

Zygoparity and sex steroid hormone profiles in bluemouth *Helicolenus dactylopterus*

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Running headline: *H. DACTYLOPTERUS* ZYGOPARITY AND SEX STEROIDS

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Abstract

Bluemouth *Helicolenus dactylopterus* (Scorpaeniformes, Sebastidae) is a commercially important deep water species with an unusual reproductive strategy. 207 individuals (103 females and 104 males) from the western Atlantic ranging from 13.9 cm to 37.5 cm total length (L_T) were analysed from September 2011 to October 2012 for gonad maturity stages and blood plasma levels of estradiol-17 β (E_2), 11-ketotestosterone (11-KT), 17,20 β -dihydroxypregn-4-en-3-one (17,20 β -P). Results confirmed the existence of an annual reproductive cycle with asynchrony between females and males and a spawning season from January to May. A pronounced peak in 17,20 β -P in October for both sexes was associated with possible mating behavior and recent copula. Levels of E_2 increased preceding the elevation of gonadosomatic index during ovarian growth, and were lower during regression and regeneration. The frequency distribution of oocyte/embryonic stages and variation of hormone levels suggest the existence of daily rhythms. Fertilization was detected between 2000–0000 and 0800–1200 hours period and spawning took place throughout the day peaking between 2000–0000 hours. The cyclic pattern of sex steroids and ovarian recruitment provides a new insight into the reproductive strategy of this species.

Key words: Reproductive strategy; estradiol-17 β ; 11-ketotestosterone; 17,20 β -dihydroxypregn-4-en-3-one.

Introduction

Teleost fishes are characterized by a diversity of reproductive strategies with the majority being oviparous ovuliparous; nevertheless ~2.5% are viviparous (Wourms, 1991). Knowledge of reproductive strategies and spawning patterns is essential for a comprehensive understanding of the population dynamics of any fish species (Rinchard & Kestemont, 2003) allowing future management and assessment of fishery resources. Scorpaeniformes are particularly interesting as their reproductive strategies vary between oviparity and viviparity (Wourms, 1991).

Bluemouth *Helicolenus dactylopterus* (Delaroche, 1809) is a benthopelagic (100-1000m) scorpaeniform with a wide distribution in the eastern Atlantic (from Norway to South Africa, and around the Azores, Madeira and the Canary islands) and in the Mediterranean (Hureau & Litvinenko, 1986). It is an important commercial species in ICES waters (~ 10,500 tons average annual landings since 2006) as bycatch of demersal trawl and as target for longline fisheries (ICES, 2012). Female and male reproductive cycles are out of phase, with internal fertilization and storage of spermatozoa in cyst-like structures inside the ovaries for several months (Muñoz *et al.*, 1999; Sequeira *et al.*, 2012a; Sequeira *et al.*, 2015). Fecundity is relatively low highlighting the vulnerability of the species to the fishery. Furthermore, fecundity appears to be of the indeterminate type in the western Iberian Peninsula (Sequeira *et al.*, 2012b; Sequeira *et al.*, 2015) and of determinate type in the northwestern Mediterranean Sea (Muñoz *et al.*, 2010). Oocyte development is centripetal (Muñoz *et al.*, 1999) and asynchronous (Muñoz *et al.*, 2010; Sequeira *et al.*,

2012b). Multiple spawning of individual females occurs in the winter-early spring (Muñoz *et al.*, 2010; Sequeira *et al.*, 2012b; Sequeira *et al.*, 2015) and consists of early celled embryos (zygoparity) wrapped in a gelatinous matrix produced by the ovarian wall and peduncular epithelia (the structure that supports each oocyte) (Sequeira *et al.*, 2011).

Considering the complexity of the reproductive cycle and spawning pattern of *H. dactylopterus*, a combined analysis of gonadal stages and sex hormones can provide insights into the physiology and timing of reproductive processes and thereby better support management decisions about the fishery. Sex steroids, produced in the gonads, are under control of pituitary gonadotrophins and regulate key processes during germ cell development. Estradiol-17 β (E₂) produced by the ovarian follicle regulates vitellogenin synthesis in the liver, which is then transported in the blood stream and incorporated into the oocyte where it is responsible for most of secondary growth and for the provision of key nutrients for the developing embryo; 11-ketotestosterone (11-KT) produced by the Leydig cells is necessary for spermatogenesis to proceed; and 17,20 β -dihydroxypregn-4-en-3-one (17,20 β -P) is the maturation inducing steroid in most fish species and is important for spermiation and milt hydration (Nagahama, 1994; Scott *et al.*, 2010; Schulz *et al.*, 2010).

The objectives of the present study were: 1) to examine morphological changes in the annual reproductive cycle of gametogenesis and spawning of male and female *H. dactylopterus*, and 2) to relate the individual levels of sex steroids (E₂, 11-KT, 17,20 β -P) in order to clarify aspects of the species reproduction and the potential role of hormones.

Materials and methods

Sampling

Helicolenus dactylopterus individuals were collected monthly on board bottom-trawlers operating along the western Atlantic (center region of Portugal 39° 21'N; 9°23'W; average depth 100 m) between September 2011 and October 2012, except April due to adverse weather conditions. Trawls lasted 1.30 to 3.00 h and time of day was recorded; five intervals of four hours between 0400 h and 0000 h were created to analyse data (there were no trawls between 0000h and 0400 h). 207 individuals (103 females and 104 males) (Table I) ranging in size from 13.9 cm to 37.5 cm in L_T were sampled.

Blood samples (~1 ml) were collected from the caudal vein with heparinised syringes. Plasma was separated by centrifugation (13 000 rpm for 5 min) and stored at -20° C until hormone analysis. Fish were tagged, stored in ice and taken to the laboratory where total length (L_T ; 0.1 mm), total and eviscerated masses (W_T and W_E ; 0.01 g), gonad mass (W_G ; 0.01 g) and sex were recorded. The gonadosomatic index (I_G) ($I_G = W_G / W_E \times 100$) was determined. In imminent spawning females, the gelatinous matrix was extracted according to Sequeira *et al.* (2011), and 1 ml of each fresh sample was observed on a light microscope for embryo staging. All gonads were preserved in 10% buffered formaldehyde immediately after sampling and maturity stages were assigned by histological examination.

Histology

Fixed fragments of the mid ovarian and testicular region were dehydrated with ethanol, embedded in metacrylate, sectioned at 3-5 μm , stained with toluidine blue and examined on a Leica DM 2000 light microscope with a Leica DFC 290 digital camera (<http://www.leica-microsystems.com/home/>).

Ovarian follicles and testicular germ cells were classified based on histological criteria (Grier, 1981; Wallace & Selman, 1981; West, 1990; Grier, 2012). Advanced oocytes and embryos in spawning females were staged according to Grier (2012) and Sequeira *et al.* (2015) as follows: secondary growth oocyte, full-grown step (SGfg), mature oocyte, eccentric germinal vesicle step (OMegv), mature oocyte, germinal vesicle migration step (OMgvm), mature oocyte, meiosis resumes step (OMmr), fertilized (f), early celled (ec), blastula (b).

The universal terminology proposed by Brown-Peterson *et al.* (2011) was used to describe gonadal maturity phases and subphases: developing (D), spawning capable (SC) (and subphase actively spawning (AS), for females), gestation (G) (and subphases fertilized (F), early celled (EC) and blastula (B), for females), regressing (RE) and regenerating (R).

Histological sections were searched for the presence of spermatozoa cysts (CSz) (prevalence of CSz = number of females with CSz/total number of females x 100) and interlamelar free spermatozoa.

Sex steroid analysis

Individual plasma samples (50 μ l) were extracted with diethyl ether (2x4 ml), the solvent evaporated under nitrogen and the residue re-suspended in 1 ml 0.05 M phosphate buffer, pH 7.6. Steroid hormones were measured by radioimmunoassay as previously described: E₂ (Guerreiro *et al.*, 2002), 17,20 β -P (Canario *et al.*, 1989) and 11-KT (Kime & Manning, 1982). The limits of detection were between 10 (E₂) and 100 (17,20 β -P and 11-KT) pg/ml.

Statistical analysis

Data were log transformed whenever necessary to meet assumptions of analysis of variance (ANOVA). One-way ANOVA was used to compare sex steroids monthly, daily and between maturity stages, followed by Duncan's honestly significant difference post-hoc test. If assumptions of ANOVA were not met even after transformation nonparametric tests (Kruskal-Wallis (*H*) and Mann-Whitney *U*-test (*U*)) were used. The latter was performed to determine statistical significance in average monthly *I*_G for both sexes. Pearson correlations between steroid levels and *I*_G were estimated.

All values are expressed as the mean \pm standard error of the mean (\pm S.E.) and statistical significance was inferred at $P < 0.05$. Statistica Software version 12 (<http://www.statsoft.com/Products/STATISTICA-Features/Version-12>) was used for all statistical analyses.

Results

Reproductive cycle

The females' I_G ranged from $0.33 \pm 0.02\%$ in June to $5.34 \pm 0.48\%$ in February, while the males' I_G varied between $0.03 \pm 0.02\%$ in May and $0.52 \pm 0.22\%$ in September. In both, the I_G changed significantly throughout the year (females: $H = 69.866$, d.f. = 10, $P < 0.001$; males: $H = 47.375$, d.f. = 10, $P < 0.001$) with higher I_G values in females between December and March ($P \leq 0.034$) and in males between July and December ($P \leq 0.043$) (Fig. 1(a)).

Females at the D stage were observed between September and January, followed until May by predominance of females at late vitellogenesis, maturation and gestation (SC and G) coinciding with the spawning period. Regarding G females, 44% were at the F subphase, 33% at B subphase and 22% at the EC subphase. Post-spawning females (Re and R) were sampled between May and October (Fig. 1(b)). In males, the D stage lasted from May until August and individuals in active spermatogenesis (SC) were observed between July and February. Males with residual spermatozoa in regression and regenerating condition (RE and R) were sampled between January and June (Fig. 1(c)).

Cysts of spermatozoa were observed at the centre of ovary around the connective tissue at the base of the ovigerous lamellae and in the interlamellar space in 84% of the females (Fig. 2). Their prevalence was over 75% between October and May. Free sperm was also observed at the base of the ovigerous lamellae and in the interlamellar space between August and January. All females sampled in October and November were in this condition. Empty cysts

were observed between February and August coinciding with spawning and post-spawning periods.

Sex steroid hormone profile

Sex steroids were analysed in 163 individuals (88 females and 75 males) (Table I).

E₂ levels ranged between 0.12 ± 0.02 ng ml⁻¹ in August and 0.97 ± 0.61 ng ml⁻¹ in February. Significant monthly changes were observed (ANOVA, $F_{10,81} = 4.565$, $P < 0.001$) with higher values between December and February compared to May through November ($P \leq 0.048$) corresponding to active vitellogenesis during the pre-spawning/spawning period. In males, the lowest level of 11-KT was measured in March (0.15 ± 0.07 ng ml⁻¹) and the highest in October (0.94 ± 0.49 ng ml⁻¹). The monthly changes were significant (ANOVA, $F_{10,75} = 2.839$, $P = 0.006$) with the period from May to December showing significantly higher values than February and March ($P \leq 0.035$) (Fig. 3(a)). Both E₂ and 11-KT started to increase one month before *I*_G. In males *I*_G and 11-KT were significantly positively correlated (Pearson's correlation, $r = 0.52$, $n = 73$, $P < 0.001$) but there was no significant correlation between *I*_G and E₂ in females (Pearson's correlation, $r = 0.21$, $n = 76$, $P = 0.075$).

For most of the year 17,20β-P varied between 0.25 ± 1.15 ng ml⁻¹ (November) and 1.17 ± 0.33 ng ml⁻¹ (July) in females, and between 0.37 ± 0.09 ng ml⁻¹ (August) and 0.88 ± 0.05 ng ml⁻¹ (March) in males. However, in October 3 in 8 females had values ≥ 9.49 ng ml⁻¹ and 2 in 5 males ≥ 3.82 ng ml⁻¹ (Fig. 3(b)). Given the high variability, the average levels in October (4.48 ± 5.22 ng

ml⁻¹ in females, and 2.45 ± 3.02 ng ml⁻¹ in males) were not statistically significant from other months. Nevertheless, plasma 17,20 β -P was significantly elevated in February compared to January, May and November (U , $P \leq 0.048$) for females and in March compared to August for males (U , $P = 0.024$).

E_2 levels varied significantly between maturation phases (ANOVA, $F_{4,80}$, $= 4.253$, $P = 0.004$) increasing from post spawning females (RE and R) to vitellogenesis and spawning (SC and G) ($P < 0.045$). 11-KT did not vary significantly although regressing males presented lower values (11-KT: ANOVA, $F_{3,75}$, $= 2.227$, $P = 0.093$) ((Fig. 4(a)). Mean plasma 17,20 β -P levels did not vary significantly between maturity stages in both sexes (females: U , $P = 0.540$; males: U , $P = 0.340$) (Fig. 4(b)).

The relative frequency of the f stage increased from 2000–0000 to 0800–1200 hours period, of the ec stage from 0400–0800 to 1200–1600 hours period and of the b stage from 0400–0800 to 2000–0000 hours period (Fig. 5(a)). In the same individuals an apparent increase in E_2 and 17,20 β -P levels was observed in females sampled between 0020–0000 and 0800–1200 hours period, and a decrease in the subsequent period between 1200–1600 hours (Fig. 5(a)). Nevertheless, these variations were not statistically significant (E_2 : ANOVA, $F_{4,70} = 1.052$, $P = 0.387$; 17,20 β -P: U , $P = 0.942$). In mature males, no pattern was observed for 11-KT (ANOVA, $F_{4,69} = 0.538$, $P = 0.709$). 17,20 β -P was only analysed for part of the day, with elevated levels registered in the 1200–1600 hours period but without statistical significance (U , $P = 0.522$) (Fig. 5(b)) due to the high variability.

To help clarify the ovarian development pattern during the spawning season, E_2 and 17,20 β -P levels were related to the stage of the two most

advanced cohorts of oocytes of spawning females (Fig. 6). A similar apparent cyclic pattern for both sex steroids could be observed. E₂ and 17,20β-P levels were lower in ovaries containing ec embryos and OMegv oocytes and higher when oocytes were at maturation stage and included OMmr oocytes. Higher levels of E₂ were observed in ovaries with OMgvm and SGfg as the two most advanced cohorts (Fig. 6). Nevertheless these variations were not statistically significant (E₂: ANOVA, F_{9,42} = 0.290, P = 0.071; 17,20β-P: H = 3.875, d.f. = 9, P = 0.920).

Discussion

This study analysed sex steroid hormones and gonadal changes during the reproductive cycle of *H. dactylopterus*. While observations of the reproductive cycle to a large extent confirmed previous observations, the hormonal profile allowed a more detailed characterization of the timing of the underlying processes. Overall, changes in 11-KT and E₂ preceded I_G, and 17,20β-P variations are suggested to be associated with oocyte maturation, ovulation and mating events.

The histological analysis and the I_G confirmed the spawning season of *H. dactylopterus* from January to May with a small delay compared to previous reports for the same geographic area (Sequeira *et al.*, 2012a; Sequeira *et al.*, 2015). Males had an out of phase and more extended active reproductive period than previously found (Muñoz *et al.*, 1999) lasting eight months. This result suggests the availability of females to be fertilized over a long period.

Indeed, 84% of the females collected had spermatozoa cysts in their ovaries with over 75% prevalence covering the period of active spermatogenesis in males and spawning in females. Moreover free spermatozoa were observed inside the ovaries for several months, suggesting the possibility of multiple copulations throughout the year. The presence of empty cysts inside the ovaries during the spawning and post-spawning periods is indicative of recent fertilization, as suggested by Vila *et al.* (2007).

From the analysis of relative frequency of the last oocyte/embryonic stage in spawning females, the increase of the relative frequency of the f stage from 2000–0000 to 0800–1200 hours period followed by the increase of the relative frequency of successive embryonic stages (ec and b) from 0400–0800 hours period onwards suggests that fertilization occurs from around dusk throughout the night. Based on the fact that blastula (b) is the most advance embryonic stage that can be observed in *H. dactylopterus* ovaries (Sequeira *et al.*, 2011; Sequeira *et al.*, 2015), females should spawn (release embryos) throughout the day reaching their maximum around dusk between 2000–0000 hours. A nocturnal preference for reproductive activity is not unusual within the same family: yellowtail rockfish *Sebastes flavidus* (Ayres, 1862) and rockfish *Sebastes inermis* Cuvier, 1829, releases their embryos during the night (Eldridge *et al.*, 1991) and copulate at dusk (Shinomiya & Ezaki, 1991), respectively.

The annual profile of E₂ and 11-KT confirmed the overall pattern of the reproductive cycle emphasizing that female and male are out of phase. A significant positive correlation between 11-KT and I_G supports the role of this steroid in active spermatogenesis (SC) (Nagahama, 1994; Barcellos *et al.*,

2002; Schulz *et al.*, 2010; Shimizu, 2014). Also, highest values of E₂ were present in vitellogenic females (SC and G). The delay of one month observed between the increase of E₂ and I_G was expected as a time interval mediates estrogen stimulation of vitellogenin production by the liver and the beginning of vitellogenesis (Takano *et al.*, 1991).

As for 17,20β-P, there was very little variation throughout the year, except in October when females were in RE/D maturation phase and males in SC. Some individuals of both sexes had high levels resulting in a peak of high variability. This variability is not unexpected since 17,20β-P is normally produced at higher levels during oocyte maturation, or in relation to sperm duct hydration and possibly mating behavior and copula (Liley *et al.*, 1986, Scott *et al.*, 2010) which are relatively short lived events (from minutes to hours). In support of this hypothesis is the fact that all females sampled in October and November had free spermatozoa in the ovaries suggesting recent fertilization. Therefore, the probability that all individuals do not go through the processes simultaneously resulted in high variation in hormone levels. A consequence of the increased variability is that larger samples would be required to achieve statistical significance.

For the same reason it was not possible to demonstrate significant daily hormone peaks. However, a daily rhythm is suggested for E₂ and 17,20β-P as both hormones had an apparent elevation between 2000–0000 and 0800–1200 hours period decreasing thereafter. These hormonal changes could be related to the secretion from two or more cohorts of oocytes under the control of gonadotrophin(s), one secreting E₂ and undergoing vitellogenesis and the other secreting 17,20β-P and undergoing oocyte maturation and ovulation. Short term

fluctuations in E₂ have been previously observed in multiple batch spawners (Takano *et al.*, 1991; Dahle *et al.*, 2003 and references therein). *H. dactylopterus* is an asynchronous multiple spawner species (87 batches per season) with a short spawning interval (1.73 days) (Sequeira *et al.*, 2015) requiring continuous vitellogenesis during the spawning period to recruit new batches into maturity, which is consistent with the present results. Parallel changes for the two hormones have been shown in goldfish *Carassius auratus* (L. 1758) (Kobayashi *et al.*, 1987). Also, levels of E₂ and 17,20β-P in relation to the two most advanced oocyte cohorts of spawning females is consistent with their roles in oocyte development and maturation in *H. dactylopterus*. Lower levels of both steroids were present in females with ec embryos-OMegv oocytes with a subsequent apparent increase of hormonal levels as the two most advanced cohorts continued to develop. The cohorts consisting of ec and b embryos can be considered to originate a “between batches” period; the levels of E₂ were lower because there are fewer vitellogenic oocytes producing it. As development proceeds more and larger mature oocytes become available for the next batch and E₂ levels increase. Lower peak concentrations of E₂ have also been detected in group-synchronous Atlantic cod *Gadus morhua* L. 1758 (Dahle *et al.*, 2003) and turbot *Scophthalmus maximus* (L. 1758) (Howell & Scott, 1989) and suggested to be characteristic of fishes with asynchronous oocyte development (Matsuyama *et al.*, 1990) as is the case of *H. dactylopterus*. The decrease of 17,20β-P after ovulation could indicate that it is the maturation inducing steroid of *H. dactylopterus*, but further studies are needed to confirm this hypothesis. In any case, 17,20β-P seems to play an

important role in both sexes, particularly in relation to sexual behavior, as levels seem to be higher during matting.

In males, few studies have analyzed daily cycles in sex steroids. However, in *H. dactylopterus*, 11-KT did not show any particular daily pattern, probably reflecting differences in the way meiosis proceeds in the two sexes.

In conclusion, the hormonal profile of sex steroids was associated with important events in the reproductive cycle of *H. dactylopterus*. This is particular relevant for a wild commercial species which lacks physiological data to interpret its peculiar reproductive strategy. Nevertheless, given the diversity of reproductive processes involved, further studies are required to understand the importance and function of gonadal steroids, namely those associated with mating behavior, gestation and continuous oocyte recruitment in this species.

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Table I. Total number and total length range of *Helicolenus dactylopterus* females and males sampled by month and subject of sexual steroid analysis.

| | Females | | | | Males | | | |
|-------|----------|---------------------------------|----------------|----------|----------|---------------------------------|-------|----------|
| | <i>n</i> | <i>L_T</i> range (cm) | E ₂ | 17,20β-P | <i>n</i> | <i>L_T</i> range (cm) | 11-KT | 17,20β-P |
| Jan | 17 | 18.7 - 23.7 | 12 | 12 | 25 | 17.9 - 24.8 | 19 | 18 |
| Feb | 19 | 17.7 - 27.0 | 19 | 19 | 2 | 20.5 - 26.4 | 2 | 2 |
| Mar | 14 | 17.0 - 22.9 | 13 | 13 | 3 | 18.8 - 23.5 | 3 | 3 |
| Apr | - | - | - | - | - | - | - | - |
| May | 7 | 19.3 - 23.9 | 6 | 7 | 6 | 20.5 - 23.0 | 6 | 6 |
| Jun | 10 | 18.6 - 20.9 | 4 | 6 | 5 | 19.6 - 22.5 | 3 | 3 |
| Jul | 5 | 17.1 - 20.4 | 3 | 4 | 13 | 13.9 - 25.2 | 7 | 7 |
| Aug | 3 | 19.0 - 19.5 | 3 | 2 | 10 | 19.6 - 24.1 | 6 | 6 |
| Sep | 4 | 21.4 - 31.5 | 4 | | 8 | 21.4 - 27.6 | 8 | |
| Oct | 8 | 20.4 - 31.9 | 6 | 8 | 5 | 20.0 - 23.7 | 5 | 5 |
| Nov | 3 | 21.6 - 29.3 | 2 | 2 | 12 | 21.2 - 37.5 | 5 | 5 |
| Dec | 13 | 18.6 - 28.6 | 8 | 8 | 15 | 19.2 - 25.2 | 11 | 11 |
| Total | 103 | 17.0 - 31.9 | 80 | 81 | 104 | 13.9 - 37.5 | 75 | 66 |

11-KT, 11-ketotestosterone; 17,20β-P, 17,20β-dihydroxypregn-4-en-3-one; E₂, estradiol-17β; *L_T*, total length; *n*, sample size

Figure captions

Fig. 1

Monthly variation of (a) gonadosomatic index (I_G) (mean \pm S.E.) (○, females; ■, males) and relative frequency of reproductive phases (□, development; ■, gestation; □, regenerating; ▣, regressing; ■, spawning capable) in *Helicolenus dactylopterus* (b) females and (c) males.

Fig. 2

Histological sections of *Helicolenus dactylopterus* female and male gonads: (a) mature female in the gestation phase, early celled subphase showing oocytes in different stages and embryos in the early celled stage (sampled in January 2012); (b) mature male in the spawning capable phase showing sexual cells in different stages (sampled in September 2011); (c) mature female presenting spermatozoa inside a cyst and freely next to the ovarian lamellae (sampled in October 2011). CSz, spermatozoa cyst; ec, early celled embryo; FSz, free spermatozoa; gv, germinal vesicle; od, oil drop; OMegv, mature oocyte, eccentric germinal vesicle step; PGca, primary growth oocyte, cortical alveolar step; Sc, spermatocyte; Sg, spermatogonia; SGe, Secondary growth oocyte, early growth step; SGfg, secondary growth oocyte, full-grown step; SGI, Secondary growth oocyte, late growth step oocyte; St, spermatid; Sz, spermatozoa.

Fig. 3

Monthly changes of plasma sex steroids (mean \pm S.E.) in *Helicolenus dactylopterus*: (a) estradiol-17 β (○) and 11-ketotestosterone (■); (b) 17,20 β -dihydroxypregn-4-en-3-one (○, females; ■, males).

Fig. 4

Steroid hormones concentrations according to *Helicolenus dactylopterus* reproductive maturity phase: (a) estradiol-17 β (□) and 11-ketotestosterone (■), and (b) 17,20 β -dihydroxypregn-4-en-3-one in females (□) and males (■). Bars represent mean \pm S.E. * denotes a significant difference from all other maturity phases ($P < 0.05$). D, development; G, gestation; R, regenerating; RE, regressing; SC, spawning capable.

Fig. 5

Daily rhythm of (a) estradiol-17 β (—) and 17,20 β -dihydroxypregn-4-en-3-one (----) in association with female relative frequency of the last oocyte/embryonic stage (□, b, blastula; ▣, ec, early celled; ■, f, fertilized; □, OMegv, mature oocyte eccentric germinal vesicle step; ▤, OMgvm, mature oocyte germinal vesicle migration step; ▥, OMmr, mature oocyte meiosis resumes step), and (b) 11-ketotestosterone (□) and 17,20 β -dihydroxypregn-4-en-3-one (■) in males. Bars represent mean \pm S.E.

Fig. 6

Rhythm of estradiol-17 β (▤) and 17,20 β -dihydroxy-4-pregnen-3-one (▥) according to the oocyte stage of the two most advanced cohorts present in ovaries of *Helicolenus dactylopterus* spawning females. Bars represent mean \pm

50 S.E. 2nd LC, second last cohort; b, blastula; ec, early celled; f, fertilized; LC, last
51 cohort; OMegv, mature oocyte, eccentric germinal vesicle step; OMgvm, mature
52 oocyte germinal vesicle migration step; OMmr, mature oocyte meiosis resumes
53 step; SGfg, secondary growth oocyte full-grown step.











