Sex steroids of black scabbardfish, *Aphanopus carbo*, in relation to reproductive and migratory dynamics

Inês Farias\textsuperscript{a,b,∗}, Elsa Couto\textsuperscript{c}, Neide Lagarto\textsuperscript{b}, João Delgado\textsuperscript{d,e}, Adelino V.M. Canário\textsuperscript{c}, Ivone Figueiredo\textsuperscript{a}

\textsuperscript{a}Department of Sea and Marine Resources, Portuguese Institute for Sea and Atmosphere (IPMA), Avenida Doutor Alfredo Magalhães Ramalho 6, Algés, 1495-165, Portugal

\textsuperscript{b}Mediterranean Institute for Advanced Studies (IMEDAE), Esporles, 07190, Spain

\textsuperscript{c}Centre of Marine Sciences (CCMAR-CIMAR), University of Algarve, Campus de Gambelas, Faro, 8005-139, Portugal

\textsuperscript{d}Regional Fisheries Directorate, Research Service (DSI-DRP), Estrada da Pontinha S/N, Funchal, 9004-562, Portugal

\textsuperscript{e}Interdisciplinary Centre of Marine and Environmental Research (CIIMAR-CIMAR), Rua Dos Bragas, Porto, 4050-123, Portugal

\textsuperscript{∗}E-mail addresses: ifarias@ipma.pt (I. Farias), ecouto@ualg.pt (E. Couto), nlagarto@ipma.pt (N. Lagarto), joao.delgado@madeira.gov.pt (J. Delgado), acanario@ualg.pt (A.V.M. Canário), ifigueiredo@ipma.pt (I. Figueiredo).

https://doi.org/10.1016/j.aaf.2020.03.006

Received 23 July 2019; Received in revised form 10 January 2020; Accepted 15 March 2020

© 2020 Published by Elsevier B.V. on behalf of Shanghai Ocean University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

Please cite this article as: Inês Farias, et al., Aquaculture and Fisheries, https://doi.org/10.1016/j.aaf.2020.03.006

1. Introduction

The black scabbardfish, *Aphanopus carbo* Lowe, 1839, is an important commercial deep-sea species captured in Portuguese waters. This benthopelagic teleost fish is widely distributed along the NE Atlantic (Allain, Biseau, & Kergoat, 2003; Bordalo-Machado et al., 2009; Ehrich, 1983), where it undergoes large-scale migrations: the smallest specimens are reported further north and the largest specimens at the southernmost limit of distribution (Farias, Morales-Nin, Lorance, & Figueiredo, 2013; Ribeiro Santos, Minto, Connolly, & Rogan, 2013). The only known spawning grounds are located off Madeira, the Canary Islands, and possibly Morocco (Figueiredo et al., 2003; Pajuelo et al., 2008; Perera, 2008, p. 71). Juveniles recruit to the fisheries off the west of the British Isles, documented to be a feeding area, where they remain some time growing (Figueiredo et al., 2003; Ribeiro Santos, Minto, et al., 2013). Black scabbardfish subsequently moves to areas off mainland Portugal where caught specimens attain larger sizes and pre-spawning individuals are seldom captured (Figueiredo et al., 2003; Neves et al., 2009). After spending some time here to feed and grow, juveniles and pre-adults move further south to the spawning areas around Madeira (Farias et al., 2013). A key question is why fishes off mainland Portugal do not develop beyond the pre-spawning stage, despite attaining sizes larger than the estimated length at first maturity (L\textsubscript{50} = 102.8 cm) (Figueiredo et al., 2003).
Fish diet, growth, reproduction, and energy consumption are closely dependent on environmental conditions, which highly constrain metabolic rate processes in the deep-sea (Merrett & Haedrich, 1997, p. 282). Understanding the role of hormonal regulation of deep-sea species' vital processes will contribute to a better knowledge of their spatial dynamics. In this context, associating alterations in gonad development with the levels of sex steroids in blood plasma has proven to be a valuable tool to comprehend the endocrine control of reproduction in some deep-sea teleosts although this is a field not fully explored (Lee & Yang, 2002; Pankhurst & Conroy, 1987; Sequeira et al., 2017; Sisneros, Forlano, Knapp, & Bass, 2004).

In teleost fish females, the follicle stimulating hormone (FSH) is responsible for the stimulation of granulosa cells to produce estradiol-17β (E2), the key hormone that induces the liver to synthesize vitelligenin and egg shell proteins, which are incorporated into the oocyte during vitellogenesis (Lubzens, Young, Bobe, & Cerdá, 2010). After the growth phase, a surge of luteinizing hormone (LH) stimulates the follicle to produce the maturation-inducing steroid – either 17,20β-dihydroxyprog-4-en-3-one (17,20β-P) or 17,20β,21-trihydroxyprog-4-en-3-one (17,20β,21-P), depending on the species – that promotes final oocyte maturation (resumption of meiosis) and ovulation (Lubzens et al., 2010; Nagahama & Yamashita, 2008).

In males, FSH regulates Sertoli cell activity to support germ cell development while LH acts on Leydig cells to promote steroidogenesis (Chauvigne, Zapater, Gasol, & Cerdà, 2014; Schulz, Sumpter, & Stacey, 2010). The key androgen in males is 11-ketotestosterone (11-KT) which stimulates germ cell proliferation and maturation, as well as the development of secondary sexual characters and the mediation of reproductive behaviours (Borg, 1994; Schulz et al., 2010). In males, 17,20β-P is responsible for endorsing the initiation of meiosis, for stimulating spermatogenesis, and for enhancing sperm motility (by alteration of the pH and fluidity of the seminal fluid) and can act as a pheromone, e.g. in goldfish (Scott, Sumpter, & Stacey, 2010). Finally, both male and female gonads produce testosterone (T) which is a precursor of E2 and 11-KT and feeds back on the pituitary gland to promote the synthesis of gonadotrophins, among other functions (Lubzens et al., 2010; Nagahama, 1994; Schulz et al., 2010).

The main objective of this study was to assess and compare the hormonal status and gonadal development stage of specimens caught off mainland Portugal and off Madeira. Black scabbardfish specimens caught by commercial fisheries are already dead when pulled on board and it was only possible to obtain blood from specimens several hours after capture or thawed. To establish how these conditions may affect hormone levels, a pilot study tested the effect of sample age and freezing on the sex steroids of Mozambique tilapia, Oreochromis mossambicus (Peters, 1852).

2. Material and methods

2.1. Pilot study

To assess the effect of the time lag between death (capture) and blood collection and the effect of freezing (specimen storage) on sex steroid levels, Mozambique tilapia (Oreochromis mossambicus) male specimens were used. The Mozambique tilapia is a freshwater cichlid that can live at temperatures ranging from 8° to 42°C and can be reared in hypertaline conditions (Froese & Pauly, 2019). Despite the biological and ecological differences between the Mozambique tilapia and the black scabbardfish, the former was used in this pilot study because specimens were readily available from a captive stock, raised from fertilised eggs at the University of Algarve and maintained in freshwater under natural annual conditions of photoperiod and water temperature (26°C) prior to these experiments. Males were chosen over females because they are expected to show less hormonal variation owing to a simpler reproductive physiology.

Fish care and experimentation complied with the national legislation for the use of laboratory animals under a Group-1 license issued by the Portuguese National Authority for Animal Health. Fish were stunned and killed by immersion in iced water and total length (TL, cm) and total weight (TW, g) were measured. Blood samples were collected in heparinised syringes from the caudal pudenile in larger specimens or from the heart in smaller specimens.

After a first sample of blood was collected from 29 specimens (control, 10), fish were randomly separated into two groups: 18 fish were stored on ice in a refrigerator (temperature 4°C) and the remaining 11 fish were frozen (temperature −20°C). A second blood sample was collected from each fish at the distinct times that defined the treatments (Table 1). Frozen fish were thawed at room temperature prior to blood collection. Blood samples were centrifuged at 4°C and separated plasma was stored at −20°C until assay.

2.2. Black scabbardfish samples

Black scabbardfish (Aphanopus carbo) specimens were collected at irregular times between 2010 and 2012 from commercial longline vessels operating off Madeira Archipelago and off mainland Portugal (Table 2). Since Aphanopus intermedius Parin, 1983 (intermediate scabbardfish) specimens are mixed with A. carbo in Madeira’s commercial landings (Stefanetti & Knutsen, 2007), specimens caught in this region were assigned to species following the morphological criteria defined by Biscoito et al. (2011). In the present study, only A. carbo specimens were used.

In Madeira, specimens were sampled between October and December from commercial port landings. This month range was selected to encompass the reproductive season. In this fishery, black scabbardfish are caught by mid-water horizontal drifting longline set below 1000 m deep and the soaking time lasts from two to four days (Bordalo-Machado et al., 2009). Specimens from mainland Portugal were collected throughout the year on board commercial vessels or from commercial port landings. In this fishery, the fishing gear is a horizontal bottom longline and the soaking time lasts from one to two

| Treatment | T (°C) | Time (days) | n | TL (cm) | TW (g) |
|-----------|--------|-------------|---|---------|-------|
| t1        | 4      | 1           | 6 | 15.2-18.9 | 61.74-103.62 |
| t2        | 4      | 2           | 5 | 15.8-17.3 | 62.09-75.65  |
| t4        | 4      | 4           | 4 | 16.5-18.9 | 66.4-105.21  |
| t15       | -20    | 15          | 6 | 15.1-19.3 | 53.7-103.52  |
| t30       | -20    | 30          | 5 | 14.6-17.2 | 50.68-81.64  |

Table 1

Experimental design and summary of Mozambique tilapia samples used in the pilot study. T, storage temperature; n, sample size; TL, total length range; TW, total weight range.

| Region | Sex | Maturity | Madeira | Mainland Portugal |
|--------|-----|----------|---------|-------------------|
| females | 1 | - | 890-1081 (13) |
| 2 | 1087-1506 (16) | 1041-1178 (6) |
| 3 | 1141-1254 (4) | - |
| 4 | 1135-1323 (7) | - |
| 5 | 1141-1291 (4) | - |
| males | 1 | - | 874-1003 (10) |
| 2 | 1117-1179 (2) | 1045 (1) |
| 3 | 1115-1177 (4) | - |
| 4 | 1122-1233 (7) | - |
| 5 | 1040-1244 (4) | - |

Table 2

Summary of black scabbardfish samples used for measuring sex steroids estradiol-17β, testosterone, and 11-ketotestosterone. Values are total length range (mm) and sample size (parenthesis) by sex and maturity stage (1–5).
days (Bordalo-Machado et al., 2009).

For each specimen, the following information was collected: total length (TL, mm), total weight (TW, g), gutted weight (UW, g), liver weight (LW, g), gonad weight (GW, g), sex, and maturity stage. Maturity stage was macroscopically assigned following the scale proposed by Gordo et al. (2000, p. 35): stage 1, immature or resting; stage 2, developing; stage 3, pre-spawning; stage 4, spawning; and stage 5, post-spawning or spent.

The gonadosomatic index (GSI) was calculated as the gonad weight as a proportion of the gutted weight; the hepatosomatic index (HSI) was calculated as the liver weight as a proportion of the gutted weight; and Fulton’s condition factor (K) was calculated as the ratio between the gutted weight and the cube of the total length. To calculate the previous reproductive indicators, whole data sets collected between 2010 and 2012 from specimens caught off Madeira (n = 1976) and off mainland Portugal (n = 692) were used.

Blood samples were collected in heparinised syringes from the specimen’s caudal vessel, which was exposed by removing the lateral musculature close to the caudal peduncle. To minimize the effect of metabolism and degradation, the collection of blood samples took place as soon after hauling as possible. In specimens caught off Madeira, blood was extracted in the laboratory from fish that had been dead and remained hooked to the longline for one to four days and was kept on ice after hauling for less than 24 h (n = 48). The exact individual time of death or hauling is not known since each vessel deploys the fishing gear more than once during each fishing trip and the fish is kept on board all together. Specimens caught off mainland Portugal had been dead and remained hooked to the longline for up to one day. Blood samples from these specimens were collected (i) on board from fresh fish immediately after hauling (n = 12); (ii) in the laboratory from fish kept on ice after hauling for less than 24 h (n = 14); or (iii) in the laboratory from thawed fish that was stored in a freezing room at −20 °C, less than one day after hauling, for 30 days (n = 4).

Blood samples were centrifuged at 4 °C to separate the plasma, which was stored at −20 °C until assay.

2.3. Steroid analysis

Blood plasma (100 μL) was extracted twice with 3 ml of diethyl ether to obtain the free steroids. Extracts were dried on a dry bath at 40 °C under nitrogen gas and suspended in 1 ml of assay buffer (0.5 M phosphate–gelatine buffer, pH 7.6). Free steroids were measured by radioimmunoassay (RIA) following the methodology described by Scott, Sheldrick, and Flint (1982). Individual plasma samples were mixed with 100 μl of distilled water and extracted twice with 4 ml of diethyl ether to obtain free steroids. Extracts were dried under nitrogen, reconstituted in 0.5 M phosphate–gelatine buffer, pH 7.6 and steroids were measured by RIA.

The estradiol-17β (E2) antiserum was purchased from Research Diagnostics (USA) and the cross-reactions (% have been reported as follows: < 0.2% for 4- pregnene-3,20-dione; < 0.2% for 11β,17β,21-trihydroxy-4-pregnene-3,20-dione; < 0.2% for 11β-androsten-3,17-dione; < 0.2% for 17β-hydroxy-4-androstene-3-one; < 0.2% for 3β-hydroxy-5-pregnane-20-one; < 0.2% for 3β-hydroxy-5-androstene-17-one; 15% for 3β-hydroxy-1,3,5(10)-estratrien-17-one; 8% for 3aβ,17α,20dihydroxy-1,3,5(10)-estratrien-16-one; 0.7% for 3,16α,17β-trihydroxy-1,3,5(10)-estratrien-3-one; < 0.2% for 3aβ,16α-dihydroxy-1,3,5(10)-estratrien-17-one (Guerrero, Fuentes, Canario, & Power, 2002).

The testosterone antiserum was kindly donated by Dr. David Kime (University of Sheffield, UK). The testosterone (T) antiserum cross-reactions were 63% for androstenedione, 35% for 11-ketotestosterone, 55% for 11-hydroxytestosterone, 40% for 5-androstane-17-ol-3-one, 31% for 5-androstane-17-ol-3-one, 12% for 5-androstane-3,17-diol, 25% for 5-androstane-3,17-diol. The 11-ketotestosterone (11-KT) antiserum cross-reactions were 20.1% for 11-hydroxytestosterone, 20.6% for testosterone, 76.9% for androstenedione, 30.1% for 11-hydroxyandrostenedione, 52% for dihydrotestosterone, 3.3% for cortisol, and 1.3% for cortisone (Kime & Manning, 1982). All samples were assayed in duplicate in a single assay. The intra-assay and inter-assay coefficients of variation were, respectively: 6.6% and 14.2% for E2, 5.0% and 8.2% for T; and 8.2% and 11.6% for 11-KT. The limits of detection were between 10 (E2) and 100 (T and 11-KT) pg/mL.

2.4. Statistical analyses

In the Mozambique tilapia pilot study, one-way ANOVA was applied for comparing T and 11-KT concentrations between t0 (control) and the time of each treatment. The variation in T and 11-KT concentration in Mozambique tilapia was estimated as the difference between the value at time t0 (control) and at the time of each treatment. One-Way ANOVA was applied for comparing the variation in T and in 11-KT between treatments. Whenever necessary, data were log10-transformed to meet the ANOVA assumptions. If ANOVA assumptions were not met after data transformation, nonparametric Kruskal–Wallis test by ranks was used instead. When there were statistically significant differences between treatments, Wilcoxon signed-rank test was applied to compare each pair of treatments.

In black scabbardfish blood plasma, one-way ANOVA was used to investigate the association between E2, T, and 11-KT concentrations and the way the specimens are preserved prior to blood collection for each sex separately. Whenever necessary, data were log10-transformed to meet the ANOVA assumptions. If assumptions were not met after data transformation, nonparametric Wilcoxon signed-rank test was used instead. When there were statistically significant differences between preservation methods, Wilcoxon test was applied to compare each pair of maturity stages.

Two-way fixed effects ANOVA was applied for analysing the effects of geographical region and maturity stage on GSI, HSI, K, and on E2, T, and 11-KT concentrations for each sex separately. Whenever necessary, data were log10-transformed to meet the ANOVA assumptions. If assumptions were not met after data transformation, nonparametric Wilcoxon signed-rank test was used instead. When there were statistically significant differences between preservation methods, Wilcoxon test was applied to compare each pair of maturity stages.

A k-means clustering method was applied to steroid (E2 and T) profile data using developing (stage 2) females both from Madeira and mainland Portugal using the R package cluster (Maechler, Rousseeuw, Struyf, Hubert, & Hornik, 2018, pp. 7–1).

The R software (R Core Team, 2018) was used for all statistical analyses and 5% significance level was adopted. Graphics were built with the R package ggplot2 (Wickham, 2009).

3. Results

3.1. Pilot study

In Mozambique tilapia, no significant differences in T concentration ($\chi^2(4, n = 28) = 3.918, p-value = 0.417$) and in 11-KT concentration ($\chi^2(4, n = 28) = 1.601, p-value = 0.809$) were found between samples collected initially and samples collected after treatment (Fig. 1).

T and 11-KT concentrations varied between the control (t0) and each treatment but the variations were not significantly different between treatments ($\chi^2(4, n = 28) = 3.918, p-value = 0.417$ and $\chi^2(4, n = 28) = 1.601, p-value = 0.809$, respectively) (Fig. 2).

3.2. Reproductive indicators in black scabbardfish

Black scabbardfish specimens caught off mainland Portugal, between 2010 and 2012, were in maturity stages 1–3 and 5 (Table 2). Immature or resting females and males (maturity stage 1) were not found among fish sampled in Madeira.

In females caught off mainland Portugal, mean GSI significantly
increased between stage 1 and stage 2 ($W = 3544$, $p$-value < 0.001). However, the increase between stage 2 and stage 3 was not statistically significant ($W = 164$, $p$-value = 0.358) (Fig. 3). The mean GSI of females caught off Madeira significantly increased from stage 2 to stage 3 ($W = 1866$, $p$-value < 0.001) and from stage 3 to stage 4 ($W = 1015$, $p$-value < 0.001), and significantly decreased from stage 4 to stage 5 ($W = 6983$, $p$-value < 0.001). Concerning males, mean GSI significantly increased from stage 1 to stage 2 ($W = 488$, $p$-value < 0.001) and from stage 2 to stage 3 ($W = 17,335$, $p$-value < 0.001) in specimens caught off mainland Portugal, and from stage 2 to stage 3 ($W = 10,395$, $p$-value < 0.001) in specimens caught off Madeira, whereas the decrease from stage 4 to stage 5 was statistically significant ($W = 8213$, $p$-value < 0.001) in specimens from Madeira. The mean GSI was significantly higher in specimens caught off Madeira than off mainland Portugal for stage 2 ($W = 23,842$, $p$-value < 2.2 × 10^{-16}$) and stage 3 females ($W = 7$, $p$-value = 0.004) and for stage 2 ($W = 11,430$, $p$-value = 0.008) and stage 3 males ($W = 8147$, $p$-value = 0.033).

Mean HSI was significantly higher in stage 2 females caught off Madeira than off mainland Portugal ($W = 33,667$, $p$-value = 0.020) (Fig. 4). Mean HSI significantly increased from stage 1 to stage 2 females caught off mainland Portugal ($W = 10,395$, $p$-value < 0.001) and from stage 2 to stage 3 females caught off Madeira ($W = 13,287$, $p$-value < 0.001), whereas it significantly decreased from stage 4 to stage 5 females caught off Madeira ($W = 5690$, $p$-value < 0.001).

Mean K significantly increased between stage 1 and stage 2 females ($W = 9041$, $p$-value < 0.001) and males ($W = 7505.5$, $p$-value = 0.002) caught off mainland Portugal, and from stage 3 to stage 4 males ($W = 7946$, $p$-value < 0.001) caught off Madeira, whereas it significantly decreased from stage 4 to stage 5 males ($W = 5828$, $p$-value < 0.001) (Fig. 5). Mean K was significantly higher in stage 2 females ($W = 57,444$, $p$-value < 0.001), in stage 2 males ($W = 18,945$, $p$-value < 0.001), and in stage 3 males ($W = 15,444$, $p$-value < 0.001) caught off Madeira than in the same sex and same maturity stage specimens caught off mainland Portugal.

### 3.3. Sex steroids in black scabbardfish

The changes of both T and 11-KT concentrations in Mozambique tilapia between the time of blood extraction and the time of measurement after preservation did not statistically differ among the different treatments applied. Therefore, the sex steroid concentrations quantified in the black scabbardfish samples were directly used in the data analyses.

In black scabbardfish specimens, no significant differences were found between the specimens preservation methods for all three analysed sex steroids: E$_2$ ($F_{2,41} = 2.851$, $p$-value = 0.069); T ($F_{2,41} = 1.789$, $p$-value = 0.175); and 11-KT ($F_{1,26} = 0.239$, $p$-value = 0.629).

In black scabbardfish females, T was not significantly different between regions ($F_{1,36} = 1.470$, $p$-value = 0.233) nor between maturity stages ($F_{1,36} = 0.830$, $p$-value = 0.368) neither was the interaction between region and maturity stage statistically significant ($F_{1,36} = 1.349$, $p$-value = 0.253) (Fig. 6). E$_2$ was significantly different between regions ($F_{1,40} = 5.717$, $p$-value = 0.022), but no significant
differences were found between maturity stages \( (F_{1,40} = 2.215, p\text{-value} = 0.145) \) neither was the interaction between region and maturity stage statistically significant \( (F_{1,40} = 2.954, p\text{-value} = 0.093) \).

11-KT was significantly different between males caught off Madeira and males caught off mainland Portugal \( (F_{1,24} = 14.364, p\text{-value} = 0.001) \), but no significant differences were found between maturity stages \( (F_{1,24} = 0.465, p\text{-value} = 0.502) \) neither was the interaction between region and maturity stage statistically significant \( (F_{1,24} = 0.389, p\text{-value} = 0.539) \) \( \) \). T in males was not significantly different between regions \( (F_{1,24} = 2.228, p\text{-value} = 0.149) \) and no significant differences were found between maturity stages \( (F_{1,24} = 0.008, p\text{-value} = 0.928) \). The interaction between factors was not analysed because factors were not crossed.

The only maturity stage within the same sex for which blood was collected in specimens caught off both Madeira and mainland Portugal areas was stage 2 females. Considering T and E\(_2\) concentrations together, stage 2 female samples were grouped into two clusters that were statistically different \( (F_{1,16} = 6.793, p\text{-value} = 0.019) \). T and E\(_2\) concentrations did not show evidence of geographic differentiation as each cluster included specimens from both regions \( \) (Fig. 7) .

4. Discussion

The pilot study with the Mozambique tilapia showed that it is possible to obtain meaningful measures of sex steroids in blood collected from fish that has been preserved refrigerated or frozen for a relatively prolonged period. Furthermore, it was assumed that the general behaviour of the hormonal steroids during refrigeration and the degradation mechanisms would follow similar patterns, although this is an area that requires more in-depth studies. In a previous study with plainfin midshipman fish, *Porichthys notatus*, blood plasma, no significant differences in E\(_2\), T, and 11-KT concentrations were found between samples collected at different times after capture (within one or 4 h) in either offshore or intertidal zones for both males and females.

---

**Fig. 3.** Black scabbardfish gonadosomatic index (GSI) (mean ± SE) of females (left) and males (right) caught off Madeira and mainland Portugal by maturity stage.

**Fig. 4.** Black scabbardfish hepatosomatic index (HSI) (mean ± SE) of females (left) and males (right) caught off Madeira and mainland Portugal by maturity stage.
Black scabbardfish blood samples were collected up to 24 h or 30 days after capture and steroid levels were not corrected. Nonetheless, black scabbardfish sex steroid levels should be regarded as relative and not absolute values. In addition to the time between death and blood collection and the above-mentioned factors that influence hormone levels, the stress of capture, which may vary with time and method of capture, can also lower sex steroid levels (Clearwater & Pankhurst, 1997; Cleary, Battaglene, & Pankhurst, 2002; Pankhurst & Conroy, 1988).

E₂ and T increased during vitellogenesis (stages 1–3), peaked at the
pre-spawning stage (stage 3), and decreased from stage 3 onwards. The same steroid profile was observed for GSI in females. Although the increase in GSI between stages 1 and 2 was not statistically significant, the concomitance between the highest levels of E2 and T and the peak in vitellogenesis (stage 3 in this species) has also been described for other teleosts (e.g., Frisch, McCormick, & Pankhurst, 2007; Hachero-Cruzado et al., 2007; Li, Liu, & Lin, 2007; Prat, Zanuy, Carrillo, de Mones, & Fostier, 1990). Furthermore, the same profile has been observed in the HSI of female black scabbardfish, demonstrating that the hepatic reserves are being consumed during the maturation process (Domínguez-Petit & Saborido-Rey, 2010; Ribeiro Santos, Minto, et al., 2013).

In fact, E2 and T are connected since T is a precursor of E2, such as other steroids (Pankhurst & Conroy, 1987), and E2 is crucial for the start of vitellogenesis because it promotes the synthesis of hepatic yolk precursors (vitellogenin) in a variety of teleost species and provides negative feedback to LH secretion (Lubzens et al., 2010; Sisneros et al., 2004; Tyler & Sumpter, 1996). Chemical and physiological changes occurring throughout female developing stage are of uttermost importance for comprehending the reproductive cycle of black scabbardfish and the differences between fish living off Madeira and fish living off the Portuguese mainland coast. To disentangle this moment in the species’ life cycle, E2 and T concentrations in developing females were analysed together through cluster analysis. The mixing of females from both areas within each cluster shows that the steroid profiles are similar between areas. Madeiran females were sampled at the spawning season and during this period it is likely that females between an early and a late development stage may concur. Fish from the group with the lowest steroid concentrations represent an earlier development stage. These fish will not have the time to mature and reach the spawning stage and will fail to spawn in the current season. It is not a case of skipped spawning, which refers to a mature fish that fails to spawn at a given year (Rideout & Tomkiewicz, 2011), but the drivers of both processes are similar. In black scabbardfish, the developing stage during early vitellogenesis proved to be the critical window for the decision to spawn or not to spawn (Neves et al., 2009; Skjæraasen et al., 2010).

The energy saved not developing into mature stages is invested in growth and in increased fecundity, similar to what happens when fish skip spawning (Jorgensen, Ermande, Øyvind, & Dieckmann, 2006). The persistence of a high proportion of relatively immature individuals in a plentiful environment may allow them to grow fast before the next reproductive cycle drives energy towards the gonads (Alonso-Fernández & Saborido-Rey, 2012; Folkvord et al., 2014; Roff, 1983). Moreover, the simultaneous occurrence of two groups of developing females with similar length, steroid levels, and reproductive indicators points to a long duration of this maturity stage.

Stage 2 females caught off mainland Portugal during the spawning period show a high degree of follicular atresia in the ovaries and do not develop into mature stages (Neves et al., 2009). The abundance of fish at the same location after the maturation process is interrupted explains why ca. 25% of individuals off mainland Portugal are larger than L50 but immature (Figueiredo et al., 2003; Neves et al., 2009; Farias et al., 2013). This phenomenon was also observed off the west of the British Isles (Ribeiro Santos, Minto, et al., 2013). The capability of specimens to migrate to the spawning grounds will depend on the completion of high energy reserves (Merrett & Haedrich, 1997, p. 282; Ribeiro Santos, Minto, et al., 2013). In fact, the relatively high HSI observed in immature males caught off mainland Portugal suggests that energy is being stored in the liver to be spent when moving to the spawning grounds. Black scabbardfish’s readiness for migration was also supported by the high content of arachidonic acid (ARA), which is demanded for long distance movements (Sargent, Bell, McEvoy, Tocher, & Estevez, 1999; Tocher, 2003), that was found in the muscle of specimens caught off mainland Portugal (Farias et al., 2014). Off the west of the British Isles, the species prepares for migration towards the south, between January and April, through an intense feeding activity on blue whiting (Ribeiro Santos, Trueman, Connolly, & Rogan, 2013).

Androgen (T and 11-KT) levels did not significantly differ among reproductive stages. Nevertheless, in males caught off Madeira, the two steroids were low in spent and regressed fish, increased during gonadal recrudescence, and peaked at the end of spermatogenesis as described in several studies (Pankhurst & Conroy, 1988; Prat et al., 1990). The significant differences between regions are expected to be a consequence of the unbalanced sampling amongst maturity stages.

The present work supports the role of sex steroids as intrinsic triggers for gonadal maturation and spawning in black scabbardfish. It also shows that it is possible to measure sex steroids in blood plasma that was collected late after death and relate the values with the dynamics of the species reproduction, overcoming some sampling constraints of deep-sea species. Given the fact that specimens used in this study were collected from commercial fisheries with a soak time greater than one day, the number of blood samples were limited because, as the time passes after capture, the blood extraction is more difficult. Moreover, the depth at which black scabbardfish specimens were collected implies that fish are boarded already dead and, subsequently, the collection of an adequate volume of blood is problematic. To overcome these difficulties, dedicated surveys are required, which in the case of deep-sea fishes are costly and do not take place in Portuguese waters. Nonetheless, the present results put in evidence that the RIA method is appropriate for the quantification of sex steroid concentration even with small blood volumes. The metabolization of sex steroids after capture was considered to be negligible or to have occurred equally in all individuals, as the relationships between hormones and reproductive stages were maintained.

Since spawning occurs during a relatively short period, for future work it would be important to have a monthly or more frequent coverage in the two regions to test if there are differences between maturity stages related with the time of the year when specimens are caught.

**Author statement**

Inês Farias performed conceptualization, methodology, investigation, formal analysis, writing - original draft, writing - review and editing, and visualization. Elsa Couto, Neide Lagarto, and João Delgado performed investigation. Adelino V.M. Canário performed conceptualization, methodology, formal analysis, writing - review and editing, and supervision. Ivone Figueiredo performed conceptualization, methodology, formal analysis, writing - review and editing, supervision, and project administration.
Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. All financial support came from public funding.

Acknowledgements

The authors are especially grateful to the master, Henrique Santos, and crew of the longliner Varamar for hosting researchers on board to collect samples off mainland Portugal, and to Sofia Vieira, Sara Reis, and the technical staff from DSI-DRP for their collaboration in collecting the samples in Madeira. We also thank Pedro Guerreiro (CCMAR-CIMAR) for support in the tilapia experiment. This work was supported by the Portuguese Foundation for Science and Technology (FCT – Fundação para a Ciência e Tecnologia) under the PhD Grant SFRH/BD/64434/2009 to I. Fa. and the project UID/Multi/04326/2013 to A.V.M.C.; the Portuguese Government Development Investments and Expenses Program (PIDDAC – Programa de Investimentos e Despesas de Desenvolvimento da Administração Central) under the EU/DG Fisheries’ Data Collection Framework (DCF); the Portuguese Fisheries Operational Program (2007–2013) (PROR – Programa Operacional Pescas), co-financed by the European Fisheries Fund (EFF) (2007–2013), under project CERTIFICA (2011–2015), MSC Certification in Portugal – Acquiring knowledge and studying the viability for its application to coastal and artisanal fisheries.

References

Allain, V., Biseau, A., & Kergoat, B. (2003). Preliminary estimates of French deepwater fishery discards in the Northeast Atlantic Ocean. Fisheries Research, 60, 185–192. https://doi.org/10.1016/S0165-7836(03)00131-6.

Alvares, F., Nardi, S., Moreira, A., Candiano, G., Conzelmann, J., Bobillier, B., & Bertoli, E. (2019). Reproductive and feeding spatial dynamics of the black scabbardfish (Aphanopus carbo, 1839) in the NE Atlantic. Aquatic Living Resources, 26, 73–79. https://doi.org/10.1007/s13592-018-0305-0.

Folkvord, A., Jørgensen, C., Korsbrekke, K., Nash, R. D. M., Nilsen, T., & Skjæraasen, J. E. (2014). Trade-offs between growth and reproduction in wild Atlantic cod. Canadian Journal of Fisheries and Aquatic Sciences, 71, 1106–1112. https://doi.org/10.1139/cjfas-2013-0600.

Frisch, A. J., McCormick, M. I., & Pankhurst, N. W. (2007). Reproductive periodicity and steroid hormone profiles in the sex-changing coral-reef fish, Plectropomus leopardus. Coral Reefs, 26, 189–197. https://doi.org/10.1007/s00338-006-0183-9.

Froese, R., & Pauly, D. (2019). FishBase. World wide web electronic publication (01/2020). www.fishbase.org.

Gordo, L. S., Carvalho, D. S., Figueiredo, I., Reis, S., Machado, P. B., Newton, A., et al. (2000). The sexual maturity scale of black scabbardfish: A macro- and microscopic approach. Oceanologia, 42, 165–174. https://doi.org/10.1007/BF02889856.

Guerrero, P. M., Fuentes, J., Canario, A. V., & Power, D. M. (2002). Calcium balance in sea bream (Sparus aurata): The effect of oestriol-17β. Journal of Endocrinology, 179(2), 377–385. https://doi.org/10.1677/joe.0.1730377.

Hachet-Cruzado, I., García López, A., Infantes, M. Vargas-Chacoff, L., Martínez-Rodríguez, G., Manero, J. M., et al. (2007). Reproductive performance and seasonal plasma sex steroid and metabolite levels in a captive wild broodstock of brill Scophthalmus rhombus I. Aquatic Research, 38, 1161–1174. https://doi.org/10.1111/j.1618-0316.2007.00178.x.

Jørgensen, C., Ernande, B., Øyvind, F., & Dieckmann, U. (2006). The logic of skipped spawning in fish. Canadian Journal of Fisheries and Aquatic Sciences, 63, 200–211. https://doi.org/10.1139/f05-210.

Kim, D. E., & Manning, N. J. (1982). Seasonal patterns of free and conjugated androgens in the brown trout Salmo trutta. General and Comparative Endocrinology, 48, 222–231. https://doi.org/10.1016/0016-6480(92)90020-x.

Lee, W. K., & Yang, S.-W. (2002). Relationship between ovarian development and plasma levels of 11-ketotestosterone and induction of oocyte maturation and spawning in the cultured female Korean spotted sea bass Latesolbus maculatus (Jeemong-eo). Aquaculture, 207, 169–183. https://doi.org/10.1016/S0044-8484(01)00728-1.

Li, G. L., Liu, X. C., & Lin, H. R. (2007). Seasonal changes of serum sex steroids concentration and aromatase activity of gonad and brain in red-spotted grouper (Epinephelus auroa). Animal Reproduction Science, 99, 156–166. https://doi.org/10.1016/j.anireprosci.2006.05.015.

Lubens, E. Y., Young, G., Bobe, J., & Cerda, J. (2009). Oogenesis in teleosts: How fish eggs are formed. General and Comparative Endocrinology, 165, 387–389. https://doi.org/10.1016/j.ygcen.2009.05.022.

Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., & Hornik, K. (2018). cluster: An R package for cluster analysis by Unsupervised Learning Methods. University of Vienna, Faculty of Sciences: Department of Animal Biology.

Pajuelo, J. G., González, J. A., Santana, J. I., Lorenzo, J. M., García-Mederos, A., & Tuset, F. (2007). Reproductive performance and seasonal plasma sex steroid and metabolite levels in a captive wild broodstock of brill Scophthalmus rhombus II. Aquatic Research, 39, 76–86. https://doi.org/10.1111/j.1618-0316.2007.00179.x.

Pajuelo, J. G., González, J. A., Santana, J. I., Lorenzo, J. M., García-Mederos, A., & Tuset, F. (2008). Biological parameters of the batalhial fish black scabbardfish (Aphanopus carbo, 1839) off the canary islands, central-eastern atlantic. Fisheries Research, 92, 206–212. https://doi.org/10.1016/j.fishres.2007.12.022.

Pankhurst, N. W., & Conroy, A. M. (1987). Seasonal changes in reproductive condition and plasma levels of sex steroids in the blue cod, Parapercis colias (Bloch and Schneider) (Mugilidae). Fish Physiology and Biochemistry, 4, 15–26. https://doi.org/10.1007/BF00728260.

Pankhurst, N. W., & Conroy, A. M. (1988). Endocrine changes during gonadal maturation and spawning in the orange roughy (Hoplostethus atlanticus Collett), a teleost from the mid slope waters of New Zealand. General and Comparative Endocrinology, 70, 262–273. https://doi.org/10.1016/0016-6480(89)90146-3.

Perera, C. S. (2008). Distribution and biology of black scabbardfish (Aphanopus carbo Lowe, 1839), report. Masters’ Thesis. The northwest of africa. University of Lisbon, Faculty of Sciences: Department of Animal Biology.

Prat, F., Zanuy, S., Carrillo, M., de Mones, A., & Fostier, E. (1990). Seasonal changes in plasma levels of gonadal steroids of sea bass. General and Comparative Endocrinology, 73, 297–304. https://doi.org/10.1016/0016-6480(90)90073-C.

Rideout, R. M., & Tomkiewicz, J. (2011). Skipped spawning in fish. Canadian Journal of Fisheries and Aquatic Sciences, 40, 1395–1404. https://doi.org/10.1139/f11-016.
Sargent, J., Bell, G., McEvoy, L., Tocher, D., & Estevez, A. (1999). Recent developments in the essential fatty acid nutrition of fish. *Aquaculture, 177*, 191–199. https://doi.org/10.1016/S0044-8486(99)00083-6.

Schulz, R. W., de França, L. R., Lareyre, J.-J., Le Gac, F., Chiarini-Garcia, H., Nobrega, R. H., et al. (2010). Spermatogenesis in fish. *General and Comparative Endocrinology, 165*, 390–411. https://doi.org/10.1016/j.ygcen.2009.02.013.

Scott, A. P., Sheldrick, E. L., & Flint, A. P. F. (1982). Measurement of 17α,20β-dihydroxy-4-pregnen-3-one in plasma of trout (*Salmo gairdneri richardson*): Seasonal changes and response to salmon pituitary extract. *General and Comparative Endocrinology, 46*, 444–451. https://doi.org/10.1016/0016-6480(82)90098-3.

Scott, A. P., Sumpter, J. P., & Stacey, N. (2010). The role of the maturation-inducing steroid, 17,20β-dihydroxyprog-4-en-3-one, in male fishes: A review. *Journal of Fish Biology, 76*, 183–224. https://doi.org/10.1111/j.1095-8649.2009.02483.x.

Sequeira, V., Couto, E., Neves, A., Vieira, A. R., Paiva, R. B., Canário, A. V. M., et al. (2017). Zygoparity and sex steroid hormone profiles in bluemouth *Helicolenus dactylopterus*. *Journal of Fish Biology, 90*, 2157–2169. https://doi.org/10.1111/jfb.13299.

Sismeros, J. A., Forlano, P. M., Knapp, R., & Bass, A. H. (2004). Seasonal variation of steroid hormone levels in an intertidal-nesting fish, the vocal plainfin midshipman. *General and Comparative Endocrinology, 136*, 101–116. https://doi.org/10.1016/j.ygcen.2003.12.007.

Skjæraasen, J. E., Nau, R. D. M., Kennedy, J., Thorsen, A., Nilsen, T., & Kjesbu, O. S. (2010). Liver energy, atresia and oocyte stage influence fecundity regulation in Northeast Arctic cod. *Marine Ecology Progress Series, 404*, 173–183. https://doi.org/10.3354/meps08486.

Stefanni, S., & Knutsen, H. (2007). Phylogeography and demographic history of the deepsea fish, *Aphanopus carbo* (Lowe, 1839), in the NE Atlantic: Vicariance followed by secondary contact or speciation? *Molecular Phylogenetics and Evolution, 42*, 38–46. https://doi.org/10.1016/j.ympev.2006.05.025.

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science*, 11, 107–184. https://doi.org/10.1080/713610925.

Tyler, C. R., & Sumpter, J. P. (1996). Oocyte growth and development in teleosts. *Reviews in Fish Biology and Fisheries, 6*, 287–318. https://doi.org/10.1007/BF00122584.

Wickham, H. (2009). *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag.