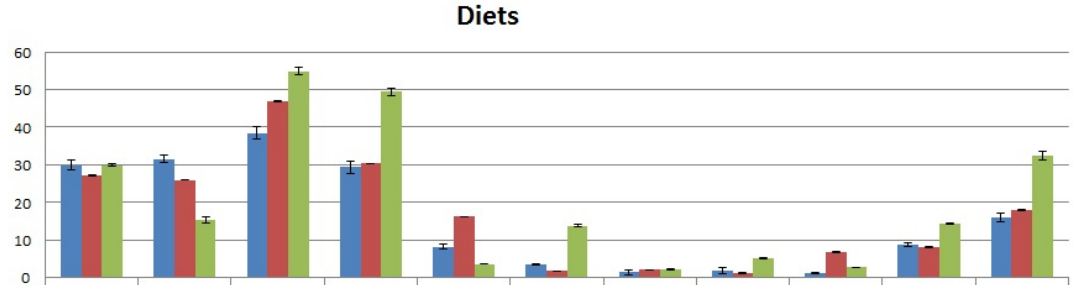
Diets oocytes	*Semi-moist diet OSM 0.710-2.033 µ*	*Dry food 2 ODF 0.778-2.118µ*	*Hake/Squid OHS 0.831-1.388µ*
14:00	1.55 ± 0.11a	1.72 ± 0.03a	1.24 ± 0.13b
16:00	15.09 ± 0.63	14.68 ± 1.50	14.46 ± 0.85
17:00	1.00 ± 0.05a	0.75 ± 0.11b	0.62 ± 0.11b
18:00	5.02 ± 0.19	4.77 ± 0.57	4.70 ± 0.26
Saturated (SAFA´s)	22.97 ± 0.57	22.17 ± 2.07	21.22 ± 1.15
16:1n-9	1.31 ± 0.04	0.98 ± 0.22	1.16 ± 0.20
16:1n-7	4.46 ± 0.39	4.49 ± 0.49	4.39 ± 0.32
18:1n-9	17.83 ± 0.34	19.41 ± 4.21	17.08 ± 1.83
18:1n-7	5.36 ± 0.38	4.78 ± 0.74	6.00 ± 0.77
20:1n-9	1.95 ± 0.27	1.94 ± 0.33	1.63 ± 0.11
Monoenoics (MUFA´s)	33.58 ± 1.10	33.91 ± 4.33	32.35 ±2.89
18:2n-6	4.45 ± 0.56b	5.58 ± 0.16a	3.25 ± 0.47c
20:4n-6	1.88 ± 0.36b	6.74 ± 1.25a	1.61± 0.99b
20:5n-3	7.57 ± 0.19b	5.49 ± 0.35c	10.72 ± 1.13a
22:5n-3	2.80 ± 0.26	2.76 ± 1.40	3.63 ± 0.33
22:6n-3	24.60 ± 1.53ab	21.32 ± 1.90b	25.48 ±1.16a
Polyunsaturated (PUFA´s)	43.46 ± 1.34	43.92 ± 4.10	46.42 ± 2.29
Σn-3	36.87 ± 1.59ab	31.35 ± 3.31b	41.31 ± 2.15a
Σn-6	6.34 ± 0.84b	12.32 ± 1.21a	4.86 ± 0.50b
n-3/n-6	5.90 ± 0.93b	2.58 ± 0.20c	8.63 ± 0.90a
DHA/EPA	3.25 ± 0.15b	3.91 ± 0.27a	2.40 ± 0.18c
EPA/ARA	4.16 ± 0.59b	0.86 ± 0.10c	6.71 ± 0.38a

Fatty acid values ≥1% of total. Values expressed in mean ±SD. Different letters within a line denote significant differences (P<0.05)

There are not significant differences in SAFAS's, MUFA's and PUFA's values of oocytes from females fed with semi-moist diet (OSM), dry food 2 (ODF) and the mixture of hake and squid (OHS) with values of SAFA´s between 21-23%, MUFA 32-34% and PUFA 43-46%. The highest content of n-3 was observed in OHS (41%), while OSM had 37% and ODF 31% of total fatty acids. In the case of n-6 PUFA the values are much higher (12%) in ODF than in OHS and OSM (5-6%). In individual fatty acids the most important saturates are 16:0 and 18:0 which represent 14-15% and 5% TFA respectively in the three kinds of oocytes. In the case of MUFA, 18:1n-9 represents the 17-19% TFA, 18:1n-7, 5-6% and 16:1n-7 the 4% in the three types of oocytes. Regarding n-3PUFA, the values of 20:5n-3 (EPA) and 22:6n-3 (DHA) have significant differences in the oocytes from females fed with the three diets. EPA has the highest value in OHS, 10.7% of total, 7.6% in OSM and 5.5% in ODF. DHA values have significant differences between OHS and ODF (25.5 and 21.3% respectively) and there are not differences between OSM and ODF and OSM and OHS.In the case of n-6 PUFA, the 20:4n-6 (ARA) level is the highest in ODF representing the 6.7% TFA in comparison with OSM and OHS with 1.6-1.9%. The 18:2n-6 represents values of 5.6% (ODF), 4.4% (OSM) and 3.2% in OHS.

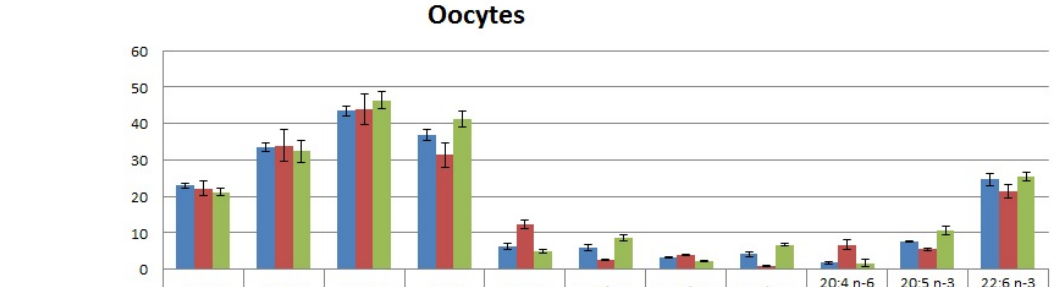
The n-3/n-6 ratio is high in OHS (8.6) because of the high amount of EPA and have values of 5.9 and 2.6 in OSM and ODF respectively. DHA/EPA ratio has values of 3.9, 3.2 and 2.4 in ODF, OSM and OHS respectively, while the EPA/ARA ratio are higher in OHS (6.7) than in OSM (4.2) and a much

lower value is obtained in ODF (0.9) because of the high value of ARA. There was a strong relationship between fatty acid composition of diets and oocytes (**Fig. 5**) as it was said above for the first experiment.



	SAFAs	MUFAs	PUFAs	Σn-3	Σn-6	n-3/n-6	DHA/EPA	EPA/ARA	20:4 n-6 (ARA)	20:5 n-3 (EPA)	22:6 n-3 (DHA)
Semi-moist diet	29,96	31,55	38,49	29,4	8,28	3,56	1,43	1,86	1,25	8,84	15,91
Dry food 2	27,1	26,01	46,89	30,31	16,25	1,87	2,19	1,19	6,9	8,19	17,94
Hake/Squid	29,92	15,25	54,83	49,44	3,58	13,81	2,28	5,22	2,73	14,27	32,55

Figure 5a. Fatty acid composition of semi-moist diet, dry food 2 and hake/squid



	SAFAs	MUFAs	PUFAs	Σn-3	Σn-6	n-3/n-6	DHA/EPA	EPA/ARA	20:4 n-6 (ARA)	20:5 n-3 (EPA)	22:6 n-3 (DHA)
Oocytes Semi-moist 0,710-2,033 µ	22,97	33,58	43,46	36,87	6,34	5,9	3,25	4,16	1,88	7,57	24,6
Oocytes Dry food 2	22,17	33,91	43,92	31,35	12,32	2,58	3,91	0,86	6,74	5,49	21,32
Oocytes Hake/Squid	21,22	32,35	46,42	41,31	4,86	8,63	2,4	6,71	1,61	10,72	25,48

Figure 5b. Fatty acid composition of oocytes from females fed with semi-moist, dry food 2 and hake/squid.

Furthermore, some samples of viable eggs of females from IEO and IGAFA broodstocks were taken out to be analysed. Lipid (%DW) and fatty acid composition of eggs are shown in **Table 13** and **Fig. 6**.

Table 13. Fatty acid profile of eggs from females fed with different diets (% TFA)

Diets	Semi-moist	Dry food	Hake/Squid
Proximate analysis (% dry matter)			
Lipids	24.65±2.14a	18.94±4.55a	9.40±0.41b
Fatty acids content (% TFA)			
14:0	1.58±0.08a	1.53±0.09ab	1.44±0.07b
16:0	15.76±0.67a	14.95±1.00b	15.06±0.47ab
18:0	4.73±0.22a	4.44±0.37b	4.16±0.14b
Saturated (SAFAs)	23.35±0.96a	21.83±1.29b	21.39±0.66b
16:1 n-9	1.40±0.18	1.30±0.18	1.32±0.07
16:1 n-7	5.01±0.48a	4.54±0.31b	4.62±0.16ab
18:1 n-9	19.43±1.67	18.14±2.56	16.97±0.47
18:1 n-7	4.97±0.18	4.67±0.90	4.37±0.07
20:1 n-9	1.70±0.08a	1.55±0.09b	1.29±0.03c
Monoenoics (MUFAs)	34.93±1.96a	35.10±3.46a	30.11±0.76b
18:2 n-6	4.75±1.04	4.67±0.61	4.13±0.09
20:4 n-6 (ARA)	1.46±0.13b	5.12±0.47a	1.41±0.08b
20:5 n-3 (EPA)	7.21±0.76b	5.74±0.39c	11.22±0.10a
22:5n-3	2.45±0.19b	2.16±0.29c	3.52±0.05a
22:6 n-3 (DHA)	23.76±2.16b	22.73±1.23b	26.17±0.83a
Polyunsaturated (PUFAs)	41.72±2.38b	43.07±2.64b	48.50±0.72a
Σn-3	35.29±3.16b	32.48±1.62c	42.64±0.83a
Σn-6	6.22±0.93b	9.79±0.91a	5.54±0.10b
n-3/n-6	5.92±1.76b	3.38±0.39c	7.73±0.31a
DHA/EPA	3.30±0.12b	3.96±0.16a	2.33±0.06a
EPA/ARA	4.92±0.22b	1.13±0.08c	7.99±0.50a

HUFA,highly unsaturated fatty acids; ARA, arachidonic acid; DHA,docohexaenoic acid; EPA,eicosapentaenoic acid

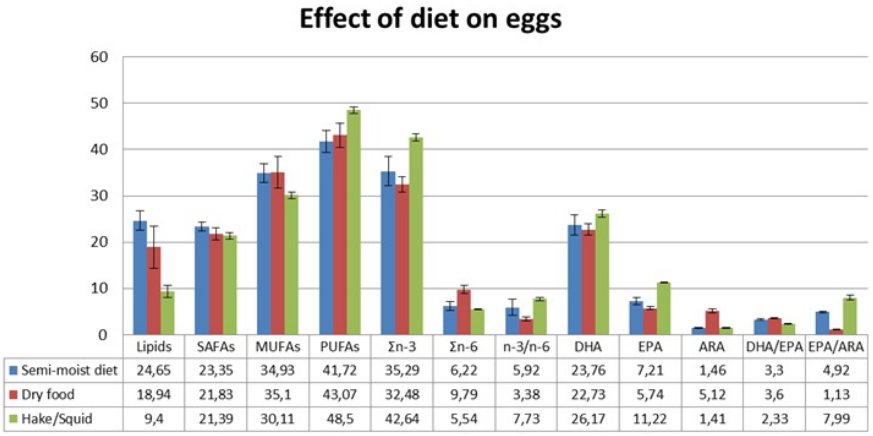


Figure 6. Lipid (% DW) and fatty acid composition (% TFA) of eggs from females fed with different diets

The total amount of lipids is higher (significant differences) in eggs from females of the IEO broodstock fed with semi-moist diet, ESM, and with dry food 2, EDF, (24.6 and 18.9% DW respectively) than in eggs from females from the IGAFA broodstock fed with hake and squid (half and half), EHS, with 9.4% of lipids which is clearly influenced by the food (hake and squid).

With respect to fatty acid composition, the total amount of PUFA's is higher in EHS (48.5% TFA) than in EDF and ESM (42-43%), on the contrary MUFA's have the lowest level in EHS (30.1%) while EDS and EDF have 35%; n-3 PUFA are higher in EHS (42.6%) than in ESM (35.3%) and EDF (32.5%) while n-6 PUFA have higher values in EDF (9.8%) than in the rest of eggs (6%). The DHA content is a little higher in EHS with values of 26.2% TFA than in the rest of the eggs (23-24%). EHS eggs have more

amount of EPA (11.2% TFA) than EDF and ESM (6-7%). In the case of ARA the highest values were found in EDF (5.1%) while ESM and EHS have values about 1-1.5%.

The tendency of these results is similar to the one obtained previously in oocytes from females fed with the same diets. A correlation was found between the fatty acid profile of wreckfish broodstock feeding and eggs fatty acid profile.

Fatty acid profile of sperm from wreckfish males

On the other hand, some samples of sperm were taken out from males of different broodstock (stock IGAFA, stock IEO and stock AF) to be characterized (velocity and motility of sperm) and to perform the fatty acid profiles. Wreckfish males produced a high volume of sperm with a high percentage of motile cells and this high speed was associated with a long swimming duration compared to other marine fish. The fatty acid profile of sperm from males of different stocks is shown in **Table 14**.

Table 14. Fatty acid composition (mean values, % TFA) of sperm from males from different wreckfish broodstocks

Stocks sperm	*Stock IGAFA*	*Stock IEO*	*Stock AF*
Saturated (SAFA´s)	32.02 ± 1.92	32.56 ± 0.19	31.71 ± 1.96
Monoenoics (MUFA´s)	10.82 ± 1.80	9.35 ± 0.79	9.81 ± 0.35
Polyunsaturated (PUFA´s)	57.15 ± 0.60	58.10 ± 0.63	56.43 ± 1.93
Σn-3	51.50 ± 0.44	48.92 ± 4.54	49.86 ± 1.49
Σn-6	5.52 ± 0.62b	8.99 ± 3.97a	6.44 ± 0.45b
n-3/n-6	10.15 ± 1.96a	6.22 ± 2.62b	7.85 ± 0.28b
DHA	36.67 ± 1.96	36.89 ± 2.96	36.47 ± 1.74
EPA	10.81 ± 1.12a	8.78 ± 1.76b	9.91 ± 0.36ª
ARA	4.94 ± 0.75b	7.84 ± 3.85a	5.91 ± 0.40b
DHA/EPA	3.45 ± 0.53b	4.37 ± 0.78a	3.71 ± 0.29b
EPA/ARA	2.49 ± 0.70a	1.35 ± 0.69b	1.70 ± 0.16b

There are not significant differences between the main groups of fatty acids in the sperm of males from the different stocks, with values of SAFA`s between 32-33%, MUFA`s 9-11% and PUFA´s 56-58% of the total fatty acids. The n-3 PUFA content varies between 49-51% of the TFA and the n-6 PUFA content is higher in sperm from IEO stock (9% TFA) than in stock IGAFA (5%) and AF (6%).

Fecundity of females and diet

Relative fecundity of females (nºeggs/Kg female) fed with semi-moist diet, dry food and hake/squid were recorded from 2015 to 2017. The relative fecundity from ten females were recorded, one from tank S1 (IEO) in 2015 and 2018 fed with semimoist diet, four from tank S2 (IEO) in 2016, 2017 and 2018 and four from IGAFA broodstock during 2016, 2017 and 2018 (**Fig. 7 & 8**)

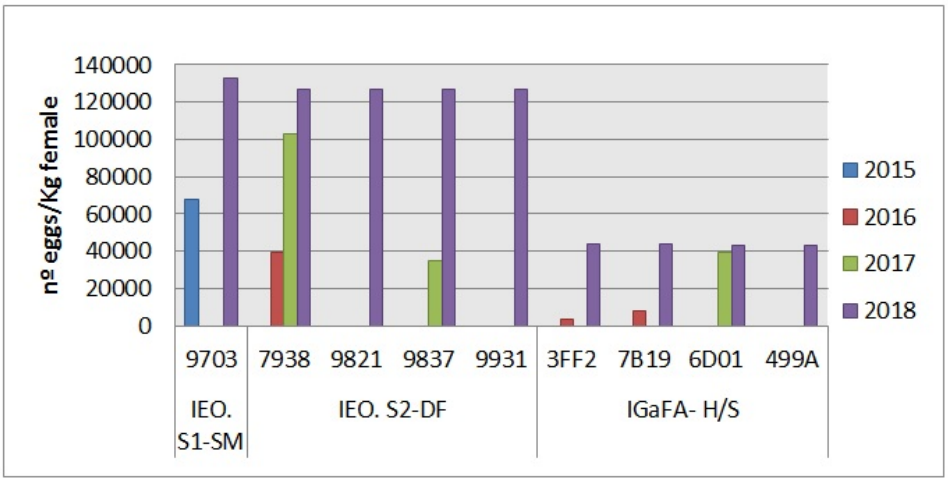


Figure 7. Relative fecundity (nº of eggs/kg of female) in females fed with different diets

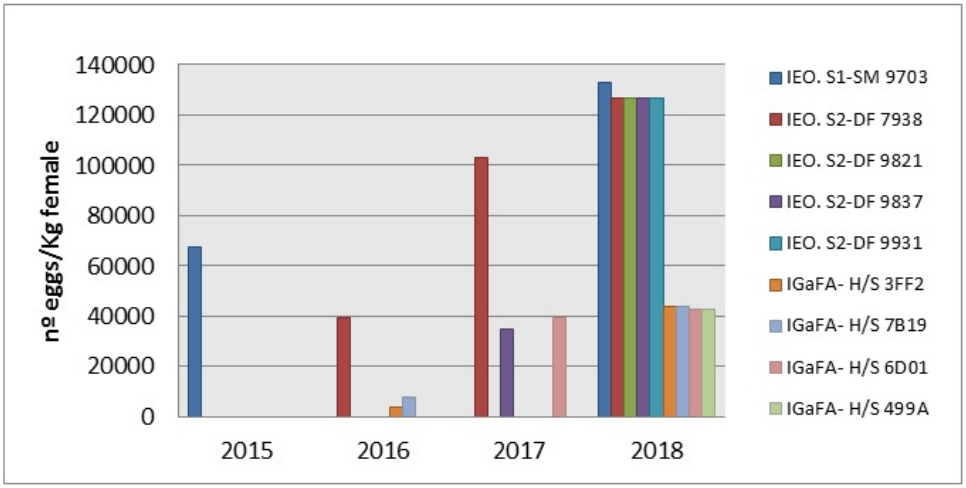


Figure 8. Relative fecundity (nº of eggs/kg of female) from 2015 to 2018

The number of spawns was also recorded from 2015-2018 in females from the IEO and IGAFA broodstock and fed with different diets (**Fig. 9**)

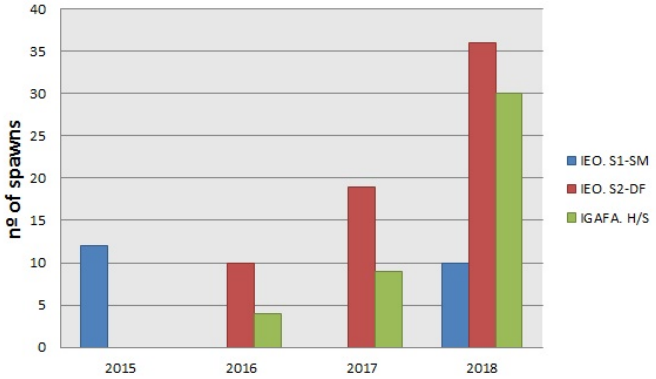


Figure 9. Number of spawns obtained from 2015 to 2018

The highest relative fecundity was observed in females fed with dry food (tank S2-IEO) with an average value of 126627.36 eggs/kg of female in 2018 with the exception of only one female from tank S1of EO broodstock fed with semi-moist diet with a value of relative fecundity of 133178.57 eggs/kg of female. Females from IGAFA broodstock had and average fecundity of 43530,06 eggs/kg of female in 2018 fed with hake/squid (half and half). In all the females from IEO and IGAFA broodstocks the fecundity have been increasing for over the years.

With respect to the number of spawns, they were increasing too for over the years in both, IEO and IGAFA broodstocks. With respect to IEO broodstock, females fed with dry food had 10 spawns in 2016 from only one female, 19 spawns in 2017 from two females and 36 in 2018 from 4 females. In the case of females from this broodstock fed with semi-moist diet, only one female spawns in 2015, no spawns were obtained in 2016 and 2017 and 10 spawns were obtained in 2018. Regarding females from IGAFA broodstock fed with hake/squid, the spawns started in 2016 from only one female (4 spawns) and they were increasing in 2017 (9 spawns) and 2018 with 30 spawns from 4 females.

Even though these results about females fecundity and number of spawns are preliminary and it is necessary to check them with data from more females, the results obtained in 2017 and 2018 suggest an influence of the broodstock diet on fecundity of females.

4.- DISCUSSION AND CONCLUSIONS

An improvement in broodstock nutrition has been shown to have a great importance in aquaculture. In the case of a new species such as the wreckfish, knowledge of its nutritional requirements is crucial to get an adequate and good quality diet ,which will contribute to its success in culture. Results from this study highlighted the correlation among broodstock diet composition and oocytes-eggs, with potential consequences on reproductive success and egg and larvae quality. Special attention was paid to fatty acid composition and in particular the essential fatty acids (EFA), EPA,DHA and ARA content since these fatty acids play a crucial role in the reproductive process of marine fish.

Furthermore, in the present work, tissues composition (muscle, liver and gonads) from wild wreckfish were analysed and the results obtained were compared with tissues from captive-reared wreckfish showing that wild fish had more proteins in muscle (84%) higher than in the captive reared muscle (78%), while the lipid content is much higher in reared fish (about 25%) than in wild fish with 7%. In the case of gonads, proteins are also higher in wild fish than in reared fish and the muscle lipid content is much higher in reared fish than in wild fish. The liver was the main lipid storage organ in both wild and reared fish having a higher content (43%) in wild fish than in reared fish (39%) and it is considered as an indicator-organ for the nutritional and physiological status of the fish (Caballero et al., 1999), because it responds directly and rapidly to the various dietary conditions created by the diet and the rearing protocol (Papadakis et al., 2009; Papadakis et al., 2013).

The predominant fatty acid in the fatty acid composition of the muscle of wild wreckfish was DHA, 26%, similar to the one obtained by Roncarati et al (2014) in the Mediterranean wreckfish with values among 24.2-25.7. Other fatty acids as oleic acid (18:1), palmitic acid (16:0), eicosapentaenoic (20:5n-3) and stearic acid (18:0) represent the major fatty acids in both Atlantic and Mediterranean wreckfish. In this study the ARA content represents 3% of the total fatty acids in muscle. The values of ARA found by Roncarati et al.(2014) in meat of Mediterranean wreckfish vary between 1-4.7%. In wild wreckfish gonads this fatty acid has values about 4% in immatures gonads and 7% and 10% in female and male mature gonads respectively.

In a recent study about comparative reproductive development in wild and captive-reared greater amberjack (*Seriola dumerili*) was found that gonads of captive-reared fish had different polar lipid contents, as well as specific lipid classes and fatty acid profiles with respect to wild individuals, significant differences were found in gonads fatty acid composition between wild and captive

specimens, particularly during the early and advanced gametogenic phases with both ovaries of captive fish displaying around 30 and 40% less DHA and ARA respectively (Zupa et al., 2017). In wreckfish in the whole fatty acids only MUFA present higher values in gonads from reared fish than developed gonads from wild fish while PUFAs, n-3 and n-6 present higher values in female gonads from wild fish than those from reared fish and with a similar tendency to the one observed in Seriola, ARA and DHA content are higher in wild wreckfish female gonads than in farmed wreckfish and consequently affect the EPA/ARA and DHA/EPA ratios with values of 3.4 and 2.4 in reared fish and among 0.8-1 and 4.3-4.8 in wild gonads respectively.

The influence of the body composition of fish, specially with regards fat on oocyte recruitment, fecundity and atresia has been documented in Atlantic cod, both in wild fish and experimentally (Karlsen et al., 1995; Kjeshu and Holm, 1994; Skajaraasen et al.,2010).

The comparison of tissues and /or eggs from wild and captive fish allows the identification of potential nutritional defficiencies, which is essential for the development of suitable broodstock diets (Migaud et al., 2013) and this strategy has been successful in many species as sea bass *Dicentrarchus labrax* (Alasalvar et al., 2002), white seabream *Diplodus sargus* (Cejas et al., 2003, 2004), greater amberjack *Seriola dumerilii* (Rodriguez-Barreto et al., 2012; Saito, 2012) and Senegalese sole *Solea senegalensis* (Norambuena et al.,2012).

A clear relationship between the fatty acid composition of wreckfish broodstock diets and the fatty acid composition of oocytes and eggs was found in this study. The highest n-3 PUFA content was observed in oocytes (41%) and eggs (43%) from females fed with hake/squid diet. This diet has 49% of n-3 PUFA while dry food 2 and semi-moist diet have 30 and 29% of TFA respectively. In the case of n-6 PUFA content represent 16% in dry food 2 and 8 and 3.6% in semi-moist and hake/squid diets respectively and these fatty acids reach values of 12 and 10% in oocytes and eggs from females fed with dry food 2 and 5-6% in oocytes and eggs from females fed with the other two diets.

ARA has been extensively studied in relation to reproductive performance, and it has been shown that moderate levels of ARA in broodstocks diets exert significant positive effects on the spawning performance, egg quality , and offspring quality of several fish species (Xu et al., 2017). ARA content was reported to be higher and EPA/ARA ratio lower in eggs and ovaries obtained from wild cod and other marine fish such as Senegalese sole (*Solea senegalensis*), than in culture broodstock. These factors were suggested to be related to higher viability in eggs from wild fish (Norambuena et al., 2012; Salze et al.,2005). ARA content is higher in oocytes and eggs from females fed with dry food (ODF and EDF) reaching values of 7 and 5% respectively, than in oocytes and eggs from females fed with semi-moist diet and hake/squid with values nearly 2% in oocytes and 1-1.5% in eggs.

Furthermore, not only the individual amounts of ARA have an effect on fish, but an interaction also exists between this and the n-3 LC-PUFA eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (Torrecillas et al., 2018). A recent study in Atlantic salmon (*Salmo salar*) showed that combined dietary inclusion of ARA and EPA improved fish performance and changed tissue fatty acid composition (Norambuena et al., 2016).

In the present study, the EPA/ARA ratio in oocytes and eggs is 0.9 in ODF and 1.1 in EDF, which are similar to the one obtained in tissues (muscle and gonads from wild fish) and much lower than they were obtained in oocytes and eggs from females fed with semi-moist diet and hake/squid with values of: 4.2 (OSM), 4.9 (ESM) and 6.7 (OHS) and 8 (EHS). Migaud et al., (2013) pointed out that due to the influence of both ARA and EPA on tissue eicosanoid production it is likely that maintaining both n-6 and n-3 LC-PUFA at values close to wild values will be beneficial to subsequent egg and larvae success.

A clear increase in relative ARA content in the ovaries in response to increasing ARA in the diets was found in a recent study done in females of Atlantic cod, ARA seems to have actively incorporated into ovarian tissue possibly due to a special function in steroid synthesis (Norberg et al., 2017). The ratio EPA/ARA in the ovaries reflected the diet, and was negatively correlated to dietary ARA content.

Regarding sperm fatty acid composition, only the n-6 present differences among the wreckfish males from different broodstock being higher in males from IEO broodstock fed with dry food 2 suggesting the influence of the diet on sperm fatty acid, but further research with more number of males would be necessary. Baeza et al., (2015) pointed out the usefulness of the development of enriched diets that may improve sperm quality which could have an impact on the reproductive abilities of European eel males, thus improving fertilization success and embryo development.

Wreckfish females fed with dry food 2 had higher relative fecundity in terms of number of eggs per kg of female, than females fed with semi-moist diet and hake/squid. The maximum number of spaws were obtained in female fed with dry food which had 36 spaws in 2018 from four females. Furthermore, the fecundity has been increasing in the four years of the study.

The reduction of the amount of lipids in diets for wreckfish broodstock with values of 12.5 and 8% in dry food 2 and hake/squid respectively seems to have a beneficial effects being a better health state of broodstock, diminishing the mortality and producing a greater number of spawns. The good quality of squid meal was also documented previously. Zohar et al., (1995) reported that squid meal contain nutritional components which are essential for successful spawning in gilthead seabream.

PUFA and in particular n-3 content in oocytes and eggs from females of IGAFA broodstock fed with hake/squid showed that the values were higher than in oocytes and eggs from females fed with the other diets as it was said above. This diet could be used as a wreckfish broodstock feed but it is recommended to reinforce with dry food 2 at certain times of the year for example in the maturation and spawning phases of the broodstock.

Some ingredients as raw krill often included in diets for sparids has a distinct quality of having and enhancing effect on feed intake compared wih fishmeal (Izquierdo et al., 2001). The spawning quality enhancement effect of raw krill has shown that both polar and nonpolar lipid fractions contain important nutritional components for red seabream stock (Watanabe et al., 1991 a, b). In wreckfish broodstocks dry food 2 that was specifically formulated for this species, included among other ingredients a big amount of squid meal (34%) and Krill meal (7.5%) and this diet exerts a beneficial effect in terms of eggs viability, biochemical composition of oocytes and eggs, fecundity, etc.

Concluding remarks

- Results obtained from tissues composition of wild wreckfish were very useful to advance the knowledge of the nutritional requirements of this species. These results and those obtained from eggs and larvae newly hatched were very useful for the formulation of a specific dry food for wreckfish broodstock.
- Comparisons between wild and reared wreckfish composition showed that fish from intensive culture have more lipids in muscle and liver than those obtained in wild fish. In contrast, protein content is higher in muscle of wild wreckfish than in reared fish and some differences were also observed in the fatty acid profile with higher values of PUFA and n-3 PUFA in wild than in reared wreckfish.
- Gonads from females of wild wreckfish have a high level of ARA (7-10 %TFA) and an EPA/ARA ratio of nearly 1.
- A clear relationship between fatty acid profile of broodstock diets (semi-moist, dry food and a mixture of hake and squid) and fatty acid profile of oocytes and eggs from females fed with the different diet was found.
- Regarding wreckfish broodstock feeding regimes, results obtained showed that most of commercial dry food have too much fat for wreckfish.
- Results obtained with dry food specifically formulated for wreckfish broodstock demonstrated that the diet must contain a big amount of proteins, low level of lipids, a high amount of n-3 PUFA and the EPA/ARA ratio must be similar to that obtained in wild females gonads. The

mixture of hake/squid (half and half) seems to be a diet with good quality because of the protein content and the big amount of n-3 PUFA (EPA and DHA) but in certain times of the year corresponding with maturation and spawning phases it is recommended to use dry food 2 because it is more appropriate to the needs of the broodstock and therefore be associated with greater success in reproduction.

- First data of fatty acid profile of sperm from wreckfish males of different broodstock were obtained.
- A relationship was found between broodstock diets and fecundity, number of spawnings of the females, etc.
- Relative fecundity (nº of eggs/Kg of female) and number of spawns per female have been increasing in females fed with dry feed over the years, from 2015 to 2018.

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