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Endocrine Correlates of Male Polymorphism and Alternative Reproductive Tactics in the Azorean Rock-Pool Blenny, *Parablennius sanguinolentus parvicornis*

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In the Azorean rock-pool blenny male sexual polymorphism occurs. Larger and older males (M+ males) fully express male secondary sex characters (SSC), particularly an anal gland that produces a sex pheromone, whereas smaller and younger sexually active males do not express SSC (M− males). Two mating tactic types can be identified among M+ males: nest-holders that establish nests and court females and floaters that move around in the breeding area and try to achieve parasitic fertilizations and/or to take over nests. Two behavioral tactic types can also be identified within M− males: satellites that are associated with particular nests and actively participate in territorial defense (when females go inside the nest to spawn they try to enter to fertilize some of the eggs) and sneakers that do not help nest holders (when spawning occurs they also try to enter the nest to fertilize eggs). It was found that M+ males have significantly higher levels of 11-ketotestosterone (KT), but not testosterone (T), than M− males [M+ male androgen levels (mean ± SE): total T = 11.6 ± 3.0 ng ml^{-1}, total KT = 4.5 ± 1.1 ng ml^{-1}; M− male androgen levels (mean ± SE): total T = 9.6 ± 1.0 ng ml^{-1}, total KT = 2.5 ± 1.1 ng ml^{-1}]. There were no differences in plasma T or KT among individuals using different mating tactics within the same male morph; that is, among M+ males, nest-holders did not differ in androgen levels from floaters [nest-holder androgen levels (mean ± SE): total T = 12.3 ± 4.4 ng ml^{-1}, total KT = 4.3 ± 1.4 ng ml^{-1}; floater androgen levels (mean ± SE): total T = 5.9 ± 0.8 ng ml^{-1}, total KT = 3.4 ± 0.3 ng ml^{-1}], and among M− males, satellites did not differ in androgen levels from sneakers [satellite androgen levels (mean ± SE): total T = 7.7 ± 1.5 ng ml^{-1}, total KT = 1.3 ± 0.3 ng ml^{-1}; sneaker androgen levels (mean ± SE): total T = 8.3 ± 1.6 ng ml^{-1}, total KT = 1.4 ± 0.3 ng ml^{-1}]. Thus, the observed differences appear to be correlated with the expression of different male morphotypes and not with the expression of different behavioral tactics within the morphotype. Androgen levels were not correlated with the behavior activity of nest-holders, except for a negative correlation between KT levels and parental behavior. Furthermore, nest-holder males that succeeded in having females spawn in their nests during the observation period had significantly lower KT levels than unsuccessful males. Since behavioral observations preceded blood sampling in time, it is suggested that these results indicate a negative relationship between KT and parental care, since successful males were parenting when blood samples were collected. Male SSC were better correlated with KT than with T and the use of total blood levels (i.e., free + conjugates) yielded higher correlation coefficients than when only the free fraction of each steroid was considered. Since conjugates are nonactive metabolites of the

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free androgen they should reflect active free steroids in a previous time. Thus, their incorporation into the hormonal measurements increases the time frame captured, and because steroids are released in a pulsatile way, this time-integrated measure can be more meaningful than the free steroids, which represent a snapshot of the hormone levels at a given point in time.

Key Words: sexual polymorphism; alternative reproductive tactics; androgens; 11-ketotestosterone; teleosts; paternal care.

INTRODUCTION

Male polymorphism associated with different reproductive tactics is a widespread phenomena in teleost fish (e.g., plainfin midshipman, *Porycthys notatus;* Bass, 1996). As a rule each male sexual morphotype uses a specific (alternative) behavioral tactic to mate. Usually a bourgeois and a parasitic tactic can be found. Bourgeois males actively compete among themselves, investing in the acquisition of mates (e.g., by defending breeding territories), whereas parasitic males exploit the investment of bourgeois males to get access to females and fertilize eggs (Taborsky, 1997). Alternative mating tactics also occur in male sexually monomorphic species with a single male morph present that can adopt different conditional behavior tactics (e.g., guppies; Evans and Magurran, 1999).

In a population of *Parablennius sanguinolentus parvicornis* (Blenniidae) from the Azores islands alternative male reproductive tactics occur, which translate into male polymorphism. Two sexually active male types can be recognized morphologically. Larger and older males develop conspicuous secondary sexual characters (SSC), namely well-developed anal glands and head humps (type M+ males), whereas smaller and younger males (0^+–1) do not express them (type M− males) (Santos, 1985; Santos *et al.*, 1995). Also, M− males have gonads that are two to three times larger relative to fish body mass (i.e., gonadosomatic indexes) than those of M+ males (Santos *et al.*, 1996). During the breeding season, from May to August (Santos, 1989), some of the M+ males establish breeding territories in which they prepare a nest in a natural crevice or under a boulder. They court females by signaling the location of the nest, circling, and leading

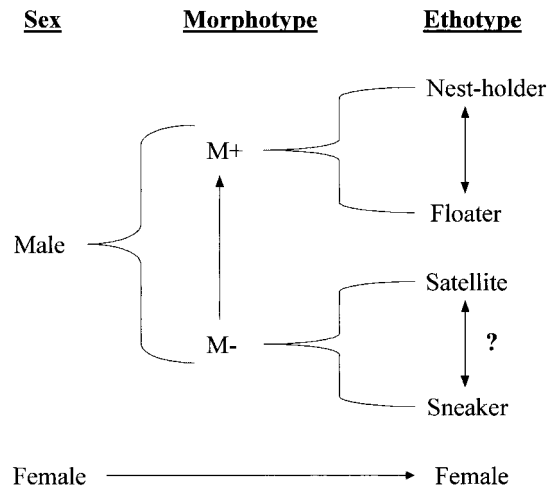

FIG. 1. Classification of male polymorphism and alternative behavioral tactics in male *P. s. parvicornis.* Arrows indicate potential transitions in behavioral tactics within the same individual.

the females, and spawning occurs inside the nest (Santos and Barreiros, 1993). Females leave the nest immediately after spawning and parental care is exclusively provided by the males that guard the eggs until hatching (Santos and Barreiros, 1993). Each nest-holder male receives in its nest eggs from more than one female and females spawn in different males' nests; thus, it is a promiscuous mating system with exclusive male parental care (Santos and Barreiros, 1993). A fraction of the M+ males do not establish a nest and instead act as floaters, also trying to sneak fertilizations or to take over a nest from a nest-holder. Thus, among the M+ males, two behavioral tactics can be recognized: nest-holders and floaters (Fig. 1).

In contrast, some M− males act as satellites of the nest-holders' territories, participating in their active defence (Santos, 1985) and when females go inside the nest to spawn they also try to enter the nest to fertilize some of the eggs (satellite males). Alternatively, some M− males are not attached to any particular nest and do not help any of the nest-holder males in territorial defence. However, during spawning these males may also try to sneak into the nest and to achieve fertilizations (sneaker males). Thus, among M− males, two behavioral tactics can also be recognized: satellites and sneakers (Fig. 1). It is not clear whether the same M− individuals can conditionally switch between acting as satellites and acting as sneakers (Fig. 1). However, M− males can become M+ males in subsequent

breeding seasons (Santos *et al.*, 1995), suggesting that male polymorphism in *P. s. parvicornis* is a conditional strategy for less competitive males to breed in their first breeding season.

This study investigates the hormonal correlates of male polymorphism in *P. s. parvicornis* by comparing circulating levels of androgens in M+ and M− males. The endocrine correlates of alternative mating tactics were also assessed, by comparing males following different behavioral tactics within and between morphs.

METHODS

Study Area and Sampling Procedures

This study was conducted in rock intertidal pools (maximum depth 0.75 m) on a flat basaltic intertidal platform at Feteira on the south coast of Faial Island, Azores (38°31' N; 28°27' W). The behavioral observations and blood collection was carried out during the breeding seasons of 1998 and 1999. Following a period of behavioral observations of each male *P. s. parvicornis* subject (that lasted from 15 to 30 days), blood was collected in the field, during low tide, immediately after capture with a hand net. Subjects were anesthetized in a bucket containing seawater and MS-222 (Argent, 1–10,000 dilution), and blood was collected from the caudal vein with a 1-ml heparinized syringe fitted with a 0.6-gauge needle, placed in 1.5-ml Eppendorf tubes on ice, and taken to the laboratory where it was centrifuged, and the plasma stored at −20°C. Subjects were also transported to the laboratory where they were anesthetized, and for each individual, the following measurements were taken: weight (to the nearest 0.1 g), standard length, and head height and body height taken at the insertion of the pectoral fins (to the nearest 0.1 mm). They were then immediately perfused transcardially with marine teleost Ringer followed by a solution of 4% paraformaldehyde in 0.1 M phosphate buffer to fix the brain tissue. The brains were then removed and processed for immunocytochemistry (not reported here). Gonads were also removed, and the length and width of the testis and testicular glands (TG) (see Patzner and Lanhsteiner, 1999, for a description of the male gonadal accessory organs of blennies) were measured with an ocular micrometer of a binocular stereoscope. The following morphometric indices were calculated: head hump = maximum head height / body height measured at the level of the insertion of the pectoral fin; condition factor (K) = body weight / standard length^3; hepatosomatic index (HSI) = 100 × liver weight / body weight; gonadosomatic index (GSI) = 100 × gonad weight / body weight; and testicular gland index (TGI) = testicular gland area / testis area. TG and testis areas were calculated from dorsal-view measurements of maximum width and length, assuming testis and TG to be ellipsoidal. The genital papillae length and width were also measured with an ocular micrometer of a binocular stereoscope and the papillae ventral-view area was calculated, assuming a rectangular shape.

Blood sampling occurred on consecutive days for similar periods and equivalent tidal regimes so that any effect of rhythms was minimized and should not have affected the main results. A total of 17 M+ males, 6 females, and 25 M− males were sampled. Of these there were behavioral observations available on 15 M+ males and on 14 M− males, which gave separation in terms of their behavioral mating tactics: 11 nest-holders and 4 floaters among M+ males, and 7 satellites and 7 sneakers among M− males.

Behavioral Observations

Behavioral observations were conducted during low tide in May–July 1998 and June–July 1999. The hydrodynamic conditions prevented behavioral observations during high tide. During the first 3–5 days of each field season a large number of fish of both sexes were captured and individually tagged with plastic beads inserted at the base of the dorsal fin, following a method described by Patzner (1984), a system successfully used in this species (Santos, 1986). Since it was difficult to capture nest-holders without disturbing the nests, most nest-holder males were individually recognized using a combination of individual marks (e.g., scars) and relative size differences. Nests were mapped and observed daily during both periods of the field study.

Two types of behavioral sampling methods (*sensu* Martin and Bateson, 1993) were used: (a) focal observations, each lasting 20 min, with an average of three observations per fish, and totaling 79 h and 40 min on

68 individuals (42 M+ males and 26 M− males); and (b) scan observations at each minute during the 20 min of the focal observation to record the identity of fish present in the area around each nest. Observations were carried out between 0933 and 1936 h. For each focal observation the following variables were recorded: number of visits by conspecifics, the categorical identity of the visitor (i.e., nest-holder, floater, sneaker male, satellite male, or female), and details of all social interactions involving the nest-holding male, including agonistic and courtship behaviors given and received and the identity of the participants. Feeding and egg fanning by the nest-holders were also recorded. The available ethogram for this species was followed (Santos and Barreiros, 1993).

Hormone Assays

Plasma samples (5–50 μl) were extracted for free and conjugated steroids (Canario and Scott, 1989; Scott and Canario, 1992), and the extracts were redissolved in phospate buffer 0.1 M, pH 7.6, containing gelatine ($1\ g \times L^{-1}$). Each steroid fraction (i.e., free, sulfate, and glucuronide) was assayed separately by specific radioimmunoassay (RIA). Specificity tables for the assays used in this study have been published elsewhere: testosterone (T) (Scott *et al.,* 1984) and 11-ketotestosterone (KT) (Kime and Manning, 1982). Depending on the volume of plasma sampled, the limit of detection of the RIA's was 200–1600 pg/ml. Intraassay and interassay coefficients of variation were, respectively, 7.5 and 12.4% for T and 8.2 and 11.6% for KT.

Total plasma hormone concentrations for each steroid were obtained by adding values obtained for each fraction. An index of the relative abundance of KT (RA-KT) was calculated as the concentration of KT over the total concentration of the measured androgens (i.e., concentration of KT + concentration of T). This index is intended as a tentative measure of T converted into KT through the biosynthetic pathway T $\rightarrow$ 11-β-hydroxytestosterone $\rightarrow$ KT.

For all statistical procedures, the software package Statistica for Windows v. 5.0 (Statsoft, Inc., Tulsa, OK) was used. Androgen blood level data were transformed (log) to fit the parametric statistic assumptions of homogeneity of variances and normality. The comparison of androgen levels among different sexual morphotypes (i.e., the two male morphs and females) and among males following different reproductive tactics was performed using ANCOVA to control for interannual variations (1998 vs 1999) in steroid levels.

RESULTS

Androgen Correlates of Male Polymorphism and Sex

M+ males did not differ from M− males or from females in T levels except for the proportion of the free T fraction relative to the conjugated fraction (% free T), which was significantly higher in M− males than in either females or M+ males (HSD post hoc test, $P < 0.05$; Table 1). Levels of free KT, KT glucuronides, and total KT were higher in M+ males than in M− males (Table 1). There were no significant differences among male morphotypes in the proportion of free KT to conjugates (% free KT) and in the concentration of KT sulfates (Table 1). The RA-KT was also significantly higher in M+ males than in M− males (Table 1).

Androgen Correlates of Male Alternative Reproductive Tactics

For the subset of males for which blood samples and behavioral observations were available, potential differences among males following different behavioral mating tactics, namely nest-holding and floating among M+ males and satellite and sneaker among M− males, were investigated (Fig. 1). There were no significant differences in T levels among males following different male reproductive tactics (Table 2). Nest-holder males and floaters had higher blood levels of free and total KT and a higher RA-KT than satellite and sneaker males (HSD post hoc test, $P < 0.05$: nest-holders = floaters > satellites = sneakers, Table 2). KT glucuronide plasma concentrations were significantly higher in nest-holders and floaters than in sneakers, and satellites had intermediate levels not significantly different from nest-holders or floaters and sneaker levels (HSD post hoc test, $P < 0.05$, Table 2).

Morphological Correlates of Male Alternative Reproductive Tactics

Males adopting different reproductive tactics differ in some morphological traits (Table 3). Nest-holders are

TABLE 1
Comparison of Androgen Plasma Concentrations (mean ± SE) among Sex Morphotypes: M+ Males, M− Males and Females

	M+ Males	M− Males	Females	ANCOVA *F*
Free T	2.24 ± 0.61 (17)	3.47 ± 0.60 (22)	0.99 ± 0.28 (6)	1.61
T sulfates	7.33 ± 2.83 (17)	4.11 ± 0.88 (22)	2.47 ± 0.11 (6)	1.66
T glucuronides	2.03 ± 0.19 (17)	2.05 ± 0.26 (22)	2.08 ± 0.31 (6)	0.10
Total T	11.6 ± 3.0 (17)	9.63 ± 1.0 (22)	5.55 ± 0.41 (6)	0.76
% Free T	18 ± 4 (17)	36 ± 5 (22)	17 ± 5 (6)	4.1*
Free KT	2.42 ± 0.89 (17)	0.60 ± 0.06 (25)	—	21.1***
KT sulfates	0.85 ± 0.23 (17)	1.48 ± 1.03 (25)	—	1.16
KT glucuronides	1.28 ± 0.14 (17)	0.45 ± 0.08 (25)	—	32.6***
Total KT	4.54 ± 1.06 (17)	2.53 ± 1.05 (25)	—	19.6***
% Free KT	41 ± 5 (17)	38 ± 5 (25)	—	0.09
RA-KT (free)	0.31 ± 0.03 (17)	0.16 ± 0.03 (22)	—	12.7**
RA-KT (total)	0.52 ± 0.05 (17)	0.21 ± 0.04 (22)	—	22.2***

Note. All hormone concentrations are given in ng / ml. Hormone concentrations were log-transformed before analysis. ANCOVA was used to control for interannual variations in steroid levels. Sample sizes for each morphotype are given in parentheses. Significance levels are $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

larger than floaters, which in turn are still significantly larger than both satellites and sneakers (Table 3). Both nest-holder and floater males have significantly lower GSI values than either satellite or sneaker males (Table 3). Nest-holder males have lower hepatosomatic indexes than the other males (Table 3). Nest-holders and floaters have larger testicular glands than satellites, with sneakers presenting intermediate values (Table 3). There are no differences among the different male classes in condition factor (Table 3). The size of the genital papillae is larger in nest-holders than in floaters and satellites, with sneakers presenting even smaller values (Table 3). The anal glands are larger in nest-holder males than in any other male type, with floaters also having larger glands than either satellites or sneakers (Table 3).

Relationship between Androgen Levels and Male Morphological Traits

Table 4 shows correlation coefficients between androgen levels and male morphological traits and illustrates the following: (a) Free KT levels were positively

TABLE 2
Comparison of Androgen Plasma Concentrations (mean ± SE) among Alternative Reproductive Tactics: Nest-Holder Males, Floater Males, Satellite Males, and Sneaker Males

	Nest-holder	Floater	Satellite	Sneaker	ANCOVA *F*
Free T	2.18 ± 0.83 (11)	0.99 ± 0.49 (4)	3.02 ± 1.49 (6)	2.71 ± 0.85 (5)	1.35
T sulfates	8.19 ± 4.25 (11)	2.23 ± 0.34 (4)	2.52 ± 0.09 (6)	3.31 ± 0.56 (5)	2.01
T glucuronides	1.88 ± 0.18 (11)	2.24 ± 0.64 (4)	2.15 ± 0.43 (6)	2.32 ± 0.9 (5)	0.51
Total T	12.26 ± 4.43 (11)	5.89 ± 0.80 (4)	7.69 ± 1.51 (6)	8.34 ± 1.56 (5)	1.04
% Free T	17 ± 5 (11)	14 ± 7 (4)	30 ± 11 (6)	29 ± 6 (5)	2.01
Free KT	2.1 ± 1.14 (11) a	1.43 ± 0.31 (4) a	0.42 ± 0.11 (7) b	0.48 ± 0.11 (5) b	5.05**
KT sulfates	0.95 ± 0.35 (11)	0.49 ± 0.21 (4)	0.23 ± 0.05 (7)	0.48 ± 0.12 (5)	1.41
KT glucuronides	1.24 ± 0.19 (11) a	1.17 ± 0.34 (4) a	0.60 ± 0.25 (7) ab	0.40 ± 0.11 (5) b	7.41***
Total KT	4.29 ± 1.40 (11) a	3.35 ± 0.31 (4) a	1.25 ± 0.27 (7) b	1.36 ± 0.27 (5) b	8.9***
% Free KT	35 ± 6 (11)	35 ± 7 (4)	35 ± 6 (7)	33 ± 7 (5)	0.04
RA-KT (free)	0.30 ± 0.04 (11) a	0.37 ± 0.04 (4) a	0.14 ± 0.03 (6) b	0.13 ± 0.01 (5) b	6.67***
RA-KT (total)	0.49 ± 0.07 (11) a	0.62 ± 0.10 (4) a	0.19 ± 0.05 (6) b	0.15 ± 0.01 (5) b	9.57***

Note. All hormone concentrations are given in ng / ml. Hormone concentrations were log-transformed before analysis. ANCOVA was used to control for interannual variations in steroid levels. Sample sizes for each morphotype are given in parentheses. Significance levels are $P < 0.01$ (**) and $P < 0.001$ (***). Significant differences among groups (HSD post hoc test, $P < 0.05$) are indicated by different letters.

TABLE 3
Comparison of Morphological Traits (mean ± SE) among Alternative Reproductive Tactics: Nest-Holder Males, Floater Males, Satellite Males, and Sneaker Males

	Nest-holder	Floater	Satellite	Sneaker	Kruskall–Wallis ANOVA
Standard length (cm)	12.1 ± 3.6 (11) a	10.1 ± 4.1 (5) b	7.9 ± 4.4 (7) c	8.4 ± 5.6 (7) c	20.9***
Head hump size (head height/ body height)	2.6 ± 0.1 (4) a	1.7 ± 0 (2) b	1.7 ± 0.4 (2) b	1.4 ± 0.2 (3) b	7.8*
Anal gland 1 (mm^2)	16.4 ± 1.8 (11) a	8.2 ± 2.2 (6) b	0.9 ± 0.2 (7) c	1.1 ± 0.2 (6) c	20.6***
Anal gland 2 (mm^2)	13.2 ± 1.4 (11) a	6.1 ± 2.1 (6) b	1.0 ± 0.4 (7) c	1.1 ± 0.2 (6) c	18.7***
Genital papillae (mm^2)	11.2 ± 1.4 (11) a	5.4 ± 0.6 (5) b	3.7 ± 1.6 (6) bc	2.6 ± 0.6 (6) c	17.3***
HSI (% body weight)	1.0 ± 0.6 (11) a	1.5 ± 0.2 (6) b	1.6 ± 0.2 (7) b	1.6 ± 0.2 (6) b	10.7*
K (weight/SL^3)	1.3 ± 0.1 (11)	1.4 ± 0.1 (6)	1.5 ± 0.1 (7)	1.5 ± 0.1 (7)	5.5
GSI (% body weight)	0.9 ± 0.1 (11) a	1.35 ± 0.3 (6) a	4.7 ± 0.2 (7) b	4.2 ± 1.1 (6) b	20.4***
TGI (% gonad area)	26 ± 3.2 (10) a	30.6 ± 7.0 (5) a	5.6 ± 1.5 (6) b	18.5 ± 9.5 (5) ab	13.5**

Note. ANOVA was used to test differences among male types. Sample sizes for each ethotype are given in parentheses. Significance levels are $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***). Significant differences among groups (HSD post hoc test, $P < 0.05$) are indicated by different letters. SL, standard length.

correlated to body size. (b) When controlling for body size, KT levels were positively correlated with the size of the anal glands and the size of the genital papillae. Larger correlations were obtained with total KT concentrations than with free KT. (c) When controlling for body size, there were no significant correlations between any of the androgens and head hump size, which is a sexually dimorphic trait. (d) Free T levels were positively correlated to gonadosomatic and hepatosomatic indices and marginally not correlated to condition factor. (e) Total T concentrations were significantly correlated only with the size of the genital papillae. It was also found that GSI and TGI were negatively correlated ($r = -0.64$, $n = 37$, $P < 0.001$), indicating that individuals with larger gonads have relatively smaller testicular glands.

Relationship between Androgen Levels and Male Reproductive Behavior

In nest-holder males, androgen levels were negatively correlated with the frequency of female visits to the nest and spawning episodes. Whereas only total T was negatively correlated with the frequency of female visits, very high negative correlations were found between free and total KT and the frequency of female spawnings received in the nest of a nest-holder subject (Table 5). There was no association between nest-holder androgen levels and courtship acts received from the females. Neither were there significant correlations between androgen levels of nest-

TABLE 4
Correlation Coefficients between Androgen Levels and Morphological Variables

	Free T	Total T	Free KT	Total KT
Standard length	−0.08 (40)	0.20 (40)	0.41** (43)	0.21 (43)
Head hump	−0.36 (20)	−0.04 (20)	0.22 (20)	0.24 (20)
Anal gland 1	0.12 (38)	0.31* (38)	0.48*** (41)	0.58*** (41)
Anal gland 2	0.13 (38)	0.33* (38)	0.51*** (41)	0.60*** (41)
Genital papillae	0.23 (38)	0.44** (38)	0.54*** (41)	0.63*** (41)
HSI	0.38* (37)	−0.11 (37)	−0.08 (40)	−0.14 (40)
K	$0.28^{P=0.08}$ (40)	0.22 (40)	−0.03 (44)	0.18 (44)
GSI	0.32* (37)	−0.03 (37)	−0.23 (40)	−0.32* (40)
TGI	−0.29 (31)	−0.03 (31)	0.11 (31)	0.22 (31)

Note. To control for spurious effects of body size, partial correlation coefficients were used for all pairs of variables that involve head hump, anal glands 1 and 2, and genital papillae size. Sample sizes are indicated in parentheses. Significance levels are $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

TABLE 5
Spearman Correlation Coefficients between Androgen Levels and Behavioral Variables in Nest-Holder Males ($N = 11$)

	Free T	Total T	Free KT	Total KT	RA − KT
Received behaviors					
Female visits	−0.06$^{P=0.06}$	−0.67*	0.13	−0.22	0.69*
Female courtship	0.29	0.39	−0.21	−0.17	−0.02
Female spawning	0.09	0.13	−0.82**	−0.83**	−0.37
Performed behaviors					
Courtship	0.06	−0.06	0.006	0.03	−0.07
Agonistic behavior	−0.50	−0.67*	−0.17	−0.46	0.14
Egg fanning	−0.09	0.12	−0.65*	−0.62$^{P=0.06}$	0.07
Feeding	0.22	0.24	0.20	0.38	−0.06

Note. Significance levels are $P < 0.05$ (*) and $P < 0.01$ (**).

holders and male agonistic or courtship behavior, except for a negative correlation between total T levels and male agonistic behavior (Table 5). There was also a significant negative correlation between egg fanning behavior performed by nest-holders and both free and total KT levels (Table 5). If nest-holder males that were observed to receive female spawnings in their nests are compared with nest-holder males that failed to mate during the observation period, successful males had significantly lower levels of free and total KT levels (Table 6). Moreover, successful males also expressed significantly higher levels of egg fanning behavior (Table 6). There were no differences in androgen levels between nest-holder males with and without associated satellites (Table 7).

TABLE 6
Differences in Androgen Levels and Egg Fanning Behavior in Nest-Holder Males That Received (i.e., Successful Males) or Did Not Receive (i.e., Unsuccessful Males) Spawnings in Their Nests

	Successful males ($N = 4$)	Unsuccessful males ($N = 7$)	Mann–Whitney test (Z)
Free T	1.38 ± 0.74	2.80 ± 1.20	0
Total T	8.14 ± 2.71	14.97 ± 6.76	−0.19
Free KT	0.58 ± 0.11	3.30 ± 1.68	−2.65**
Total KT	2.14 ± 0.15	5.95 ± 2.00	−2.07*
RA − KT	0.24 ± 0.04	0.35 ± 0.06	−0.94
Egg fanning (acts/h)	28.28 ± 15.41	2.71 ± 2.10	−2.08*

Note. All hormone concentrations are given in ng/ml. Significance levels are $P < 0.05$ (*) and $P < 0.01$ (**).

DISCUSSION

This study has established that blood plasma levels of KT provide a good androgen correlate of male polymorphism. There is a positive association of higher KT levels with the male morphotype actively competing for access to mates (i.e., M+ males), and this is suggestive of an activational role for KT mediating (plasticity hypothesis of Moore, 1991) the transition of M− males to M+ males. The competition for the monopolization of mates by M+ (i.e., bourgeois males *sensu* Taborsky, 1997) males usually involves investment in SSC, expression of reproductive aggression (e.g., breeding territoriality), and courtship behavior. Of these M+ male displaying traits, only the sizes of the anal gland (1st and 2nd anal fin ray) and the genital papillae, but not the mating behaviors, were highly correlated with KT blood concentrations.

TABLE 7
Differences in Androgen Levels in Nest-Holder Males with and without an Associated Satellite Male

	With an associated satellite male ($N = 6$)	Without an associated satellite male ($N = 5$)	Mann–Whitney test (Z)
Free T	3.21 ± 1.38	0.96 ± 0.45	−0.73
Total T	9.99 ± 2.44	14.9 ± 9.8	0
Free KT	3.16 ± 2.06	0.83 ± 0.19	−0.55
Total KT	5.65 ± 2.47	2.66 ± 0.63	−0.91
RA − KT	0.34 ± 0.06	0.24 ± 0.04	−1.09

Note. All hormone concentrations are given in ng/ml.

Interestingly, head hump size, which is a sexually dimorphic trait (unpublished data), does not show a significant correlation with either KT or T levels when body size is taken into account. This result, together with the fact that nest-holders present higher head humps than the rest of the male types, suggests that nest acquisition might induce the development of the head hump, which could function as a badge of status. Thus, the fact that no correlation has been found between androgen levels and head hump size does not prove that this is not an androgen-dependent character. Also, no clear differences were found both in KT and in T circulating concentrations among different mating tactics within the same male morph; that is nest-holder M+ males did not differ in androgen levels (or in RA-KT) from floaters, and satellites did not differ from sneakers. Available evidence from other species from different teleost families (e.g., Salmonidae, Labridae, Centrarchidae, Batrachoididae) with alternative mating tactics (see review by Brantley *et al.*, 1993) also point, as in the present study, to higher levels of KT in bourgeois than in parasitic males. Furthermore, all those species have alternative reproductive tactics that result in the expression of male polymorphisms. In contrast to what happens for KT, a clear pattern for T is not found in the available literature (Brantley *et al.*, 1993), with bourgeois males having higher levels in some species, lower levels in others, and not significantly different concentrations than parasitic males in even other species. Another possibility is that observed differences in KT among male morphotypes may result from different social stimuli experienced by the two male morphotypes but not by males differing in behavioral tactics within the same morphotype. In fact it has been shown for a number of vertebrates (see Wingfield *et al.*, 2000) including fish (e.g., Cardwell and Liley, 1991; Oliveira *et al.*, 1996) that androgen levels are very sensitive to the social environment in which the animal lives, especially to social challenges. However, since nest-holders and their respective satellite males are exposed to the same number of territorial intrusions and satellites are more involved in territorial defence than the nest-holders themselves (i.e., satellites have a significantly higher share of territorial defence than their respective nest-holder; Santos, 1985), this possibility is highly unlikely. The dependency of male morphotypes on androgen is further supported by observations in the cichlid *Oreochromis mossambicus*, in which alternative mating tactics are expressed by the same male morphotype. The dominant courting males have higher urinary concentrations of KT and T than subordinate males that adopt pseudo-female behavior and try to achieve sneaker fertilizations (Oliveira *et al.*, 1996; Oliveira and Almada, 1998a). The dominant males of tilapia also show a higher expression of SSC (although there is no discontinuous trait distribution that would suggest the occurrence of male polymorphism) and territorial behavior and display a color pattern different from that of subordinates (Oliveira and Almada, 1998b), which indicates that, although the adoption of a given mating tactic may be driven by social (conditional) factors, the expression of male sexual morphotypes is largely androgen dependent. Further experimental studies in *P. s. parvicornis* will be needed to clarify the causal relationship between the higher KT levels and the bourgeois tactic.

KT also appears negatively correlated with relative gonad mass, which results from the fact that bourgeois males, which have higher KT blood concentrations, have smaller GSI values than parasitic males, which have lower KT levels. The difference in relative gonadal investment in bourgeois and parasitic males may be explained by sperm competition theory (Taborsky, 1998). During spawning bourgeois males achieve a closer proximity to the females than parasitic males, and they can also control the timing of the spawning (Taborsky, 1998). Thus, bourgeois males need less sperm than parasitic males to successfully fertilize the eggs. This result does not necessarily mean that KT is negatively associated with gonadal investment. In fact, in blenniids seasonal variation of GSI is positively correlated with the seasonal variation in KT levels (R. F. Oliveira and A. V. M. Canário, unpublished data for *Lipophrys pholis*), which suggests that KT may in fact play a role in gonadal investment in this family. On the other hand, the positive association of KT with the expression of SSC suggests that KT may be playing a major role in the expression of SSC during the breeding season.

T appears positively associated with relative gonad mass and less associated than KT with the gonadosomatic index, suggesting different roles for the two steroids, with T potentially being less involved in the expression of SSC and more involved in gonadal investment. Moreover, T also shows a positive correla-

tion with the relative size of the liver, which in blenniids is an organ in which fat reserves are deposited before the breeding season (Podroschko *et al.*, 1985) and which are subsequently consumed during the breeding period, when nest-holder males reduce feeding and lose weight (Santos *et al.*, 1996). Thus, T may also be involved in resource allocation for reproduction at a wider organismal level by promoting the deposit of fat reserves in the liver. It is interesting to note that the hepatosomatic index is significantly lower in nest-holders than in the other male types, which may reflect a reproductive cost of this tactic, since nest-holders have fewer opportunities for feeding than other breeding males and thus the depletion of the liver fat reserves may be more acute. Similar results have been shown for another blenniid in which nest-holders also had lower relative liver mass and condition factor values (Gonçalves and Almada, 1997). Curiously, in *P. s. parvicornis* the physical condition factor does not show significant differences among male types, which suggests that, although nest-holders suffer a depletion of their liver fat reserves, they manage to keep their physical condition during the breeding season.

Morphometric data show that nest-holders are the largest males and that floaters are significantly larger than either satellites or sneakers. Nest-holder males also show the highest expression of the anal glands, with floater males having larger glands than the M− male types (i.e., satellites and sneakers). The genital papillae are also largest in nest-holders and larger in floaters than in sneakers, with intermediate values in satellites. The testicular gland is larger in both M+ male types (i.e., nest-holders and floaters) than in satellites and has intermediate values in sneakers. In general, these results conform to the described differences (Santos *et al.*, 1996) between M+ and M− males regarding the above morphological traits.

The concentration of total (free + sulfate + glucuronide fractions) androgen gives higher correlation coefficients with SSC than when any of the steroid fractions are correlated separately. Similarly, when comparing steroid levels between sex morphotypes (Table 1) and alternative mating tactics (Table 2), higher significance probabilities are obtained in ANCOVA with total androgen levels. Although conjugated steroids are considered to be generally physiologically inactive (Kime, 1993), these results suggest that total steroid concentrations are more closely correlated with biological activity than the generally used free steroid concentrations. A possible explanation for these results could be that the active steroid environment is controlled not only by the dynamics of secretion in glandular tissue but also by conjugation / deconjugation in target tissues (Cuevas *et al.*, 1992). Differences in clearance rates for the various steroid fractions could also contribute to lower correlations when the various fractions are analyzed separately (Parks and Le Blanc, 1998). However, the role of conjugation in steroid function in physiology and behavior in fishes requires further investigation.

In contrast with the positive correlations of androgens to SSC, there were no significant correlations between androgen levels and behavior in nest-holders, except for a negative correlation between total androgen levels and the expression of agonistic behavior. Moreover, there was a negative association between androgen levels in nest-holders and success in attracting females to spawn, as indicated by the frequency of female visits to each males' nest (negatively correlated with T levels) and by the number of spawnings per nest (negatively correlated with KT levels). This is a surprising result, considering the known role of androgens on the expression of sexually selected traits such as SSC and courtship behavior (Borg, 1994) and the positive correlations of androgens with SSC found in the present study. A likely explanation for these results is that the collection of blood samples did not coincide with the occurrence of the behaviors that we were attempting to correlate. Blood samples were collected only at the end of a 1-month period of behavioral observations on focal nest-holder males, to avoid disturbing them. At this stage successful males were actively caring for eggs and their lower levels of KT could be attributed to the well-established negative relationship between androgens and parental care (e.g., Knapp *et al.*, 1999).

Although satellite males share the defence of the breeding territory, nest-holder males with an associated satellite did not differ in androgen levels from nest-holders without an associated satellite. This result may indicate that, although nest-holder males with an associated satellite are challenged less often by other male intruders (R. F. Oliveira, unpublished data), the presence of the satellite male is still stimulating the endocrine system of the nest-holder male.

In conclusion, whereas androgen levels, KT in particular, show a clear pattern among male morphotypes and are correlated with SSC and specific behaviors, there are no differences in androgen levels among males performing alternative tactics within a morphotype.

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