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ORIGINAL ARTICLE

Biometric parameters and biochemical composition of wild wreckfish (*Polyprion americanus*)

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ABSTRACT

Wreckfish (*Polyprion americanus*) is considered to be a good candidate for European aquaculture. The main biometric parameters were recorded from 86 wild-caught fish (2.6-18 kg of total weight) for 14 months and the gonadosomatic index (GSI), hepatosomatic index (HIS) and viscerosomatic index (VSI) were obtained. Biochemical analyses of muscle, liver and gonads from wild wreckfish showed that wreckfish has a high level of protein in muscle, 84% of dry weight (DW), and low level of lipids (7% DW). Capture season, sex, size or weight of the fish did not have a strong effect on the variability observed in the levels of proteins, lipids and fatty acids of the different tissues. Reared wreckfish have more lipids in mature gonads than wild fish, which may be due to a more lipid-rich diet. However, some polyunsaturated fatty acids (PUFA) such as arachidonic acid (ARA) and docosahexaenoic acid (DHA) reached higher values in wild female gonads than in reared gonads, which could indicate the wreckfish has high requirements in these fatty acids. This study obtained valuable data on the biology and biochemical composition of wreckfish to estimate its nutritional requirements, which could be useful for the future of this species in aquaculture.

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Polyprion americanus; aquaculture; biometric parameters; biochemical composition

Introduction

The wreckfish (*Polyprion americanus* Bloch & Schneider, 1801) is a deep-water fish with a very wide distribution and characterized by an extended pelagic juvenile phase (Sedberry et al. 1999; Ball et al. 2000). It can potentially reach 2 m in length and 100 kg in weight (Roberts 1989), being one of the largest Polyprionidae species. It is a voracious carnivore and accepts inert food easily. The reproductive maturation of wreckfish occurs at an age of 5–10 years in captivity (Peleteiro and Brunzón 2014) and although this slow maturation may be a problem for broodstock development and management, its long juvenile stage is an advantage from the aquaculture viewpoint, allowing for commercialization before sexual maturity. The growth of this species is strongly influenced by sex, and females reach larger sizes than males (Rodríguez et al. 2017), as observed in many other marine fish species.

Considering the good growth performance, good adaptation to captivity (Machias et al. 2003; Papandroulakis et al. 2004; Rodríguez Villanueva et al. 2014), high market value and consumer acceptance internationally, the wreckfish is one of the selected species for the diversification of marine species culture. Despite the high potential for aquaculture of wreckfish, there are only few references on the biology (Wakefield et al. 2013) and culture in captivity of this species (Peleteiro and Brunzón 2014; Rodríguez Villanueva et al. 2014; Martínez-Vázquez et al. 2016; Rodríguez et al. 2017). More recently, some studies on its reproduction, describing the gametogenesis of both males and females and its relationship with blood plasma levels of sex steroids have been performed (Papadaki et al. 2018). Natural spawns with high fertilization and hatching rates of the eggs and promising results in larvae feeding sequence in a recirculating aquaculture system (RAS) were achieved (Pérez Rial et al. 2019).

Aquaculture of groupers is carried out in tropical and subtropical areas throughout the world, but most production is in Asia. It is estimated that there are at least 47 grouper species, 36 *Epinephelus*, plus 15 grouper hybrids that have been trialled or are currently cultured (Rimmer and Glamuzina 2019). Based on data provided to the FAO, almost 155,000 tonnes of grouper were produced in 2015. Problems in larval

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culture are still the mayor difficulty in the development of mass juvenile production of groupers. hapku (*Poyprion oxygeneios* Schneider & Forster, 1801) is a high-value commercial species found in the waters of the Southern Hemisphere and its life cycle was completed in 2013, but the key production bottleneck is the highly variable and often very low fingerling survival (Khan et al. 2019).

Studies of wreckfish nutrition in captivity are scarce and a limited amount of information is available about feeding habits of wild population (Brick Peres and Haimovi 2003), feeding rates (Papandroulakis et al. 2004) or from results obtained in other close species such as the hapuku (Anderson et al. 2012). Proximate composition, fatty acid profile and cholesterol content of wild Mediterranean wreckfish were performed (Roncarati et al. 2014). In a recent study about comparative reproductive development in wild and captive-reared greater amberjack (*Srioloa dumerili* Risso, 1810), it 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 gonad fatty acid composition between wild and captive specimens, particularly during the early and advanced gametogenic phases (Zupa et al. 2017). Lenas et al. (2011) found higher total lipid content in muscle (4.3-fold) in farmed sea bass (*Dicentrarchus labrax* L.) than in wild ones, while docosahexaenoic (DHA) was dominant in wild specimens, and Cejas et al. (2004) described that the relative percentage of specific fatty acids differed in captive and wild mature females of white seabream (*Diplodus sargus*), showing that the most noteworthy difference was the lower proportion of arachidonic acid (ARA) and the higher proportion of eicosapentaenoic acid (EPA) in liver and muscle of captive fish with respect to those of wild fish.

The influence of the body composition of fish, especially with regard to fat levels on oocyte recruitment, fecundity and atresia has been documented in Atlantic cod, *Gadus morhua* (Linnaeus, 1758), both in wild fish and experimentally (Kjesbu and Holm 1994; Karlsen et al. 1995; Skjaeraasen et al. 2010). The comparison of tissues and/or eggs from wild and captive fish allows the identification of potential nutritional deficiencies, which is essential for the development of suitable broodstock diets (Migaud et al. 2013). This strategy has been successful in many species, such as sea bass (Alasalvar et al. 2002), white seabream (Cejas et al. 2003, 2004), greater amberjack (Rodríguez-Barreto et al. 2012; Saito 2012) and Sengalese sole (*Solea senglensis* Kaup, 1858) (Norambuena et al. 2012).

The information in this study will contribute to the knowledge of wreckfish biology. Some biometric parameters were obtained and some indexes, i.e. gonadosomatic (GSI), hepatosomatic (HSI) and viscerosomatic (VSI) as well as perivisceral fat (PVF) were determined. The evaluation of the biochemical composition of tissues of wild fish and the comparison of mature gonads composition between wild and reared fish are basic information on this species and will help to improve the knowledge of its nutritional requirements. This will be useful towards obtaining a specific diet for wreckfish, which is essential in the development of the aquaculture of this species.

Materials and methods

Sampling procedure

Samples of wild wreckfish were obtained from February 2014 to April 2015. A total of 86 fish were sampled in Vigo harbour market (Galicia, NW Spain) from the Azores (North Atlantic, Portugal), according with the availability in the fish market. Total length (cm), perimeter (cm) and total and eviscerated weight (kg) were recorded in the market itself for each fish. Viscera were collected and taken to the laboratories of two facilities, Centro de Investigacións Mariñas (CIMA) and Instituto Español de Oceanografía (IEO), to perform the rest of the measurements. Gonads, liver, stomach, intestine and perivisceral fat were isolated and weighed to determine respective indexes: gonadosomatic index (GSI = gonad weight (g) × body weight^{-1} (g) × 100); hepatosomatic index (HSI = liver weight (g) × body weight^{-1} (g) × 100) and viscerosomatic index (VSI = visceral weight (g) × body weight^{-1} (g) × 100). Perivisceral fat (PVF = perivisceral fat weight (g) × body weight^{-1} (g) × 100) was collected and its percentage of total weight was determined.

Samples from gonads ($n = 58$) were preserved to be studied through histological analysis to determine sex and maturation stage, and 28 females and 30 males were identified. Samples collection of white epaxial muscle ($n = 24$), liver ($n = 55$) and immature gonads ($n = 55$) were carried out for biochemical analysis. Furthermore, 17 samples of male gonads and 15 of female gonads from fish previously identified by sex were analysed separately.

Additionally, some samples of mature gonads were collected and analysed: six from wild fish (four females from Vigo harbour market and two males from Canary Islands market), and 10 from reared wreckfish from females of different broodstocks: Instituto Español de

Oceanografía (IEO), Instituto Galego de Formación en Acuicultura (IGAFA), Aquarium Finisterrae de A Coruña (AF) and Acuario O Grove.

Samples analysis

Before performing the biochemical analyses, all the samples were stored at −80°C and freeze dried. The biochemical analyses of samples of tissues of wreckfish were carried out for each individual in triplicate by the following methods.

Proteins were determined by the Bradford method (Bradford 1976) in a UV spectrophotometer Lambda 35 (Perkin Elmer, Beaconsfield, UK). The extraction of total lipids was carried out following the Bligh and Dyer method with chloroform:methanol (2:1) and gravimetric determination (Bligh and Dyer 1959, modified by Fernández-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 600 Autosampler 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 SP^{TM}-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).

Regarding the histological analysis, the gonads were fixed in 2.5% glutaraldehyde in 0.025 M and processed using conventional histological procedures. Subsequently, semi-thin sections (1–2 µm thick) were placed on polylysinated microscopy slides (Poly-L-Lysine, SIGMA) and stained with Toluidine-blue (toluidine 1%–Borax 1%). From each part, two cross sections were obtained and from each cross section, one field was used to quantify the different cell types.

Observations were made with an Olympus Provis X70 microscope with a 40× objective and equipped with automatic image analysis software (Microimage, Cybernetics Average). Fields were photographed with a digital Olympus DP10 camera. Maturation stages for microscopic examination were assigned comparing with macroscopic maturation studies: Stage I: immature; Stage II: developing; Stage III: developed; Stage IV: spawning; and Stage V: spent (Wakefield et al. 2010).

Statistical analyses

Data are reported as mean values ± standard deviations (SD). Statistical analysis was done with the statistical Minitab program, 17 version. Data were submitted to a one-way ANOVA and when the tests showed significance, individual means were compared using Tukey's test. When the data did not meet the ANOVA test requirements, they were analysed with non-parametric methods (Kruskal–Wallis). Significant differences were considered at $P \leq 0.05$.

Additionally, three principal component analyses (PCA) were performed for muscle ($n = 18$), liver ($n = 55$) and gonad ($n = 32$) to detect variations in proteins, lipids and fatty acids composition of different tissues, sex, season of capture, body size and weight, and to identify the parameters that were most responsible for the observed variation. After verifying the absence of outliers, the variables were standardized and the PCAs were performed using the *dudi.pca* function of the *ade4* package of the fee software R Project for Statistical Computing (https://r-project.softonic.com/).

Results

Biometric parameters

The values of biometric parameters (min–max and mean ± SD) for all individuals are shown in Table 1. In addition, all the variables were analysed for the individuals in which sex was characterized, and the results obtained showed significant differences ($P \leq 0.05$) in GSI data between males (0.10 ± 0.1) and females (0.28 ± 0.2), which was also related to an initial degree of maturity (stages I–II–III) in some individuals (see histological analysis). The same result was recorded for males/females in total length (73.91 ± 5/78.51 ± 7.1 cm), perimeter (54.5 ± 3.9/57.53 ± 6.8 cm), total weight (6.29 ± 1.4/7.74 ± 2.5 kg), eviscerated weight (6.18 ± 1.6/7.54 ± 3.4 kg) and gonad weight (11.94 ± 16.4/27.98 ± 26.0 g). For all these parameters, females had significantly higher levels than those observed in males. There was high variability between individuals in stomach, intestine and liver weight, HSI and VSI, but no significant differences ($P > 0.05$) were found between sexes.

Perivisceral fat was collected throughout the entire sampling period (14 months) finding animals with a percentage of 0 up to almost 3.5% fat with respect to body weight, but no differences related to sex or capture season were recorded.

The relationship between weight of individuals and GSI, HSI, VSI for males ($n = 30$) and females ($n = 28$) and

Table 1. Biometric parameters (min–max and means ± SD) for all individuals of wild caught wreckfish ($n = 86$) and for males ($n = 30$) and females ($n = 28$) for individuals in which sex was characterized.

Biometric Parameters	All individuals ($n = 86$)		Males ($n = 30$)	Females ($n = 28$)
	Min–Max	Mean	Mean	
Total length (cm)	54–98	75.36 ± 7.4	73.91 ± 5.0*	78.51 ± 7.1*
Standard length (cm)	48–99	65.99 ± 7.7	65.83 ± 7.7	68.34 ± 6.6
Perimeter (cm)	40–81	55.13 ± 6.1	54.5 ± 3.9*	57.53 ± 6.8*
Total weight (kg)	2.6–18.0	7.25 ± 2.2	6.29 ± 1.4*	7.74 ± 2.5*
Eviscerated weight (kg)	2.4–16.0	6.73 ± 2.5	6.18 ± 1.6*	7.54 ± 3.4*
Perivisceral fat (g)	0–339.3	70.42 ± 71.9	84.40 ± 79.1	66.34 ± 80.2
Stomach weight (g)	54.2–457.2	147.98 ± 72.1	132.97 ± 46.8	189.29 ± 113.3
Intestine weight (g)	34.2–274.0	94.48 ± 61.6	108.93 ± 65.3	126.00 ± 70.0
Gonadal weight (g)	0.47–117.6	14.60 ± 19.1	11.94 ± 16.4*	27.98 ± 26.0*
Liver weight (g)	29.3–516.3	95.91 ± 65.7	73.52 ± 46.8	104.71 ± 92.5
Intestine length (cm)	61–144	96.48 ± 14.0	98.08 ± 10.7	103.75 ± 13.7
HSI	0.59–3.11	1.27 ± 0.5	1.16 ± 0.5	1.29 ± 0.5
VSI	2.40–16.02	7.26 ± 2.1	5.24 ± 2.6	4.54 ± 2.8
GSI	0.01–0.84	0.17 ± 0.1	0.10 ± 0.1*	0.28 ± 0.2*

The asterisk (*) indicates the existence of significant differences between males and females ($P \leq 0.05$).

PVF for males ($n = 25$) and females ($n = 21$) is shown in Figure 1.

Histological analyses

Of the identified wreckfish females and males, 97% were immature (stages I, II, III) (Wakefield et al. 2010). Histological analysis of the female gonads of 60–80 cm length and < 8 kg weight fish (61% of sexed females), showed developed oogonia in small cysts and primary oocytes, stage I (Figure 2A). For females with length 81–91 cm and weight ≥8 kg (36% of sexed females), vitellogenic oocytes were observed, stage II (Figure 2B), and in one female captured in October 2014, with 91 cm length and 14.2 kg weight, the ovary contained many large vitellogenic and with cortical alveoli oocytes, stage III (Figure 2C) (Papadaki et al. 2018). Only a female with 18.0 kg weight and 98 cm length, captured in May 2014, showed mature gonads.

For males around 56 and 72 cm in length and <6 kg in total weight (30% of sexed males), the gonads contained clusters of spermatogonia (Sg) and spermatocytes (Sc) which were irregular in shape and densely stained, stage I (Figure 2D). From individuals measuring 73–82 cm in total length (67% of sexed males) and 6–10 kg body weight, the gonads contained all other stages of spermatogenesis: spermatocyte (Sc), spermatids (St), stage II (Figure 2E). Only one spermiating male (Sz), stage IV (Figure 2F) with 10.5 kg weight and 83 cm length was captured, in September 2014.

Tissues composition

Biochemical analysis, proximate composition and fatty acid profile (Table 2) show that wild wreckfish have a high level of proteins in muscle, 84.41% of dry weight (DW) and low level of lipids (6.92% DW). A high variability among individuals was found in liver and gonad composition. The average values of protein and lipids in liver were 38.16 and 39.34%, respectively, and in gonads the protein content reached 46.87% and the lipids were 26.31%. Analyses of male and female gonads were carried out separately (Table 3), showing a significantly higher amount of proteins in female (55.95% DW) than in male gonads (43.81% DW). Lipid levels varied between 22.07% in females and 29.98% in males.

Regarding fatty acid composition in muscle (Table 2), polyunsaturated fatty acids (PUFA), saturated fatty acids (SAFA) and monounsaturated fatty acids (MUFA) fractions had average values of 39.08, 28.83 and 32.09% of total fatty acids (TFA), respectively. DHA represents a very high content (26.38% TFA), EPA 4.55% and ARA 3.11%. DHA + EPA represented more than 30% of TFA in the muscle of wild wreckfish.

In liver, the average value of PUFA was 16.66% of TFA, SAFA content reached 26.48% and MUFA 55.64%. DHA level reached 8.69% of TFA, EPA 2.9% and ARA 1.48%. All the values have shown a high variability among individuals.

With respect to fatty acid profile of immature gonads (Table 3), even though no significant differences were found in fatty acid content between male and female gonads, the PUFA content was slightly higher in female gonads (31.51% TFA) than in male gonads (26.7%), n-3 and n-6 PUFA represented 25.74% and 5.43% in female gonads and 21.91 and 4.4% TFA in male gonads. DHA, EPA and ARA reached values of 16.73, 4.82 and 4.44% TFA, respectively, in females and 14.37, 4.29 and 3.47% in males. The EPA/ARA ratio was 1.4 in female and male immature gonads.

A principal component analysis was performed to determine the variation in the composition of

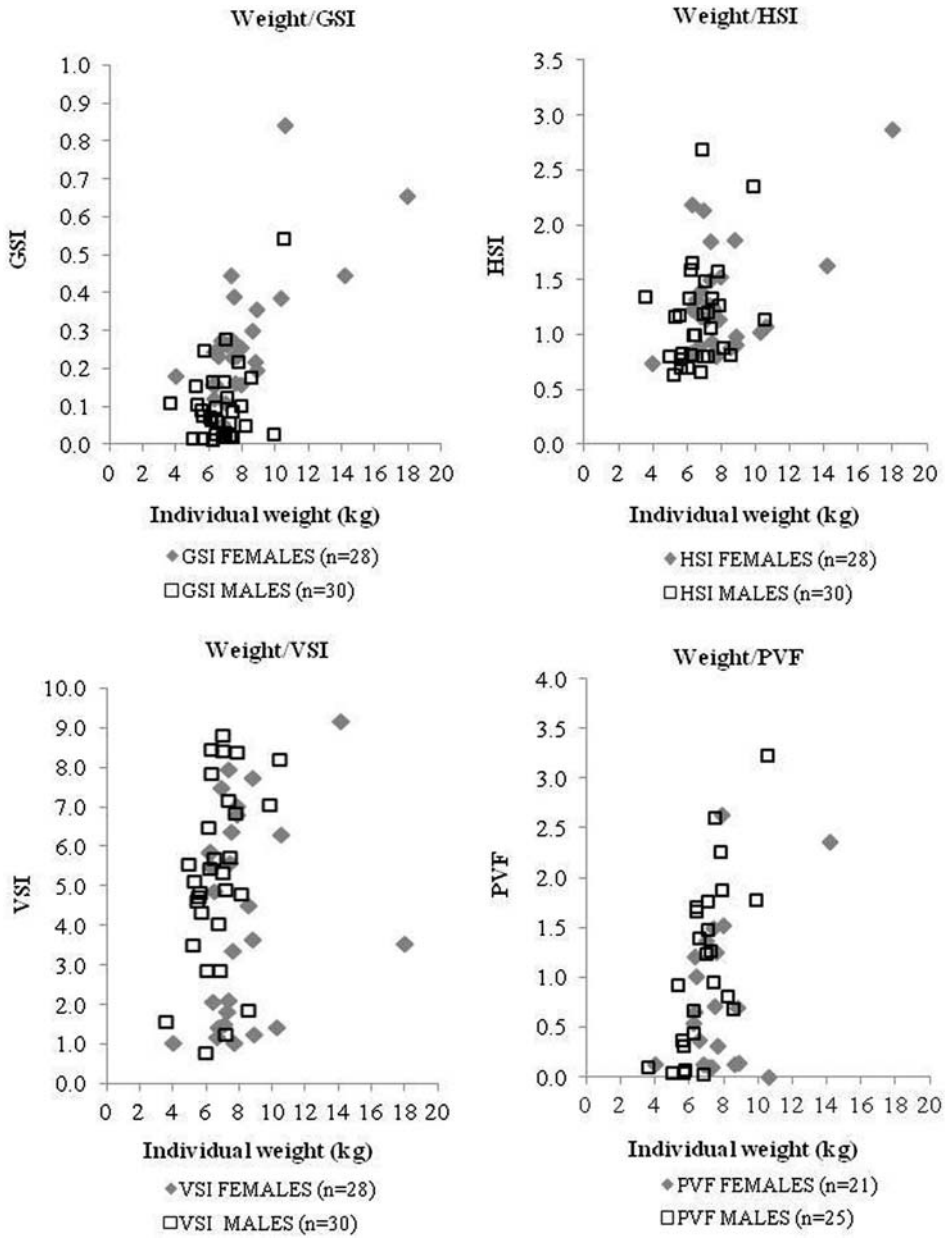

Figure 1. Relationship between weight of individuals and gonadosomatic index (GSI), hepatosomatic index (HSI), viscerosomatic index (VSI), and perivisceral fat (PVF) for males and females.

protein, lipids and fatty acids of the different organs and identify the parameters that most affected this variability. The highest protein (>80%) and lipid (>36%) levels were observed in muscle and liver respectively, in both sexes and for any season of the year (Table 4). Significant differences in gonad protein levels have been reported between males (47.10%) and females (63.28%), but were only recorded between January and June. In muscle of males, higher SAFA levels (TFA) in the first semester (29.90%) than in the second (27.54%) were obtained. The same trend was observed for n-6, which decreased from 4.33% to 3.42% of TFA between the same periods. For the rest of the variables, no significant differences were observed between sexes and season, and the levels were similar to those previously described in Tables 2 and 3.

The PCA shows how the variables studied are organized for the different tissues based on the values determined by the first two principal components of the analysis. These axes (PC1 and PC2) represent 49.5% and 16.8% of the observed variability for muscle, 54.6% and 15.9% for liver and 52.8% and 13.1% for gonads, respectively (Figure 3, left). In general, all the variables of the fatty acid analysis were highly positively correlated with each other

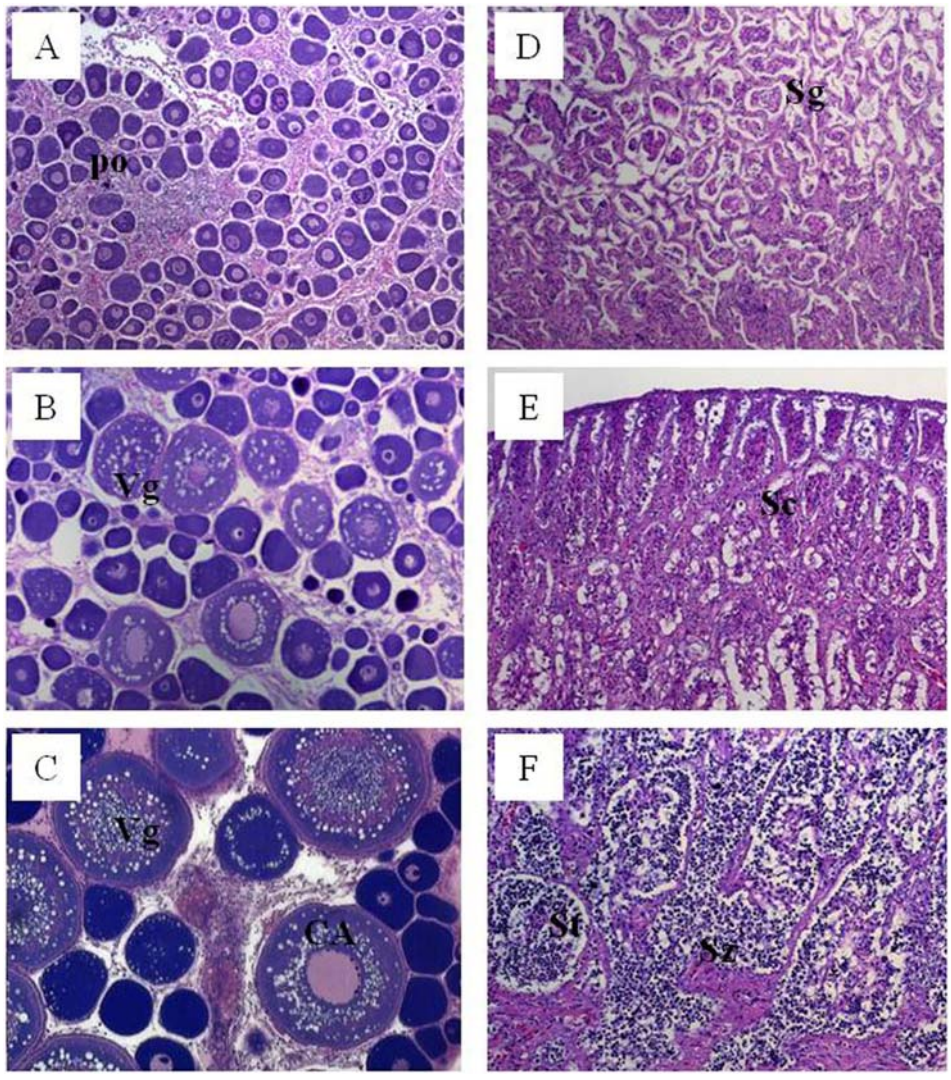

Figure 2. Histological sections of gonads samples of wild females (left column; A, B, C) and males (right column; D, E, F). Primary oocytes (po), vitellogenic oocytes (Vg) and cortical alveoli (CA) oocytes in females, and spermatogonias (Sg), spermatocytes (Sc), spermatids (St) and spermatozoa (Sz) in male gonads.

(PUFA, n-3, n-6, ARA, EPA, DHA and SAFA) and negatively with MUFA in the three tissues, and their variability is mainly explained by the first component of the analysis (PC1). EPA in muscle and saturated fatty acids (SAFA) in liver and gonads did not show this trend and were poorly explained by PCA. A similar result was observed with lipids and proteins in muscle, while they were negatively correlated in liver and gonads. Weight and size also appeared positively related and close to the correlation circle in muscle and liver, and their variability is strongly explained by the second axis (PC2). The capture season (PERIOD) and sex were the least represented variables for the three tissues and their variability is poorly explained by the PCA. Likewise, the individuals studied did not show any determined clustering pattern and were homogeneously distributed throughout the area delimited by the two axes (Figure 3, right). The PCA did not reveal a strong effect of the capture season, sex, size or weight of the fish on the variability observed in the contents of proteins, lipids and fatty acids of the different tissues. This consolidates the explanation mentioned above for the gonads, where no significant difference was observed in the concentration of fatty acids related to sex (Tables 3 and 4).

Proximate composition and fatty acid composition of mature gonads are shown in Table 5. The results showed a high value of proteins (62.78% in females and 44.02% in males) and the lipid content represented 20.73% in females and 13.21% in males in wild gonads, n-3 PUFA values reached 34.57% in wild females and 39.88% in male gonads, having a large amount of DHA (25–31%), EPA represented 5.35% in both females and males and ARA content was 7.07%

Table 2. Protein, lipid (% DW) and fatty acids profile (%TFA) of muscle ($n = 24$), liver ($n = 55$) and gonads ($n = 55$) of wild wreckfish.

	Muscle	Liver	Immature Gonads
Proximate analysis			
Proteins	84.41 ± 7.3	38.16 ± 12.9	46.87 ± 14.1
Lipids	6.92 ± 3.4	39.34 ± 15.0	26.31 ± 14.4
Fatty acid composition			
14:00	2.08 ± 0.4	1.91 ± 0.6	2.81 ± 0.8
16:00	19.37 ± 0.8	17.79 ± 3.9	17.87 ± 1.9
17:00	1.04 ± 0.2	1.09 ± 0.4	1.21 ± 0.2
18:00	5.89 ± 0.6	5.37 ± 1.2	6.35 ± 1.4
Σ SAFA	28.83 ± 1.3	26.48 ± 5.2	28.76 ± 2.8
16:1 n-9	0.47 ± 0.1	1.12 ± 0.4	0.67 ± 0.1
16:1 n-7	5.10 ± 1.2	8.58 ± 3.5	6.11 ± 1.2
18:1 n-9	16.68 ± 3.5	30.79 ± 7.0	21.29 ± 3.3
18:1 n-7	4.01 ± 1.0	8.25 ± 1.9	5.14 ± 0.9
20:1 n-9	2.16 ± 0.8	3.32 ± 1.2	3.46 ± 2.6
Σ MUFA	32.09 ± 5.4	55.64 ± 10.7	42.40 ± 8.9
18:2 n-6	0.97 ± 0.1	0.96 ± 0.4	0.96 ± 0.2
20:4 n-6 (ARA)	3.11 ± 0.8	1.48 ± 0.9	3.70 ± 2.3
20:5 n-3 (EPA)	4.55 ± 0.7	2.90 ± 1.5	4.55 ± 1.5
22:6 n-3 (DHA)	26.38 ± 3.3	8.69 ± 5.4	15.56 ± 4.8
Σ PUFA	39.08 ± 4.4	16.66 ± 8.9	28.84 ± 8.5
Σn-3	34.51 ± 3.7	14.01 ± 7.7	23.80 ± 6.8
Σn-6	4.08 ± 0.8	2.44 ± 1.3	4.66 ± 2.3
n-3/n-6	8.50 ± 1.2	5.48 ± 1.7	5.44 ± 2.0
DHA/EPA	5.69 ± 1.2	2.92 ± 1.0	3.35 ± 0.8
EPA/ARA	1.54 ± 0.4	2.02 ± 0.7	1.49 ± 0.6

Values expressed in mean ± SD.

Table 3. Protein, lipid (%DW) and fatty acid profile (%TFA) of immature gonads ($n = 32$) of wild wreckfish.

	Male Gonads	Female Gonads
	Proximate analysis	
Proteins	43.81 ± 11.0**b**	55.95 ± 13.9**a**
Lipids	29.98 ± 14.8	22.07 ± 16.7
	Fatty acid composition	
14:00	2.93 ± 1.0	2.62 ± 0.7
16:00	18.33 ± 2.7	17.06 ± 0.7
17:00	1.16 ± 0.3	1.29 ± 0.3
18:00	6.43 ± 1.6	6.50 ± 1.5
Σ SAFA	29.38 ± 3.9	27.98 ± 1.2
16:1 n-9	0.73 ± 0.2	0.64 ± 0.1
16:1 n-7	6.14 ± 1.4	5.58 ± 1.1
18:1 n-9	21.07 ± 4.3	21.25 ± 3.0
18:1 n-7	4.90 ± 1.0	5.00 ± 0.8
20:1 n-9	4.18 ± 3.6	3.03 ± 1.7
Σ MUFA	43.92 ± 12.3	40.51 ± 6.2
18:2 n-6	0.92 ± 0.2	0.99 ± 0.2
20:4 n-6 (ARA)	3.47 ± 2.3	4.44 ± 2.7
20:5 n-3 (EPA)	4.29 ± 1.9	4.82 ± 1.2
22:6 n-3 (DHA)	14.37 ± 6.9	16.73 ± 2.3
Σ PUFA	26.70 ± 11.7	31.51 ± 5.5
Σn-3	21.91 ± 9.6	25.74 ± 3.5
Σn-6	4.40 ± 2.3	5.43 ± 2.7
n-3/n-6	4.94 ± 1.7	5.39 ± 2.4
DHA/EPA	3.12 ± 1.0	3.52 ± 0.6
EPA/ARA	1.41 ± 0.5	1.45 ± 0.8

Values are expressed in mean ± SD. Different letters (a, b) denote significant differences between males and females ($P \leq 0.05$; one-way ANOVA; Tukey).

and 10.10% of total fatty acids in female and male wild gonads, respectively.

In relation to composition of female mature gonads, the values of proteins from wild fish were 62.78% and from reared fish 58.11%. Lipid content was 27.97% and 20.73% in gonads from reared and wild fish, respectively (Table 6). Regarding fatty acid content, PUFA values reached 42.47% in wild female gonads and 36.49% in gonads from reared fish with n-3 PUFA being a little higher in wild (34.57%) than in reared gonads (30.42%), and DHA levels of 25.12% and 18.2% TFA were observed in wild and reared female gonads, respectively. The same trend was detected in n-6 PUFA content which represented 7.57% and 5.55% in wild and reared gonads.

Significant differences were found between female gonads from wild and captive-reared wreckfish, in some individual fatty acids as 14:0 and 17:0 (both represented a small percentage of total fatty acids) and 18:1n-9 which reached 20.72% and 15.45% TFA in captive-reared and wild gonads respectively. 18:2n-6 (linoleic acid, LA) represents 2.82% in captive female gonads, which was higher than the amount obtained in wild gonads (0.50% TFA). More remarkable were the significant differences found in some PUFA as EPA and ARA, with EPA values being higher in mature gonads from reared (7.75%) than in wild (5.35%) wreckfish. Conversely, ARA content was higher from wild (7.07%) than in reared (2.73%) female gonads. DHA/EPA and EPA/ARA had values of 2.44 and 3.40 in reared gonads and 4.7 and 0.77 in wild gonads.

Discussion and conclusions

The wreckfish is expected to be suitable for aquaculture due to its fast growth, being a large finfish, marketed at a large size and the possibility to process it into a range of products to provide the consumer with both a greater diversity of fish species and new value-added products (Mylonas and Robles 2014).

The macroscopic appearance and microscopic structure of wreckfish gonads were described for immature specimens of this species and the gonochoric character and late maturation of the species is confirmed (Roberts 1989; Peres and Klippel 2003; Wakefield et al. 2010).

The data obtained during this study indicate that the most important amount of wreckfish marketed in NW Spain (Galicia) is immature. Individuals ($n = 86$) with weights between 2.6 and 18 kg and with a wide range of sizes between 54 and 98 cm were studied. Only two mature specimens during the sampling period, from February 2014 to April 2015, were found: a mature male in September 2014 of 10.5 kg and a female of 14.2 kg in October 2014. This wide difference in sizes in wreckfish that came from the

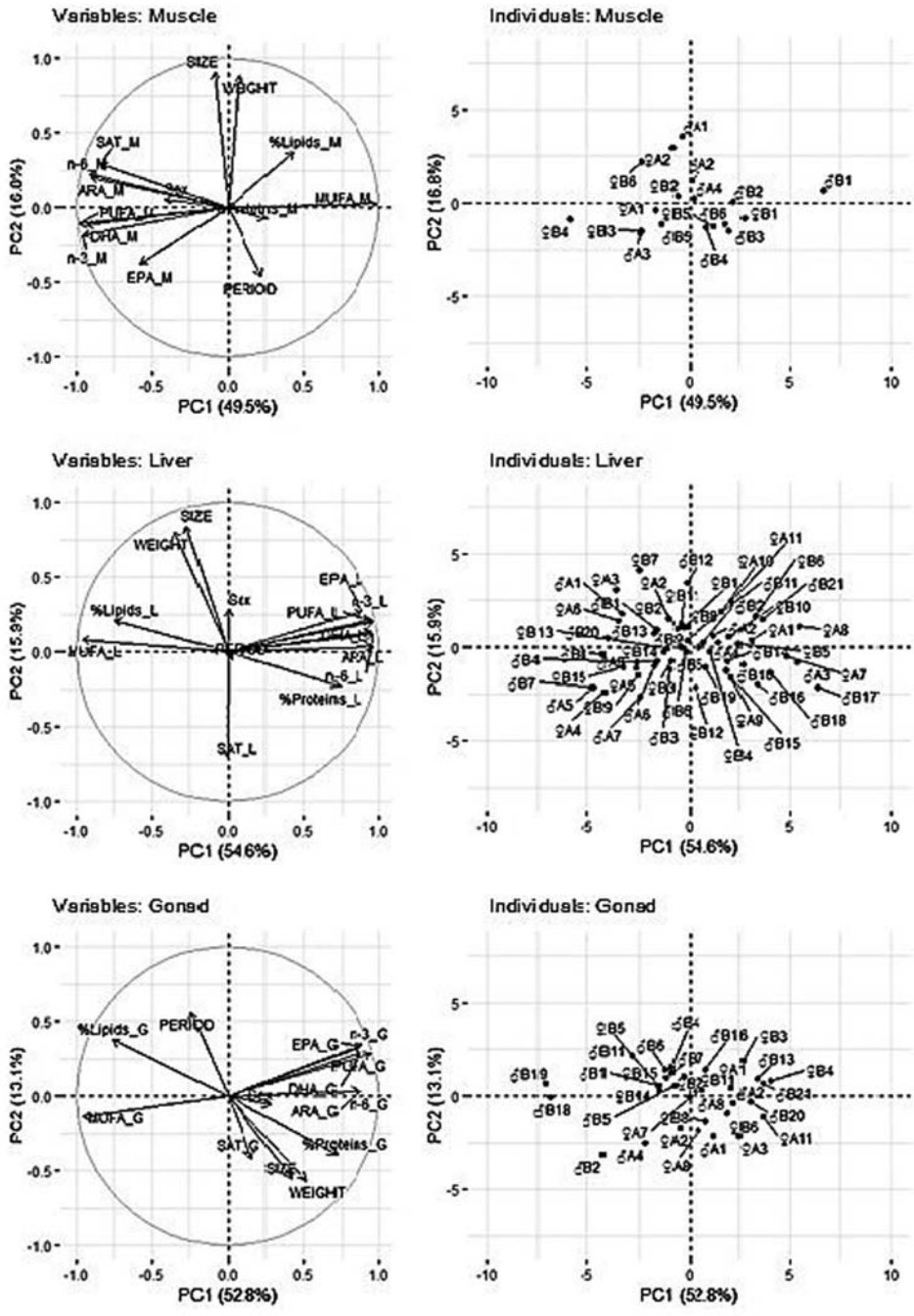

Figure 3. Distribution of the most significant proteins, lipids, fatty acids and variables (left) and cases (right) along the two first principal components (PC) of the Principal Component Analysis (PCA). For each tissue, the variables are identified with a specific epithet: Muscle (_M), Liver (_L) and Gonad (_G). The individuals are identified with a code that contains the sex (male = ♂ or female = ♀), the season that it was captured (A = January–June; B = July–December) and the number (*n*).

Azores fisheries may be due to the different fishing gear used for capture (Sedberry et al. 1996, 1999). In addition, the low GSI values confirm the immaturity status of the specimens, with values that average 0.10 in males and 0.28 in females. Wakefield et al. (2013) found that specimens in more advanced stages of gonad maturation have GSI values of between 4.0 and 10.0 for males and females, respectively. Furthermore, they found that the minimum sizes in mature specimens were 93.0 and 82.5 cm for females and males, respectively. In our sampling, the only mature fish were a female and a male of 98 and 83 cm in total length.

Regarding morphometric parameters, significant differences in total weight and total length were found between sexes. In captive fish, the difference in

Table 4. Values obtained for the different variables studied organized by tissue, capture season (A = January–June; B = July–December) and sex (male = ♂ or female = ♀).

Period	Sex	% Proteins	% Lipids	Σ PUFA	Σ SAT	Σ MUFA	Σ n-3	Σ n-6	ARA	EPA	DHA	Body Size	Weight
Muscle (M)													
A	♂ (*n* = 4)	88.77 ± 7.6	5.45 ± 1.0	39.71 ± 2.7	*29.90 ± 0.3	30.39 ± 3.0	34.64 ± 2.2	*4.33 ± 0.3	3.22 ± 0.3	4.40 ± 0.4	26.77 ± 2.7	77.25 ± 5.8	7.83 ± 2.3
	♀ (*n* = 2)	81.09 ± 0.4	12.55 ± 9.8	40.38 ± 0.9	29.40 ± 0.8	30.23 ± 0.1	35.54 ± 0.9	4.72 ± 0.1	3.79 ± 0.0	4.41 ± 0.2	27.61 ± 1.1	78.25 ± 5.3	7.09 ± 1.2
B	♂ (*n* = 6)	89.16 ± 4.1	8.34 ± 3.1	36.14 ± 5.5	*27.54 ± 0.8	36.31 ± 6.1	32.28 ± 4.9	*3.42 ± 0.6	2.50 ± 0.6	4.23 ± 0.7	24.47 ± 3.9	75.42 ± 1.9	6.80 ± 0.5
	♀ (*n* = 6)	85.28 ± 7.6	6.22 ± 1.4	40.66 ± 5.4	29.19 ± 1.7	30.15 ± 7.0	36.07 ± 4.4	4.39 ± 1.2	3.40 ± 1.2	5.06 ± 0.7	27.09 ± 4.5	77.50 ± 4.3	7.11 ± 0.9
Liver (L)													
A	♂ (*n* = 8)	36.94 ± 12.4	41.36 ± 13.4	14.37 ± 9.4	26.97 ± 2.5	58.67 ± 8.2	11.87 ± 7.9	2.26 ± 1.5	1.33 ± 0.9	2.60 ± 1.9	7.13 ± 4.9	75.31 ± 4.7	7.32 ± 1.7
	♀ (*n* = 11)	38.24 ± 17.3	40.52 ± 19.0	18.69 ± 9.9	25.49 ± 3.5	55.82 ± 9.8	15.77 ± 8.6	2.65 ± 1.4	1.60 ± 0.9	3.27 ± 1.9	9.80 ± 5.8	77.01 ± 5.0	7.60 ± 1.1
B	♂ (*n* = 21)	36.20 ± 11.8	41.27 ± 16.4	17.88 ± 7.7	25.58 ± 3.0	56.53 ± 8.4	15.11 ± 6.5	2.60 ± 1.2	1.57 ± 0.9	3.25 ± 1.4	9.25 ± 4.6	75.24 ± 4.0	6.93 ± 1.4
	♀ (*n* = 15)	38.09 ± 11.4	36.38 ± 8.7	16.12 ± 5.9	26.34 ± 3.6	57.54 ± 5.9	13.64 ± 5.2	2.33 ± 0.5	1.40 ± 0.5	2.78 ± 1.0	8.42 ± 3.6	78.03 ± 4.5	7.65 ± 1.8
Gonad (G)													
A	♂ (*n* = 4)	47.10 ± 6.4**b**	23.67 ± 6.6	28.91 ± 9.0	30.92 ± 4.7	40.17 ± 4.8	23.56 ± 7.9	4.88 ± 1.3	3.74 ± 1.5	4.78 ± 0.9	15.76 ± 6.3	78.75 ± 4.2	8.21 ± 1.8
	♀ (*n* = 6)	63.28 ± 12.1**a**	17.84 ± 8.3	31.48 ± 5.0	28.00 ± 0.8	40.52 ± 4.9	25.55 ± 3.0	5.60 ± 2.3	4.68 ± 2.5	4.76 ± 1.1	16.94 ± 2.2	78.42 ± 5.2	7.62 ± 1.2
B	♂ (*n* = 13)	42.79 ± 12.0	31.92 ± 16.2	26.01 ± 12.6	28.91 ± 3.8	45.08 ± 13.8	21.40 ± 10.3	4.25 ± 2.6	3.33 ± 2.5	4.19 ± 2.1	13.99 ± 7.3	76.04 ± 3.4	6.98 ± 1.3
	♀ (*n* = 9)	47.44 ± 15.3	27.06 ± 20.1	31.44 ± 20.1	27.92 ± 1.4	40.64 ± 6.9	26.02 ± 3.8	5.09 ± 3.0	4.04 ± 3.0	5.01 ± 1.4	16.51 ± 2.4	78.00 ± 3.9	7.25 ± 0.9

Values are expressed in mean ± SD. Different letters (a, b) in the same column denote significant differences between males and females, while an asterisk indicates significant differences related to capture period (A/B) for the same sex (a, b / * = $P \leq 0.05$).

Table 5. Protein, lipid (%DW) and fatty acid composition (% TFA) of mature gonads ($n = 6$) of wild wreckfish.

	Female Gonads	Male Gonads
	Proximate analysis	
Proteins	62.78 ± 9.1	44.02 ± 3.8
Lipids	20.73 ± 4.8	13.21 ± 3.1
	Fatty acid composition	
14:00	1.15 ± 0.4	0.93 ± 0.2
16:00	17.27 ± 2.9	19.63 ± 0.8
17:00	1.08 ± 0.1	0.68 ± 0.2
18:00	6.42 ± 0.5	7.69 ± 0.2
Σ SAFA	26.30 ± 3.0	29.24 ± 0.4
16:1 n-9	1.15 ± 0.7	0.38 ± 0.1
16:1 n-7	3.97 ± 2.0	1.57 ± 0.6
18:1 n-9	15.45 ± 5.5	9.58 ± 1.9
18:1 n-7	6.84 ± 3.7	4.24 ± 1.0
20:1 n-9	1.70 ± 0.2	1.99 ± 0.1
Σ MUFA	31.23 ± 11.7	19.37 ± 3.9
18:2 n-6	0.50 ± 0.4	0.59 ± 0.1
20:4 n-6 (ARA)	7.07 ± 1.4	10.10 ± 1.1
20:5 n-3 (EPA)	5.35 ± 1.5	5.35 ± 0.1
22:6 n-3 (DHA)	25.12 ± 7.4	31.40 ± 4.9
Σ PUFA	42.47 ± 9.7	51.39 ± 3.6
Σn-3	34.57 ± 8.9	39.88 ± 4.9
Σn-6	7.57 ± 1.2	10.69 ± 1.1
n-3/n-6	4.54 ± 0.8	3.81 ± 0.9
DHA/EPA	4.70 ± 0.2	5.87 ± 0.9
EPA/ARA	0.77 ± 0.2	0.54 ± 0.1

Values are expressed in mean ± SD.

Table 6. Protein, lipid (%DW) and fatty acid composition (% TFA) of mature female gonads from wild ($n = 4$) and captive-reared ($n = 10$) wreckfish.

	Wild	Captive-reared
	Proximate analysis	
Proteins	62.78 ± 9.1	58.11 ± 8.8
Lipids	20.73 ± 5.2	27.97 ± 13.5
	Fatty acid composition	
14:00	1.15 ± 0.4**b**	2.50 ± 0.8**a**
16:00	17.27 ± 2.9	16.21 ± 0.9
17:00	1.08 ± 0.1**a**	0.78 ± 0.2**b**
18:00	6.42 ± 0.5	5.28 ± 1.0
Σ SAFA	26.30 ± 3.0	25.10 ± 1.5
16:1 n-9	1.15 ± 0.7	0.83 ± 0.3
16:1 n-7	3.97 ± 2.0	5.45 ± 1.1
18:1 n-9	15.45 ± 5.5**b**	20.72 ± 2.4**a**
18:1 n-7	6.84 ± 3.7	5.82 ± 1.4
20:1 n-9	1.70 ± 0.2	2.34 ± 0.7
Σ MUFA	31.23 ± 11.7	38.41 ± 4.8
18:2 n-6	0.50 ± 0.4**b**	2.82 ± 1.7**a**
20:4 n-6 (ARA)	7.07 ± 1.4**a**	2.73 ± 1.5**b**
20:5 n-3 (EPA)	5.35 ± 1.5**b**	7.75 ± 1.6**a**
22:6 n-3 (DHA)	25.12 ± 7.4	18.20 ± 6.0
Σ PUFA	42.47 ± 9.7	36.49 ± 5.3
Σn-3	34.57 ± 8.9	30.42 ± 6.1
Σn-6	7.57 ± 1.2	5.55 ± 2.1
n-3/n-6	4.54 ± 0.8	6.39 ± 3.2
DHA/EPA	4.70 ± 0.2**a**	2.44 ± 0.9**b**
EPA/ARA	0.77 ± 0.2**b**	3.40 ± 2.0**a**

Values are expressed in mean ± SD. Different letters (a, b) denote significant differences between wild and captive reared fish ($P \leq 0.05$).

growth was demonstrated based on sex (Papandroulakis et al. 2004; Rodríguez et al. 2017), as observed in many other marine species where the growth is strongly influenced by the sex. In wild wreckfish, this sex-dependent morphometric difference has been

demonstrated in fisheries and exploitation studies (Sedberry et al. 1999; Wakefield et al. 2013).

It is noteworthy that regarding the great variability in terms of stomach contents, PVF and HSI, none of the three parameters is related to either the time of capture or sex, or any other parameter studied. It is possible that it is related to the transition from pelagic to demersal life, which takes place before sexual maturation, at 70–80 cm in length (Sedberry et al. 1999), and involves important morphological changes (Machias et al. 2003).

The high quality of wreckfish is well known with a high level of protein (84%) and low level of lipids (7%) in wild wreckfish muscle. In the case of mature female gonads, the protein content is 63% and 58% of dry weight in wild and reared fish and lipid content is 28% and 21% in gonads from reared and wild fish, respectively.

The liver was the main lipid storage organ reaching high values with a high content in wild wreckfish (39%). It is considered as an indicator-organ for the nutritional and physiological status of 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, 2013).

The predominant fatty acid of the muscle of wild wreckfish was DHA, 26%, similar to the results obtained by Roncarati et al. (2014) for the Mediterranean wreckfish with values between 24.2–25.7%. Other fatty acids, such 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 immature gonads and 7% and 10% in female and male mature gonads, respectively.

The PCA analysis demonstrated that there is no relationship between the proteins, lipids and fatty acids content of the wreckfish tissues (muscle, liver and gonads) with sex, weight, size or capture season. The mobilization of proteins and PUFA from the liver to the gonads is an important mechanism during gametogenesis (Sargent et al. 2002). Because the spawning season of the species takes place in the North Atlantic mainly between January and May (Sedberry et al. 2006), the significant differences observed in protein levels between female (63.28%) and male (47.10%) gonads during the first half of the year suggest the presence of some maturing female samples. The high protein level corresponds to that observed in the mature female gonads of wild wreckfish (62.78%) analysed in this work. Since most of the fish used in this study were young or immature and it has not been possible to take many samples from large individuals, more studies involving a wide range of sizes should be carried out to draw general conclusions for this species.

In wreckfish, with regard to whole fatty acids, only MUFA presented higher values in gonads from reared fish than developed gonads from wild fish while PUFA, n-3 and n-6 showed slightly higher values in female gonads from wild fish than those from reared fish. ARA and DHA content reached values of 7.07% and 25.12% TFA in wild wreckfish female mature gonads, higher than in farmed wreckfish (2.73% and 18.20%), with this representing a decrease of 60% and 28%, respectively; this consequently affects the EPA/ARA and DHA/EPA ratios with values of 3.4 and 2.4 in reared fish and between 0.8 and 4.7 in wild gonads. A similar trend was observed in female greater amberjack gonads between captive reared and wild fish where values displayed around 40% and 30% less ARA and DHA, respectively (Zupa et al. 2017).

It is known that diet is a very important factor influencing fish fatty acid composition (Cowey and Sargent 1972) and in marine fish, dietary lipids and in particular polyunsaturated fatty acids (PUFA) play a critical role in the successful production of high-quality gametes and eggs (Izquierdo et al. 2001; Sargent et al. 2002). Improvement of knowledge of wild fish composition is important as this often provides key information for formulation of specialized broodstock diets (Migaud et al. 2013). The high content of some polyunsaturated fatty acids, specifically ARA and DHA, found in gonads of wild specimens suggests that these fatty acids should play an important role in the diet of this species. Addition of ARA to the broodstock diet appears to have a species-specific optimum level (Bell and Sargent 2003). 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, than in culture broodstock. These factors were suggested to be related to higher viability in eggs from wild fish (Salze et al. 2005; Norambuena et al. 2012).

Gonads from females of wild wreckfish have high levels of ARA (7–10% TFA) and EPA/ARA is 0.8. The EPA/ARA ratio in oocytes and eggs from females is 0.9 and 1.1, respectively (Linares et al. 2018), which are similar to the values obtained in muscle and

gonads from wild fish. Given the influence of both ARA and EPA on tissue eicosanoid production it is likely that maintaining both n-6 and n-3 PUFA at values close to wild values will be of benefit to subsequent egg and larvae success (Migaud et al. 2013).

Wreckfish is considered one of the new/emerging fish species whose domestication represents a challenge, with the aim of contributing to diversification of the European aquaculture industry. The results obtained about the biology and biochemical composition of wild fish together with others regarding the results of natural spawning in captivity (Pérez Rial et al. 2019) are expected to be important starting points towards the achievement of the inclusion of wreckfish in aquaculture. Further research is required to overcome the essential and documented bottlenecks in the culture of wreckfish, such as reproduction control and larval rearing, in order to produce appropriate numbers of juveniles to launch commercial production.

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Disclosure statement

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