Fecundity and sex steroid profile in boarfish, *Capros aper*

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Abstract

The boarfish *Capros aper* is one of the most commonly discarded non-commercial species in ICES subdivision VIII in the Atlantic. An increasing interest in this fishery and an incomplete knowledge on the status of the stock justified the present investigation focused on the determination of fecundity type and its estimation, supported by the sex steroid profile of oestradiol-17β (E_2), 11-ketotestosterone (11-KT) and 17,20β-dihydroxypregn-4-en-3-one (17,20β-P). Results demonstrated that *C. aper* has indeterminate fecundity with a mean relative batch fecundity during the spawning peak of 50 oocytes g^{-1} eviscerated weight (WE) and a mean relative annual fecundity of 4,020 oocytes g^{-1} WE. E_2 variations throughout the year justified the existence of, at least, two important spawning events, at the beginning of the year and in the summer, with levels increasing from females with growing oocytes in the developing phase to spawning capable phase. Higher E_2 levels were also found from 2000-2400 h and 0800-1200 h suggesting more intense vitellogenesis activity during the night and the morning, in contrast to 17,20β-P which was higher between 1200-2000 h suggesting a more intense spawning activity during this period.

Key words: Reproductive strategy; Indeterminate fecundity; Western Atlantic.

Running head: *Capros aper* fecundity and sex steroids

Introduction

The boarfish *Capros aper* (L.1758) is a small pelagic planktivorous shoaling species found between 0 and 600 m deep, and with a wide distribution in the Eastern Atlantic, from Norway to Senegal, including the Mediterranean and oceanic island waters (ICES, 2014). *C. aper* was considered rare in the northern NE Atlantic (Coad *et al*. 2014), but since the 1970s its catches in pelagic and demersal fisheries has been steadily increasing over the Biscay area and more recently in the southern Celtic Sea (O'Donnell *et al*. 2012; Coad *et al*. 2014; Stange 2016), possibly as a result from population expansion (ICES 2012; O'Donnell *et al*. 2012; Coad *et al*. 2014).

Although *C. aper* has been one of the most commonly discarded non-commercial species in the Atlantic (Fonseca *et al*. 2005; Borges *et al*. 2008), since 2001 it is being targeted by Irish, Danish and UK-Scottish vessels in the northern NE Atlantic, mainly in ICES subdivision VIII, for reduction to fishmeal for the aquaculture market (ICES 2012; Stange 2016). After a sharp increase in landings since 2006 with a maximum value of 137 503 tonnes in 2010, a total allowable catch (TAC) has been set in EU waters consecutively since 2011, although since 2014 that has not been caught (ICES 2018). Currently, there is a management strategy on course aiming to achieve sustainable exploitation of *C. aper* fishery (ICES 2018).

The increased abundance of *C. aper* in the Northeast Atlantic that justified this new fishery has been suggested to be a result of enhanced recruitment due to an increase in water temperature during the spawning season (Blanchard and Vandermeirsch 2005). The unknown status of the stock led to a spur of investigations on the species biology (Lopes *et al*. 2006; White *et al*. 2011; Hüssy *et al*. 2012a; Hüssy *et al*. 2012b; Coad *et al*. 2014), but the reproductive strategy is far from being understood. The available information indicates that *C. aper* spawns between June and July/August in Irish waters (Quéro, 1986; Farrell *et al*., 2012), and throughout the year with a peak in summer in the western Portuguese coast C. aper spawns (Sequeira *et al*. 2015). Fecundity was suggested to be indeterminate by both studies based on the lack of a hiatus between pre-vitellogenic and vitellogenic oocytes, asynchronous oocyte development, the ability to spawn repeatedly over nine months in captivity conditions (Farrell *et al*. 2012), short spawning interval and generalized atresia and high/massive levels of atresia at the end or even during the spawning season (Sequeira *et al*. 2015) indicating the indeterminate fecundity pattern, but no specific study was carried on to prove it.

Fecundity measurements are particularly important to explore the reproductive dynamics and the spawning energetics of fish stock and to estimate its annual reproductive output and consequently how this is linked to recruitment (Ganias *et al*. 2015). As the methodology to be used to estimate fecundity dependents on the type (determinate or indeterminate) (Ganias *et al*. 2015), the investigation of the following four lines of evidence are required: (i) the stage-specific and monthly-specific variation of oocyte size-frequency distribution; (ii) the seasonal variation in the percentage of different oocyte classes during the spawning season (i.e previtellogenic/early vitellogenic and advanced vitellogenic oocytes); (iii) seasonal variation in the mean diameter of the advanced vitellogenic oocytes; and (iv) seasonal atresia (Hunter *et al*. 1992; Walker *et al*. 1994; Murua and Saborido-Rey 2003; Ganias *et al*. 2014). Moreover, the profile of sex steroids [e.g. estradiol-17β (E_2), 11-ketotestosterone (11-KT), 17,20β-dihydroxypregn-4-en-3-one (17,20β-P)] can be of value as they are responsible for controlling several reproductive functions including gametogenesis and maturation (Kobayashi *et al*. 2002; Stacey 2003). Thus, E_2 levels during vitellogenesis are generally positively correlated with oocyte diameter and its decrease in post-vitellogenic oocytes signals progress towards oocyte final maturation through the action of 17,20β-P (Nagahama *et al*. 2008). However, the short duration of this process in many species makes it difficult to detect the hormone during broad sampling approaches (Pankhurst 2008; Scott and Canario 1987). In males, 11-KT in some species correlates positively with spermatogenesis and in other cases it peaks at spermiation, while the peak of 17,20β-P varies across species (Schulz *et al*. 2010; Scott *et al*. 2010).
The objective of the present study was: (1) to define the fecundity type of *C. aper* from the western Atlantic Portuguese coast based on the lines of evidence indicated above; (2) to estimate fecundity of the species; and (3) to relate the sexual cycle with the annual hormonal profiles of plasma sex steroids (E_2, 11-KT, 17,20β-P).

Materials and methods

Sampling

A total of 453 *C. aper* by-catch individuals (284 females; 165 males were used. They were collected monthly on board of bottom-trawlers operating along the western Atlantic rocky bottom (centre of Portugal 39° 21'N; 9°23'W; 90-500 m deep) between

September 2011 to October 2012, excluding April (Table 1). Time was registered for each trawl event.

Table I. Number of *Capros aper* females and males sampled (overall and by month) and total length (L_T) range observed, caught along the western Atlantic.

Months	Females		Males		Total
	N	L_T range (cm)	N	L_T range (cm)	
Jan	29	11.2-16.8	26	10.4-14.3	55
Feb	40	7.8-16.2	26	8.1-14.4	66
Mar	25	11.4-14.4	12	10.6-15.2	37
Apr	-	-	-	-	-
May	26	8.3-14.8	7	11.1-16.2	33
Jun	32	9.2-16.7	17	11.3-14.9	49
Aul	13	11.9-13.7	3	11.7-14.9	16
Aug	15	12.3-14.2	20	10.4-14.0	35
Sep	32	12.5-17.0	13	11.2-16.0	45
Oct	28	12.3-14.5	17	12.1-15.6	45
Nov	23	11.2-14.2	12	11.1-13.6	35
Dec	21	11.0-14.0	16	10.8-13.6	37
Total	284	7.8-16.8	169	8.1-16.2	453

Blood samples (~1 ml) were collected onboard from the caudal vein or from the heart with heparinised syringes. Plasma was separated by centrifugation (13 000 rpm for 5 min) and stored at -20° C until analysis. Fish were tagged and stored on ice for posterior analysis in the laboratory, where total length (L_T; 0.1 mm), total and eviscerated weights (W_T and W_E; 0.01 g), gonad weight (W_G; 0.01 g) and sex was recorded within 800 h and 0000 h of hauling. Gonads were preserved in 10% buffered formaldehyde immediately after sampling. Maturity phases of all individuals were assigned by histological examination according to the universal terminology proposed by Brown-Peterson *et al*. (2011); individuals analysed were in the developing (D) and the spawning capable phases (SC); the actively spawning subphase (AS) was only used for females.

Fecundity type

C. aper ovaries are joined over two-thirds (Farrell *et al*. 2012) which makes it difficult to test for homogeneous distribution of oocytes as recommended for fecundity estimation by stereology (Ganias *et al*. 2014). As an alternative, the analysis was conducted using approximately 0.10 g subsamples taken from the separated tissue of the

posterior part of the right ovary lobe of each female (Lowerre-Barbieri and Barbieri 1993).

To investigate the first three lines of evidence to determine the fecundity type (see Introduction), the gravimetric method combined with a computer-aided image analysis system was used [the fourth line of evidence, i.e. the intensity of atresia was previously reported by Sequeira *et al*. (2015)]. A 125-µm mesh sieve was used as this was the threshold mean diameter fixed for *C. aper* cortical alveoli oocytes (Sequeira *et al*. 2015). Oocyte separation was performed using needles and forceps on a watch glass dish under a stereomicroscope Nikon SMZ 745T coupled with a Leica DFC 290 digital camera for image acquisition. All oocytes were automatically measured and counted using ObjectJ plugin (https://sils.fnwi.uva.nl/bcb/objectj/) of the freeware image processing software ImageJ (Schneider *et al*. 2012); each image was individually checked and manually corrected to include oocytes not automatically detected and elimination of those incorrectly marked. The number of each oocyte type in the subsamples was counted and the total number of oocytes in the whole ovary was calculated by multiplying the sum of the number of oocytes in the subsamples divided by the sum of the subsample weights multiplied by the weight of the ovaries (Ganias *et al*. 2014).

To analyse the stage-specific and monthly-specific variation of oocyte size-frequency distribution, 65 females (11.2–17.0 cm L_T; 5 D, 60 SC, 55 of which AS) were used. To analyse the seasonal variation in the percentage of different oocyte classes during the spawning season and in the mean diameter of the advanced vitellogenic oocytes, 71 AS females (11.2–17.0 cm L_T) were used. The diameter of previtellogenic and early vitellogenic oocytes range between 125 and 250 µm and the advanced vitellogenic and mature oocytes between 250 and 650 µm (Sequeira *et al*. 2015).

Fecundity estimation

Absolute batch fecundity (ABF) was estimated using the hydrated oocyte method (Hunter and Macewicz 1985). A total of 49 AS (11.70–17 cm L_T) females with ovaries containing hydrated oocytes and no signs of new post-ovulatory follicles were chosen after histological confirmation. From each ovary, three subsamples around 0.10 g were selected and the gravimetric method combined with a computer-aided image analysis system was used. Methodological procedures followed those used for the determination of the fecundity type, but with a 500-µm mesh sieve taking into consideration the larger

hydrated oocytes (mean diameter 640.354 ± 90.705 μm; Sequeira *et al*., 2015). Each sample was photographed, and hydrated oocytes were counted using the 'cell counter' plugin of freeware image processing software ImageJ (Schneider *et al*. 2012). ABF was calculated by extrapolating the density of hydrated oocytes in the subsample to the gonad weight, and relative batch fecundity (RBF) was calculated as the ratio ABF/WE. The possible correlations between ABF and TL and WE were also investigated.

S values used in the present study are those from Sequeira *et al*. (2015). Usually, relative annual fecundity (RAF) is estimated as the product of RBF by the spawning fraction (S) by the duration of spawning season in days. Since *C. aper* spawns all year round in the western Atlantic Portuguese waters with a spawning peak between June and August, relative fecundities were calculated separately for the spawning peak and for the rest of the year and added up to obtain RAF.

Sex steroid analysis

Plasma sex steroids were analysed throughout the year for both sexes, according to the maturation phase (D and SC) and along the time of day, with four intervals of four hours each between 0800 h and 0000 h (no trawls were taken between 0000 h and 0800 h). Individual plasma samples (96 females and 42 males) were extracted (50 μl) and analysed by radioimmunoassay as previously described (Sequeira *et al*. 2017).

Statistical Analysis

Analysis of variance (ANOVA) followed by the Unequal N Honest Significant Difference (HSD) test was used to test for significant statistical differences in the seasonal variation in the number of previtellogenic/early vitellogenic oocytes and the advanced vitellogenic oocytes and in the mean diameter of the advanced vitellogenic oocytes during spawning season.

To compare plasma sex steroids profiles throughout the year, according to maturity stage and time of day, an ANOVA followed by Duncan's test (whenever ANOVA assumptions were met) or nonparametric tests, Kruskal-Wallis H and Mann-Whitney U-test, were used.

All values were expressed as the mean±standard error of the mean (± S.E.) and statistical significance was inferred at $P < 0.05$. The Statistica Software version 13.2 was used for all statistical analyses.

Results

Fecundity type

From the analysis of the stage-specific and monthly-specific variation of the oocyte size-frequency distribution, no hiatus between the pre-vitellogenic and vitellogenic oocytes was observed. Hiatus was only observed between advanced vitellogenic and hydrated oocytes which separate in imminent spawning females (Figs 1 and 2). No dominant cohort progressing through time was evident in the oocyte size-frequency distribution of *C. aper* throughout the year among spawning females.

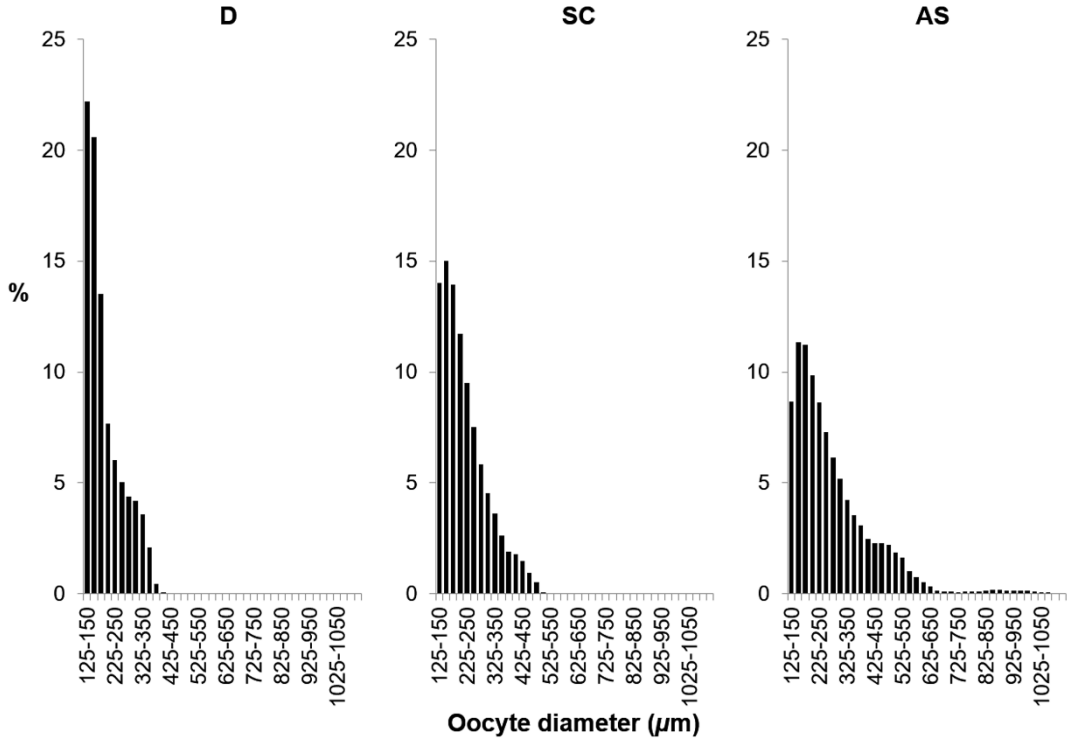

Fig. 1. Oocyte size-frequency distribution for developing (D), spawning capable (SC) and actively spawning (AS) females of *Capros aper*.

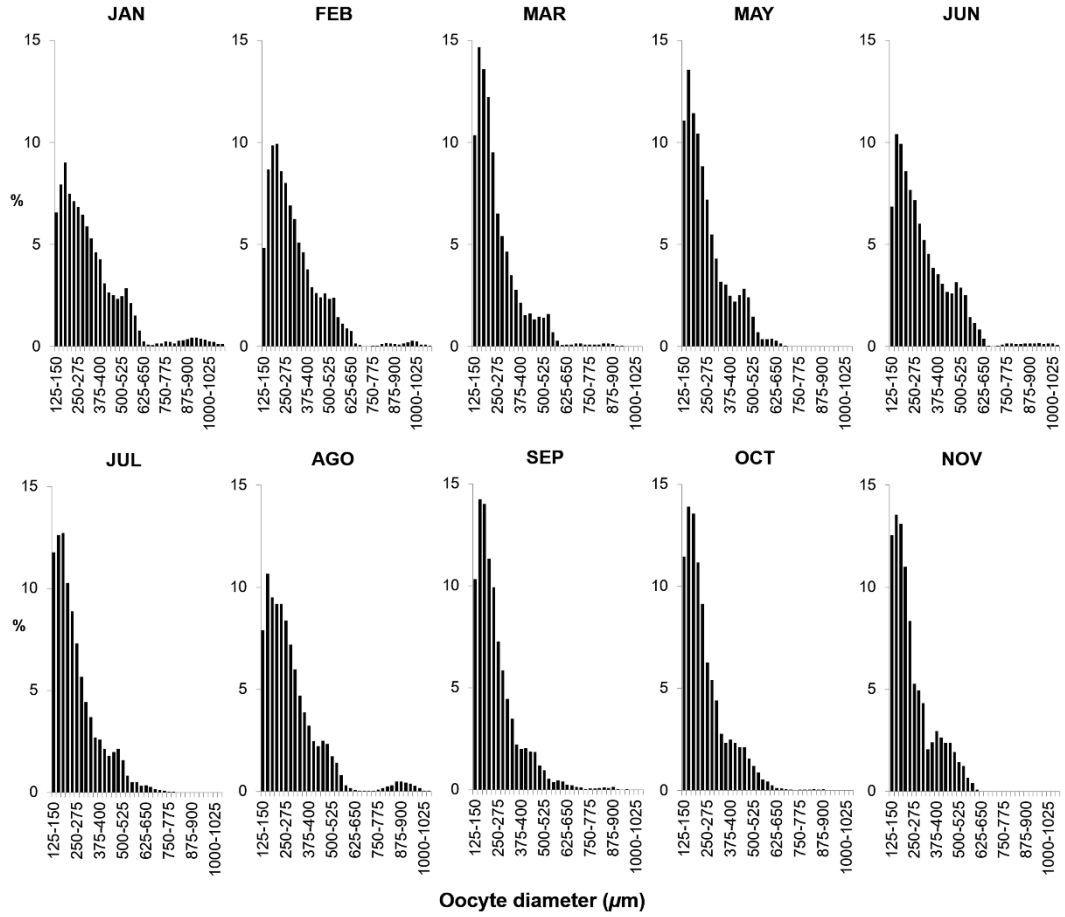

Fig. 2. Oocyte size frequency distribution of *Capros aper* actively spawning females**.**

The number of previtellogenic/early vitellogenic oocytes showed statistically significant differences throughout the year with higher values observed in May and July compared to January, February, March and October (ANOVA, $F_{9,48} = 6.249$, $P < 0.001$, HSD, $P < 0.044$). The mean diameter of the previtellogenic/early vitellogenic oocytes did not varied significantly throughout the year (ANOVA, $F_{9,50} = 1.85$, $P > 0.05$). Considering the advanced vitellogenic oocytes, neither the number (ANOVA, $F_{10,55} = 1.695$, $P > 0.05$) or the mean diameter (ANOVA, $F_{9,49} = 1.83$, $P > 0.05$) showed statistical monthly differences throughout the year (Fig. 3).

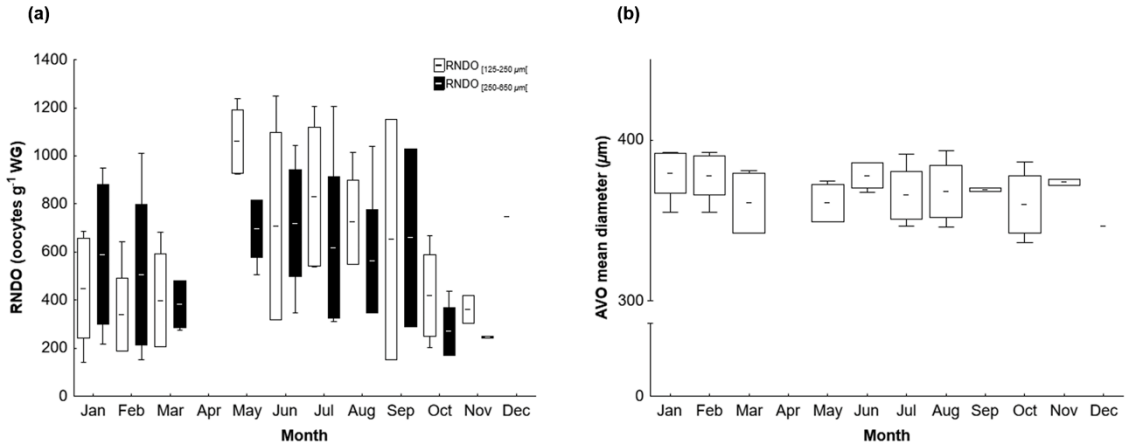

Fig. 3. Monthly variation of (a) the relative number of developing oocytes (RNDO) in previtellogenic/early vitellogenic stage ($RNDO_{[125\text{-}250\ \mu m[}$) and advanced vitellogenic stages ($RNDO_{[250\text{-}650\ \mu m[}$), and of (b) the mean diameter of advanced vitellogenic oocytes (AVO; between 250 and 650 µm) in *Capros aper* spawning capable females.

Fecundity estimation

ABF varied between 223 (at 12.0 cm L_T) and 8,694 (at 16.7 cm L_T) hydrated oocytes, and statistically significant positive correlations between ABF and L_T (Pearson's correlation, r = 0.640, n = 49, $P < 0.001$) and ABF and W_E were observed (Pearson's correlation, r = 0.776, n = 49, $P < 0.001$) (Fig. 4).

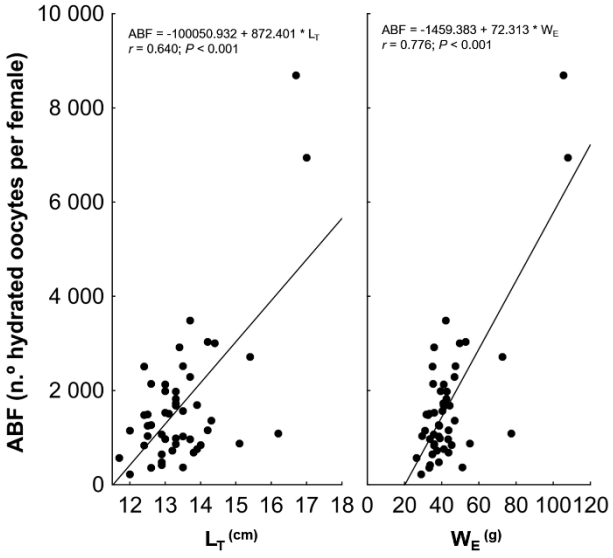

Fig. 4. Linear relation between absolute batch fecundity (ABF) and total length (L_T) and eviscerated weight (W_E) in *Capros aper* females.

The RBF varied between 11 and 83 oocytes g^{-1} W_E; the mean RBF during the spawning peak was 50 oocytes g^{-1} W_E and 31 oocytes g^{-1} W_E for the remaining of the year.

For the spawning peak and the other months of the year, the mean estimated values of S were 0.59 and 0.28 respectively (Sequeira *et al*. 2015), and RAF for each period was 2,695 and 1,325 oocytes g^{-1} W_E respectively, resulting in an annual combined RAF of 4,020 oocytes g^{-1} W_E.

Sex steroid hormone profile

E_2 levels ranged between 0.14 ± 0.04 ng ml^{-1} in November and 1.33 ± 0.40 ng ml^{-1} in July, with significantly higher values observed in July compared to October, November and December, and in August compared to November (E_2: H = 35.721, d.f. = 10, P < 0.05). 17,20β-P varied between 0.36 ± 0.19 ng ml^{-1} in August and 1.38 ± 0.81 ng ml^{-1} in May, with September values statistically different from January and March (17,20β-P: H = 27.354, d.f. = 10, P < 0.05) (Fig. 5a). In males, 11-KT levels varied between 0.14 ± 0.05 ng ml^{-1} in October and 0.52 ± 0.26 ng ml^{-1} in September, and 17,20β-P between 0.47 ± 0.13 ng ml^{-1} in October and 0.94 ± 0.10 ng ml^{-1} in February, but changes were not significant in either case (11-KT: H = 13.202, d.f. = 9, P > 0.05; 17,20β-P: H = 8.069, d.f. = 7, P > 0.05) (Fig. 5b).

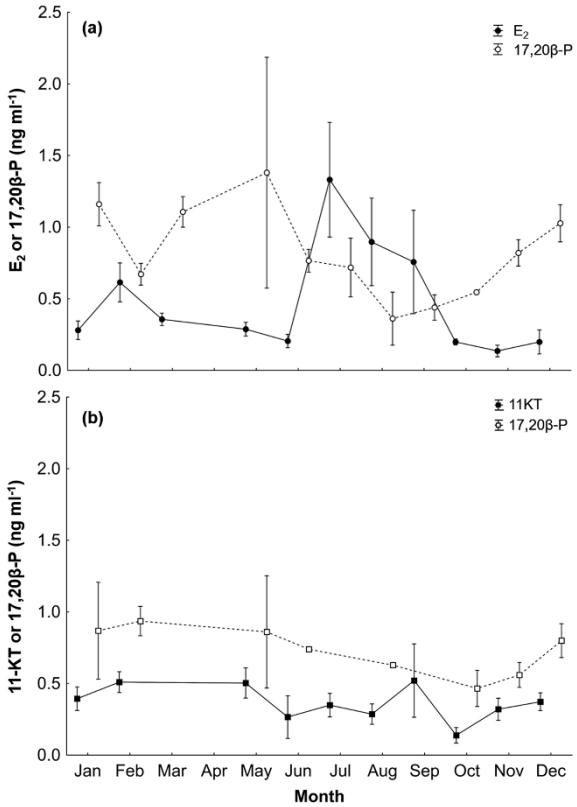

Fig. 5. Monthly changes of mean ± S.E. plasma sex steroids concentrations in (a) females and (b) males of *Capros aper*. E_2, Oestradiol-17β (2); 11-KT, 11-ketotestosterone; 17,20β-P, 17,20β-dihydroxypregn-4-en-3-one; D, development, SC, spawning capable.

Statistically significant differences in E_2 levels were observed between maturity stages (E_2: U, $P < 0.001$) increasing from D to SC females, but not in 17,20β-P (17,20β-P: U, $P > 0.05$) (Fig. 6a). In males, levels of both 11-KT and 17,20β-P did not vary significantly (11-KT: U, $P > 0.05$; 17,20β-P: U, $P > 0.05$) (Fig. 6c).

No statistically significant differences were recorded in sex steroid levels throughout the day (E_2: H = 9.325, d.f. = 4, $P > 0.05$; 17,20β-P: H = 1.270, d.f. = 4, $P > 0.05$; 11-KT: H = 6.684, d.f. = 4, $P > 0.05$; 17,20β-P: H = 8.058, d.f. = 4, $P > 0.05$), although levels of 17,20β-P in males were borderline with an apparent high in the period 2000-0000 h (Fig. 6b, d).

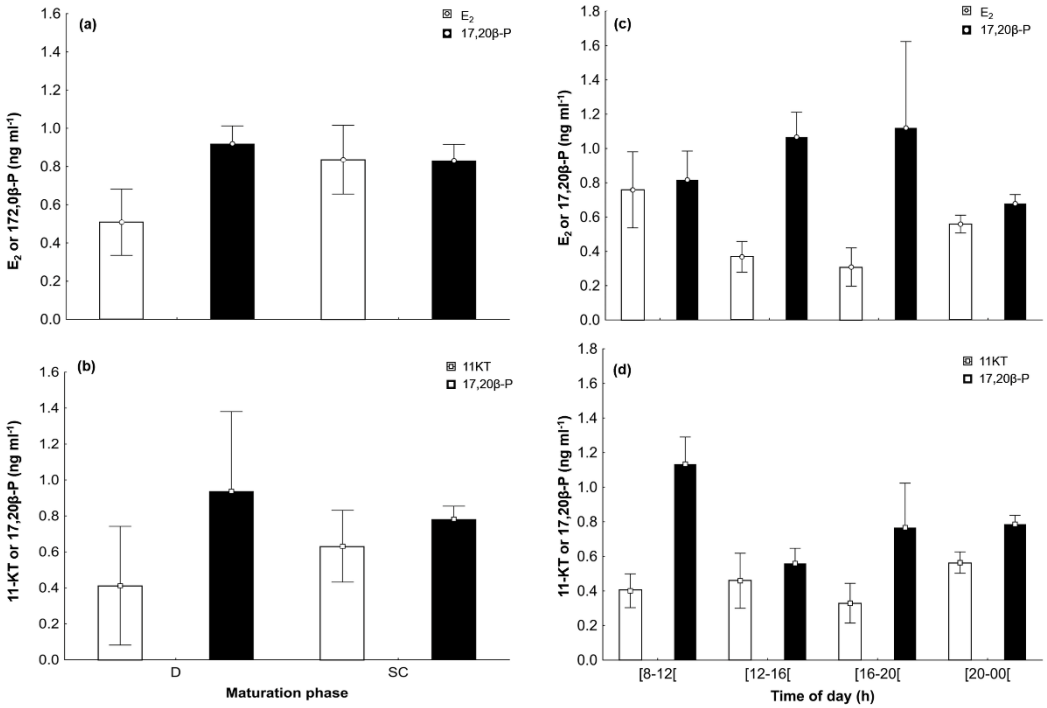

Fig. 6. Variation in mean ± S.E. plasma sex steroids concentrations with reproductive maturity phase in (a) females and (b) males and with time of day in (c) females and (d) males of *Capros aper*. E_2, Oestradiol-17β (2); 11-KT, 11-ketotestosterone; 17,20β-P, 17,20β-dihydroxypregn-4-en-3-one; D, development, SC, spawning capable.

Discussion

The present study demonstrates that in Portuguese waters *C. aper* has an indeterminate type of fecundity with an annual estimated fecundity of 4,020 oocytes g^{-1} WE which are unevenly spawned throughout the year. The asynchronous reproductive pattern is reflected in mostly unchanged steroid levels in blood plasma, at least for the temporal scales used.

Although previous studies had already suggested *C. aper* to have indeterminate fecundity (Farrell *et al*. 2012; Sequeira *et al*. 2015), this is the first one that took into account the four lines of evidence that should be investigated to convincedly demonstrate the reproductive strategy (Hunter *et al*. 1992; Walker *et al*. 1994; Murua and Saborido-Rey 2003; Ganias *et al*. 2014).

As *C. aper* spawns in Portuguese waters throughout the year with a peak in the summer (Sequeira *et al*. 2015), the analysis of the fecundity type forced an extended study to cover, whenever possible, all year round.

As so, and considering the first line of evidence, no hiatus was found in the oocyte size frequency distribution between pre-vitellogenic/early vitellogenic and advanced vitellogenic oocytes by stage and month, indicating continuous recruitment. Regarding the second line of evidence, no significant differences were observed in the number of advanced vitellogenic oocytes during the spawning season and in the number of pre-vitellogenic/early vitellogenic oocytes during the spawning peak. Significant differences were found in the number of pre-vitellogenic/early vitellogenic oocytes between spawning peak months and other months of the year which should be related with a more intense activity during spawning peak, as expected. Considering the third line of evidence, there was no statistically significant seasonal variation of the mean diameter of the advanced vitellogenic oocytes throughout the year. Finally, high prevalence of atresia throughout the year (in over 82% of the females) with relevant percentages (11% to 62%) had already been previously established (Sequeira *et al*. 2015).

Thus, the results are in line with the four criteria that establish indeterminate fecundity in *C. aper*, confirming the suggestion of Farrell *et al*. (2012). This is also supported by the asynchronous development of secondary growth follicles, the protracted spawning season with the discontinuous reproductive cycle, a maximum gonadosomatic index of 5.6% in the western Portuguese coast, much lower than the values expected for synchronous, determinate, total-spawners (Tyler and Sumpter 1996)

The estimate for annual fecundity was 4,020 oocytes g^{-1} WE with a reduction of batch size of 38% (50 to 31 oocytes g^{-1} WE) from the peak spawning period to the other

months. Since no studies on the fecundity of this species or other species of the same family are available, comparisons can only be made with indeterminate fecundity batch spawners from other families. For example, these values are the lowest compared with the European anchovy, *Engraulis encrasicolus* (L. 1758) (200 oocytes g^{-1}, Bay of Biscay; Motos, 1996), the Atlantic sardine, *Sardine pilchardus* (Walbaum 1792) (399 hydrated oocytes g^{-1} of mature females, west coast off Portugal; Nunes *et al*. 2011), the Baltic sprat, *Sprattus sprattus* (L. 1758) (85-165 eggs g^{-1} ovary free weight, Baltic Sea; Haslob *et al*. 2013), the Atlantic horse mackerel, *Trachurus trachurus* (L. 1758) (212 oocytes g^{-1}, Atlantic Iberian waters; Ganias *et al*. 2017), and the mesopelagic myctophids such the glacier lanternfish, *Benthosema glaciale* (Reinhardt, 1837) (1031 oocytes g^{-1} gonad-free weight, Balearic Islands, western Mediterranean; García-Seoane *et al*. 2014). The apparently low batch fecundity of *C. aper* in the present study is probably compensated with a fraction of the population always in spawning activity throughout the year and a short spawning interval during the spawning peak (1.84 days) resulting in a spawning frequency of 50 times during this period. A low relative batch fecundity such as the one found in the present study could raise some concern on the sustainability of the fishery, particularly in Irish waters where *C. aper* seems to spawn only in the summer (Farrell *et al*. 2012) and the intense fishery over the past 20 years is observed, justifying a specific study to ascertain fecundity estimation there.

There were positive correlations between ABF, L_T and W_E, suggesting that body size constraints fecundity in *C. aper*, which could also justify the apparently low batch fecundity and the need for a more protracted spawning season. The variability of RBF between the spawning peak and the other months of the year could be related to other female attributes other than size, such as the nutritional status. Also, environmental conditions (e.g. food abundance, availability, consumption, temperature, fish density, and biomass) could also play a key role in influencing potential fecundity (Lambert 2008) which could justify the different reproductive strategy presented by *C. aper* between the Portuguese and Irish coasts.

The higher levels of E_2 observed during the spawning peak (June-August) extending until September with a smaller peak also observed in February corroborate previous observations regarding *C. aper*'s reproductive cycle and gonadosomatic annual evolution in the Portuguese coast. Although in Irish waters female *C. aper* were observed to spawn only in June and July (Farrell *et al*. 2012), in the Portuguese waters a percentage of the females are continuously spawning (average 44%) with over 90% of

them between June and August and 51% in February (Sequeira *et al*. 2015). E_2 levels also increased significantly from D to SC females which was expected given the well-established role of this steroid and the development of vitellogenesis (Nagahama 1994). In contrast, 11-KT levels varied little throughout the year and no discernible statistical differences between months and maturation stages (D and SC). This could be related to the fact that the spawning capable phase in males was dominant all year round with few individuals in the D phase observed (Sequeira *et al*. 2015).

Significant daily hormone peaks were not observed for either females or males, possibly reflecting the asynchronous pattern of oocyte development in a relatively short developmental period (estimated to be approximately 2 days) and the dominance of SC males, respectively.

As with the other sex steroids, no specific pattern was found for 17,20β-P, neither throughout the year nor in relation to gamete developmental stage, nor the time of day. Most likely this is related to the short period of release of the hormone which makes statistically improbable to sample individuals at the right stage when levels are elevated. Another possibility is that the hormone is quickly metabolized and/or released making it undetectable by our radioimmunoassay. These and other possibilities in different species have been extensively discussed by Scott *et al*. (1987; 2010). Nevertheless, in males' levels were statistically borderline with an apparent high in the period 2000 h-0000 h.

The present study established *C. aper* as a species with indeterminate fecundity type and made the first estimation of fecundity, an important parameter to evaluate its reproductive potential. The loose pattern of sex steroids with no clear established peaks further supports the asynchronous pattern of oocyte development and frequent spawning. Further studies with more frequent sampling are needed to clarify the daily spawning rhythm of this species and the role of hormones in the process.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Declaration of funding

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