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Androgen levels and social interactions in breeding males of the peacock blenny

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Nest-holder male *Salaria pavo* have lower circulating concentrations of 11-ketotestosterone (KT) at the beginning of the breeding season than at its peak. At that peak density of nesting males was higher as were the number of visits of sneaker males to nests and of agonistic interactions between nest-holders and sneaker males. There was no difference between the two dates either in the frequency of male–male interactions or in the frequency of courtship episodes. Thus, higher plasma levels in nest-holders might be explained by a more intense sneaking pressure at the peak of the breeding season. At that peak, nest-holders had higher plasma levels of KT and a higher testosterone (T) to KT metabolization index in the gonads than did floater males. Both nest-holders and floaters had higher levels of KT and T in the testicular gland than in the testis. The levels of both androgens in the testicular gland, but not in the testis, were correlated with circulating concentrations of KT. These results suggest that the testicular gland is the major source of circulating KT in blenniids. Nest-holders had higher metabolization indexes than floaters both in the testis and in the testicular gland, which suggests that nest holding status promotes the conversion of T into KT.

Key words: challenge hypothesis; paternal care; androgens; 11-ketotestosterone; teleosts.

INTRODUCTION

Androgens play an important role in the maturation of the gonads, the development of secondary sex characters and the expression of reproductive behaviour in male teleosts (Liley & Stacey, 1983; Borg, 1994). Testosterone (T) and 11-ketotestosterone (KT) are the major androgens (Kime, 1993). Circulating plasma levels of KT are always higher in males whereas females in most species have similar to higher levels of T (Borg, 1994). Moreover, KT is usually the androgen found at the highest plasma concentrations in males during the breeding season. Furthermore, when tested, KT had more pronounced effects than T on the differentiation of secondary sex characters [e.g. sonic motor system

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in the plain midshipman *Porichthys notatus* Girard 1854 (Brantley *et al.*, 1993*a*) kidney hypertrophy in the three-spined stickleback *Gasterosteus aculeatus* L. 1758 (Borg *et al.*, 1993)] and on the expression of male reproductive behaviour [i.e. territoriality, nest construction and courtship in *G. aculeatus* (Borg, 1987); pre-spawning behaviour and parental anti-predator defence in the bluegill *Lepomis macrochirus* Rafinesque, 1819 (Kindler *et al.*, 1991)].

Recent studies have shown that the levels of sex steroids, including androgens, are influenced by the social environment. For example, the presence of ovulated females induces a rise in sex steroid and gonadotrophin (GtH) levels and an increase in milt production in male salmonids (Liley *et al.*, 1986, 1993; Rouger & Liley, 1993; Olsen & Liley, 1993). Chemical signals from the urine are perceived by the males' olfactory system triggering the neuroendocrine response (Liley *et al.*, 1993; Scott *et al.*, 1994). In the Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852), males are sensitive to the maturation stage of females, courting ovulated females more intensively (Silverman, 1978), an effect that also seems to be mediated by chemical signals emitted by receptive females (Falter & Dolisy, 1989). In this species, courtship is also accompanied by elevation of the levels of KT in males (Borges *et al.*, 1998).

Also agonistic interactions and male–male competition may induce an endocrine response in the participating individuals, especially in the case of the androgens. Levels of KT are elevated in terminal phase males of the stoplight parrotfish *Sparisoma viride* (Bonnaterre, 1788) subjected to experimental territorial intrusions (Cardwell & Liley, 1991), and are correlated with territorial density and the frequency of agonistic interactions in the demoiselle *Chromis dispilus* (Pankhurst & Barnett, 1993). In the Mozambique tilapia and in rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), dominant males have higher levels of both KT and T than do subordinate males (Liley & Kroon, 1995; Oliveira *et al.*, 1996). These results have been interpreted as an adaptation for the individuals to adjust their agonistic motivation to changes in their social environment. Thus, male–male interactions would stimulate the production of androgens and the levels of androgens would be a function of the stability of the social environment in which the animal is placed. This hypothesis was first proposed by Wingfield (1984) and is known as the challenge hypothesis (Wingfield *et al.*, 1987, 1990).

This paper investigates the relationships among circulating (KT) and gonadal androgen levels (T, KT) and social interactions in breeding males of the peacock blenny *Salaria pavo* (Risso, 1810).

During the breeding season, males of this species defend a territory in which they prepare a nest in a natural crevice. Males court females by signalling the location of the nest, circling and leading the females to it. Spawning occurs inside the nest, where adhesive eggs are deposited. Males also guard, clean and aerate the eggs until they hatch. Thus, this mating system is promiscuous with exclusive male parental care (Fishelson, 1963; Patzner *et al.*, 1986). There is a marked sexual dimorphism. Males are larger than females (Gonçalves *et al.*, 1996), exhibiting a variety of secondary sexual characters, especially during the breeding season: head crest, a pheromone-producing anal-gland in the first two rays of the anal fin and a typical nuptial colouration (Fishelson, 1963; Laumen *et al.*, 1974; Patzner *et al.*, 1986; Patzner & Seiwald, 1987).

Although *S. pavo* conforms to the basic blenniid breeding pattern (i.e. males guard eggs in a cavity where females spawn) the study population occurring at Ria Formosa (Southern Portugal) shows a number of particular features. Males form nest aggregations that are so dense that the territories are virtually absent (Almada *et al.*, 1994). At least at the peak of the breeding season, nest space becomes very scarce, becoming a limiting factor for female spawning which leads to sex role-reversal in courtship (Almada *et al.*, 1995). Indeed, at that time males, despite retaining the capacity to court when they have no eggs in the nest, assume a passive role in courtship. Females, on the other hand, compete aggressively among themselves, court nest-holder males actively and display a nuptial coloration (Almada *et al.*, 1995). Male sexual polymorphism occurs with some smaller and younger sexually active males acting as sneakers and lacking the typical male ornaments (Gonçalves *et al.*, 1996). The scarcity of nesting substrates in this population also leads to the occurrence of floaters among larger and older males, which are sexually mature but not able to establish successful nests.

In the present study, the plasma KT levels of nest-holding males were documented at the beginning and at the peak of the breeding season, when potentially these males were facing different social environments in terms of intrusion rates and courtship episodes. To assess this, interactions occurring at nests were observed, and the differences in plasma (KT) and gonadal (T, KT) androgen levels of nest-holding and floater males were investigated.

MATERIALS AND METHODS

STUDY AREA AND SAMPLING PROCEDURE

The study site was a coastal lagoon with permanent connection to the sea on the south Portuguese coast at Ria Formosa Natural Park (36°59′ N; 7°51′ W). Clam culturists use a number of artificial materials to delimit their clam fields such as tiles, stones, sand bags and bricks. *S. pavo* males use brick holes as nests, breeding from May to September (Almada *et al.*, 1994).

Male peacock blennies were collected during low tide early in May, at the beginning of the breeding season, and in late June, at the peak of the breeding season (mean nest surface covered by eggs: May=25%, June=60%; Oliveira *et al.*, 1999). Both samples were taken from the same location to avoid microhabitat variations that could influence the results. Since *S. pavo* has a special conservation status in Portugal (Instituto da Conservação da Natureza, 1993) sample size was kept to a minimum. Only eight males were sampled in May and 10 in June. In this population, alloparental care of eggs occurs and, when a nest-holder male is removed experimentally from its nest, floater males will take over and continue egg care (unpubl. data). In the June sample, males that took over vacant nests, from which the nest-holder had been removed deliberately in the previous day-time tide, were collected to compare steroid levels between nest-holder and males that took over the nests (i.e. former floater males hereafter named floaters). Thus, five nest-holders and five floaters were captured in June.

Since field conditions did not permit direct blood sampling immediately after capture, fish were transported alive to a field station <30 min away. Then males were anaesthetized in a bucket containing sea water and MS-222. For each individual, the following measurements were taken: weight (to the nearest 0·1 g), standard length, head height and body height (to the nearest 0·1 mm) taken at the insertion of the pectoral fins. Blood was collected within 30 min to 1 h after capture. Tails were severed and blood was allowed to drip into heparinized tubes. After centrifugation, plasma was separated and stored at −20° C until extraction. Gonads were dissected in individuals collected in June, separating testis from accessory organs, i.e. testicular gland (see Patzner &

Lahnsteiner, 1999 for a description of the male gonadal accessory organs of blennies). Testis and testicular gland (TG) were weighed and both structures were homogenized in 1 ml of saline teleost ringer and stored at − 20° C until extraction.

The following morphometric indexes were calculated: crest size=head height : body height; gonadosomatic index (I_G)=100*gonad weight (body weight)$^{-1}$; anal gland index (I_{AG})=100*anal gland weight (body weight)$^{-1}$; testicular gland index (I_{TG})=testicular gland area : testis area. TG and testis areas were calculated from dorsal view measurements of maximum width and length with the ocular micrometer of a binocular stereoscope, assuming testis and TG to have an approximate ellipsoidal shape.

BEHAVIOURAL OBSERVATIONS

Behavioural observations were conducted by snorkelling during high tide on the same days and locations where individuals were sampled during low tide. Since individuals were not tagged individually it was not possible to conduct behavioural observations on the males that were collected later and sampled for androgen hormones. For each nest focal observation, the following variables were recorded: number of visits and sex of the visitor (i.e. male, female or sneaker); number of agonistic interactions and identity of the participants (i.e. nest-holder *v*. male, female or sneaker); number of courtships and sex of the initiator (i.e. female or sneaker). Twenty-eight such nest focal observations of 20 min each were conducted in May and 17 in June. The recorded data on social interactions was used as an indicator of the social environment that the sampled individuals were facing in May and in June.

On the same low tides in which males were sampled, two transects (20 m each) were made along ridges delimiting the clam fields and all hard substrata found were inspected for fish. Sex was determined in each fish by inspection of the genital papillae and the cavity in which it was captured was checked for the presence of eggs. The proportion of breeding males in the population was calculated as the percentage of nest-holders (i.e. captured in a cavity with eggs) out of the total number of fish present in the transects (n=42 in May and n=64 in June). The percentage of nesting males was used as an indicator of nest density in the population in May and in June.

HORMONE ASSAYS

Plasma samples (5–50 µl) were extracted for free and conjugated steroids using described methods (Canário & Scott, 1989; Scott & Canário, 1992). Testis and TG homogenates were extracted first with butanol. After evaporation of the butanol, the residue was redissolved in 200 µl distilled water and free and conjugated steroids were extracted in the same way as the plasma samples. All extracts were redissolved in phosphate buffer 0·1 M, pH 7·6, containing gelatine (1 g l^{-1}) for RIA. Specificity tables have been published from the RIAs used in this study: T (Scott *et al*., 1984) and KT (Kime & Manning, 1982). The limit of detection of the RIAs was 200–1600 pg ml^{-1} for blood plasma (depending on plasma volume sampled) and 10 pg gland^{-1}. Intra-assay and interassay precision (coefficient of variation) were, respectively, 7·5 and 12·4% for T and 8·2% and 11·6% for KT.

STATISTICAL ANALYSIS

To examine differences in KT plasma concentrations, a planned comparisons test was used which controls the significance level for re-testing the same data set (e.g. nest-holders in June were tested twice: *v*. nest-holders in May and *v*. floaters in June) (Winer *et al*., 1991). The same test was used to compare body weight, standard length and head crest size.

The differences between the behavioural variables measured in May and in June were analysed using Mann–Whitney U-tests because the behavioural data did not meet the parametric assumptions.

An androgen metabolization index was computed as the concentration of KT over the total concentration of the measured androgens (i.e. concentration of KT+concentration of T). This index represents the relative amount of KT in each gonadal structure and is a tentative measure of T metabolization into KT as T is in the biosynthetic pathway of

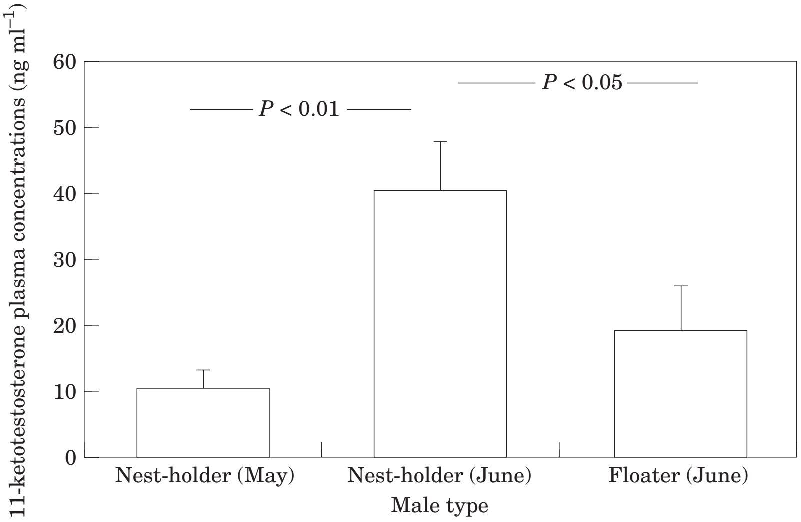

FIG. 1. Temporal variation (May and June) of the mean plasma concentrations of KT in nest-holders and the mean levels for floaters at the peak of the breeding season (June). Bars represent S.E.; P values refer to planned comparisons statistics.

KT. Although this index takes into account only one of the two KT biosynthetic pathways (i.e. androstenedione→T→11-β-hydroxy-testosterone→KT) it is the most meaningful one since it includes the two most active androgens in teleost fish.

Testosterone and KT levels and the metabolization index in the testis and in the testicular gland were compared between the two male types sampled in June using a two-way ANOVA. The two independent variables used were male type (nest-holder *v.* floater) and gonadal structure (testis *v.* testicular gland). ANOVA assumptions of normality and homogeneity of variances were checked by visual evaluation of normal probability plots and by the Levene's test respectively. Differences between nest-holders and floater males in June concerning the I_{G}, I_{TG} and I_{AG} were examined using t-tests after the data were checked for parametric assumptions. Statistics were computed using the software package Statistica for Windows v. 5.0 (copyright Statsoft, Inc., 1985).

RESULTS

TEMPORAL VARIATION OF 11-KETOTESTOSTERONE PLASMA CONCENTRATIONS AND SOCIAL INTERACTIONS

Nest-holder males had lower plasma concentrations of KT at the beginning of the breeding season than at its peak (Fig. 1). Furthermore, nest-holders had higher plasma concentrations of KT than floater males at the peak of the breeding season (Fig. 1).

There were no differences in standard length or body mass, either between nest-holders in May and in June or between nest-holders and floaters in June (Table I). However, nest-holders had higher head crests than floaters (Table I).

To investigate the relationship between the temporal variation in KT plasma levels and potential variations in the social environment in which the nest-holder males were collected, data are presented on a number of behavioural activities sampled at the beginning and at the peak of the breeding season (Table II). The percentage of nest-holder males (i.e. nest density) in the studied area increased significantly from the beginning to the peak of the breeding season (Table II).

TABLE I. Mean (± S.E.) values for body size (weight and L_S) and for the development of the head crest in the different male types (nest-holders at the beginning of the breeding season; nest-holders at the peak of the breeding season; and floaters at the peak of the breeding season)

	Nest-holders, May (NHM)	Nest-holders, June (NHJ)	Floaters, June (F)	Planned comparisons NHM *vs* NHJ ($F_{1,15}$, P)	Planned comparisons NHJ *vs* F ($F_{1,15}$, P)
n	8	5	5	—	—
Body weight (g)	16·5 ± 1·5	14·2 ± 0·9	11·5 ± 0·9	1·53, NS	1·64, NS
L_S (cm)	10·4 ± 0·3	9·9 ± 0·2	9·5 ± 0·4	0·86, NS	1·09, NS
Crest size (HH: BH)	1·47 ± 0·04	1·48 ± 0·06	1·31 ± 0·03	0·04, NS	6·65, $P<0·05$

TABLE II. Temporal variation in the social environment of nest-holders during the breeding season. Significance values refer to the Mann–Whitney U-test except for nest density in which case a test to compare two proportions was used. All the behavioural activities are expressed as a frequency (i.e. acts per 20 min per nest holder male)

	May (n=28 focal nests)	June (n=17 focal nests)	Significance
Nest density (% nest-holders)	38·1 n=42	73·4 n=64	$P<0·001$
Male visits	1·03 ± 0·25	1·12 ± 0·29	NS
Female visits	3·04 ± 0·63	4·21 ± 0·96	NS
Sneaker visits	2·13 ± 0·57	6·90 ± 1·47	$P<0·001$
Interactions with males	0·23 ± 0·11	0·28 ± 0·11	NS
Female courtship	2·41 ± 0·57	3·23 ± 1·14	NS
Sneaker courtship	0·04 ± 0·03	0·44 ± 0·36	NS
Interactions with sneakers	0·12 ± 0·07	0·68 ± 0·22	$P<0·01$

From the social interactions recorded, only the number of sneaker visits to nests and the number of nest-holder sneaker agonistic interactions were significantly different between the two periods considered, being higher in June (Table II). This suggests that nest-holders are more exposed to sneaking attempts during the peak of the breeding season.

GONADAL ANDROGEN LEVELS IN NEST-HOLDER AND FLOATER MALES

Floater males have lower gonado-somatic indexes than nest-holders (floaters: n=5, x ± S.E.M.=0·59 ± 0·08% *v*. nest-holders: n=5, x ± S.E.M.=0·94 ± 0·07%, *t*-test: t=3·16, P=0·01). Neither the development of the anal gland nor the testicular gland index were different between these two types of males (I_{AG}: nest-holders: n=5, x ± S.E.M.=0·2 ± 0·05% *v*. floaters: n=5, x ± S.E.M.=0·2 ± 0·006%, *t*-test: t=0·2, P=0·84; I_{TG}: nest-holders: n=5, x ± S.E.M.=12·6 ± 0·09% *v*. floaters: n=5, x ± S.E.M.=16·5 ± 0·10%, *t*-test: t= − 0·62, P=0·55). The concentrations of both androgens did not differ significantly between nest-holders and floaters,

TABLE III. Two-way analysis of variance to test the effects of male type (nest-holder *v.* floater) and gonadal structure (testis *v.* TG) on androgen concentrations (T and KT) and on KT metabolization index

	T ($F_{1,16}$, P)	KT ($F_{1,16}$, P)	Metabolization index ($F_{1,16}$, P)
Male type	0·14, NS	1·70, NS	17·1, $P<0·001$
Gonadal structure	20·13, $P<0·001$	23·4, $P<0·001$	2·62, NS
Interaction	0·05, NS	1·51, NS	0·59, NS

TABLE IV. Gonadal androgen levels (mean ± S.E.) in nest-holder and floater males

	Nest-holders ($n=5$)	Floaters ($n=5$)
T testis (ng g^{-1} tissue)	12·1 ± 1·6	15·4 ± 2·8
T TG (ng g^{-1} tissue)	109·3 ± 27·8	123·1 ± 36·1
KT testis (ng g^{-1} tissue)	5·3 ± 0·6	4·8 ± 1·1
KT TG (ng g^{-1} tissue)	41·5 ± 7·4	26·3 ± 9·3
Testis metabolization index KT: (KT+T)	30·7 ± 1·2	23·2 ± 2·7
TG metabolization index KT: (KT+T)	28·8 ± 2·8	17·8 ± 3·9

either in the testis or in the testicular gland (Tables III and Table IV). Although the metabolization rate of testosterone into 11-keto-testosterone, as expressed by the relative concentration of KT to T KT : (KT+T), was significantly higher in nest-holders than in floater males (Tables III and IV). The concentrations of both androgens were significantly higher in the testicular gland than in the testis (Tables III and IV) which suggests a major role for this gland as a gonadal source of androgens. Moreover, the plasma concentrations of KT are correlated significantly with both the T and KT levels in the testicular gland (T: $n=10$, $r=0·71$, $P=0·02$; KT: $n=10$, $r=0·68$, $P=0·03$) but not in the testis (T: $n=10$, $r=0·24$, $P=0·49$; KT: $n=10$, $r=0·39$, $P=0·26$).

DISCUSSION

Nest-holder males had higher plasma concentrations of KT at the peak of the breeding season than at its beginning. The density of nests in the breeding area and the number of sneaker male intrusions were also higher at the peak of the breeding season. At the peak of the breeding season, nest-holders had higher circulating levels of KT and a higher metabolization index than floaters.

The seasonal variation in nest-holder androgen levels can be explained either by endogenous variation of the reproductive state or by environmental variations such as temperature or the social context.

Since there were no significant differences in the nest-holder gonado-somatic index between May and June the variations in androgens levels between these two months cannot be explained clearly by variations in the baseline reproductive state. Temperature is known to affect androgen secretion also (Kime & Heyder, 1983; Manning & Kime, 1985). It can either promote or inhibit androgen secretion depending on the species. In cold water species such as *O. mykiss*, testosterone formation showed an optimum at low temperatures (Manning & Kime, 1985) whereas in warm water species, such as *O. moassambicus*, high temperatures were more effective in promoting androgen secretion (Kime & Hyder, 1983). The peacock blenny is a Mediterranean species that breeds in spring and summer and published results indicate that a combination of either a long photoperiod (20L : 4D) with low temperature (8° C) or a short day (6L : 18D) with high temperature (17° C) caused oogenesis and spermatogenesis to stop in individuals from a Mediterranean population (Papitsch *et al*., 1981). On the other hand, only conditions of both long photoperiod and high temperature were effective on the gonadal maturation of both males and females (Papitsch *et al*., 1981). Thus, its steroidogenesis is expected to respond positively to increased temperatures. A rise in water temperature was detected in the Ria Formosa study site from May 1997 to June 1997 (monthly averages: May=19·7° C; June=21·0° C). Thus, the possible effect of temperature to explain the present results cannot be ruled out but it seems very unlikely since the observed variation in water temperature (1·3° C) was much smaller than the effective temperature treatments reported in the literature (e.g. 7° C in *O. mossambicus*, Kime & Heyder, 1983).

According to the 'challenge' hypothesis proposed by Wingfield and co-workers, the variation in androgen levels should be associated more closely with the temporal patterns of aggressive behaviour than with changes in reproductive physiology (Wingfield, 1984; Wingfield *et al*., 1987, 1990). This hypothesis generates a number of predictions, two of which can be addressed with the present data.

Firstly, males that establish territories in more populated areas may have higher levels of androgens, since potentially they will be exposed to a higher number of agonistic interactions than males that breed in a less populated area (Ball & Wingfield, 1987; Beletsky *et al*., 1990, 1992; Pankhurst & Barnett, 1993). This prediction should be taken with caution since an increase in population density might be accompanied by mechanisms to avoid aggression. In breeding aggregations of *S. pavo* nest densities are so high that the territories are virtually absent (Almada *et al*., 1994). Although the nest density increased from the beginning of the breeding season to its peak, the number of agonistic interactions among males did not increase significantly between the two dates. Thus, the increase in nest density does not seem to be the causal explanation for the higher levels of androgens experienced by nest-holders at the peak of the breeding season. However, intrusions from sneakers did increase between the beginning and the peak of the breeding season. Thus, the elevated androgen levels in nest holders at the peak of the breeding season might be a response to this increased sneaking pressure. However, this suggestion should be taken with caution since blood could not be collected from the same males that were observed.

Secondly, during territorial establishment or during social status acquisition the levels of androgens should be higher than in the subsequent phase of territoriality when territories are already established. In the stoplight parrotfish territorial males have higher androgen levels (both T and KT) than bachelor males. However, in bachelor males which were allowed to establish territories, androgen concentrations after one week of territory establishment increased to levels above those found in established territorial males (Cardwell & Liley, 1991). Moreover, peaks of androgens could be induced also in established territorial males by experimental intrusions of other males. In the present study, nest-holders had higher levels of KT than floaters sampled one day after being allowed to establish a nest. This does not support the prediction from the 'challenge' hypothesis. However, this result may be explained by the fact that one day was not enough time for floaters to increase their androgen levels in response to nest holding. Thus, KT levels measured in floaters one day after allowing them to become nest-holders may be viewed as representative of the baseline levels of this male type. Also, floater males had smaller gonads than nest-holders and shorter head crests. Thus, the higher levels of androgens in established nest-holders may be viewed as playing a role in activating spermatogenesis and the development of the head crest. The head crest may function as a badge of status in this species since it did not vary in nest-holders along the breeding season but it shows a difference between established nest-holders and floaters that were given access to a nest. Thus, nest acquisition may be the key factor to the expression of the head crest and KT may be playing a role in activating this process. In fact, there is a large body of literature that shows that most secondary sex characters in teleost fishes are androgen dependent, and that KT is the most potent androgen in inducing the differentiation of these characters (e.g. sonic motor system in *Porichthys notatus*, Brantley *et al*., 1993*a*; median fins in *Betta splendens,* Leitz, 1987; gonopodium differentiation in Poecillids, Liley & Stacey, 1983).

Thus, the present data suggest a response of plasma levels of KT in *S. pavo* nest-holder males to the social challenges imposed by sneakers during the peak of the breeding season. They also show an association between androgen levels and social status in this species.

Blenniids have a testicular gland that is a gonadal accessory structure closely attached to the testis located in a ventral position (Patzner & Lahnsteiner, 1999). In *S. pavo* sperm has to pass through the testicular gland when travelling from the testis to the spermatic ducts and it has been proposed that the testicular gland is involved in sperm maturation (Lahnsteiner *et al*., 1990). It has been shown also that it has steroidogenic activity being the major source of steroids in *S. pavo* gonads (Reinboth & Becker, 1986; A. V. M. Canario, unpubl. data). In this species male alternative mating tactics occur with smaller and younger males in the population mimicking the behaviour and the nuptial colouration of females in order to sneak fertilization (Gonçalves *et al*., 1996). In species with male sexual polymorphism the bourgeois males have higher levels of KT than the parasitic morphotype (Brantley *et al*., 1996) which suggests they have lower levels of circulating KT which would allow them not to express the male secondary sex characters and to mimic female behaviour and colouration.

The metabolization index of T into KT was higher in nest-holders than in floater males both in the testis and in the testicular gland. A possible explanation for this result could be that a physiological consequence of nest-holding status would be to activate 11-β-hydroxlase leading to a higher conversion of T into

KT. In the Siamese fighting fish *Betta splendens* Regan 1910, keeping the fish at high densities inhibits territorial and reproductive behaviours and also blocks the conversion of T into 11-oxy-steroids (Leitz, 1987). Thus, a differential role for the two androgens in the control of social and reproductive behaviour in teleosts is a plausible hypothesis that needs further investigation.

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References

Almada, V. C., Gonçalves, E. J., Santos, A. J. & Baptista, C. (1994). Breeding ecology and nest aggregations in a population of *Salaria pavo* (Pisces: Blenniidae) in an area where nest sites are very scarce. *Journal of Fish Biology* **45,** 819–830.

Almada, V. C., Gonçalves, E. J., Oliveira, R. F. & Santos, A. J. (1995). Courting females: ecological constraints affect sex roles in a natural population of the blenniid fish *Salaria pavo. Animal Behaviour* **49,** 1125–1127.

Ball, G.F. & Wingfield, J. C. (1987). Changes in plasma levels of luteinizing hormone and sex steroid hormones in relation to multiple-broodedness and nest-site density in male starlings. *Physiological Zoology* **60,** 191–199.

Beletsky, L. D., Orians, G. H. & Wingfield, J. C. (1990). Steroid hormones in relation to territoriality, breeding density, and parental behavior in male yellow-headed black-birds. *Auk* **107,** 60–68.

Beletsky, L. D., Orians, G. H. & Wingfield, J. C. (1992). Year-to-year patterns of circulating levels of testosterone and corticosterone in relation to breeding density, experience, and reproductive success of the polygynous red-winged blackbird. *Hormones and Behavior* **26,** 420–432.

Borg, B. (1987). Stimulation of reproductive behaviour by aromatizable and non-aromatizable androgens in the male three-spined stickleback, *Gasterosteus aculeatus* L. In *Proceedings of the 5th Congress of European Ichthyologists* (Kullander, S. O. K. & Fernholm, B., eds), pp. 269–271. Stockholm: Swedish Museum of Natural History.

Borg, B. (1994). Androgens in teleost fishes. *Comparative Biochemistry and Physiology C* **109,** 219–245.

Borg, B., Antonopoulou, E., Andersson, E., Carlberg, T. & Mayer, I. (1993). Effectiveness of several androgens in stimulating kidney hypertrophy, a secondary sexual character, in castrated male three-spined stickleback, *Gasterosteus aculeatus. Canadian Journal of Zoology* **71,** 2327–2329.

Borges, R. A., Oliveira, R. F., Almada, V. C. & Canário, A. V. M. (1998). Short-term social modulation of 11-ketotestosterone urinary levels in males of the cichlid fish *Oreochromis mossambicus* during male-female interaction. *Acta Ethologica* **1,** 43–48.

Brantley, R. K., Marchaterre, M. A. & Bass, A. H. (1993*a*). Androgen effects on vocal muscle structure in a teleost fish with inter-sexual and intra-sexual dimorphism. *Journal of Morphology* **216,** 305–318.

Brantley, R. K., Wingfield, J. C. & Bass, A. H. (1993*b*). Sex steroid levels in *Porichthys notatus*, a fish with alternative reproductive tactics, and a review of the hormonal base for male dimorphism among teleost fishes. *Hormones and Behavior* **27,** 332–347.

Canario, A. V. M. & Scott, A. P. (1989). Conjugates of ovarian steroids, including 17a,20b-dihydroxy-4-pregnen-3-one (maturation-inducing steroid), accumulate in the urine of a marine teleost (plaice; *Pleuronectes platessa*). *Journal of Endocrinology* **127,** R1–R4.

Cardwell, J. R. & Liley, N. R. (1991). Androgen control of social status in males of a wild population of stoplight parrotfish, *Sparisoma viride* (Scaridae). *Hormones and Behavior* **25,** 1–18.

Falter, U. & Dolisy, D. (1989). The effect of female sexual pheromones on the behaviour of *Oreochromis niloticus, Oreochromis mossambicus* and hybrid males (Pisces, Cichlidae). *Koninklijk Museum voor Midden-Afrika, Annalen Zoologische Wetenschappen* **257,** 35–38.

Fishelson, L. (1963). Observations on littoral fishes of Israel. I. Behaviour of *Blennius pavo* Risso (Teleostei, Blenniidae). *Israel Journal of Zoology* **12,** 67–80.

Gonçalves, E. J., Almada, V. C., Oliveira, R. F. & Santos, A. J. (1996). Female mimicry as a mating tactic in males of the blennid fish *Salaria pavo. Journal of the Marine Biological Association of the United Kingdom* **76,** 529–538.

Instituto da Conservação da Natureza (1993). *Livro Vermelho dos Vertebrados de Portugal*, Vol. 3: *Peixes Marinhos e Estuarinos*. Lisboa: Instituto da Conservação da Natureza.

Kime, D. E. (1993). ‘ Classical ’ and ‘ non-classical ’ reproductive steroids in fish. *Reviews in Fish Biology and Fisheries* **3,** 160–180.

Kime, D. E. & Hyder, M. (1983). The effect of temperature and gonadotropin on testicular steroidogenesis in *Sarotherodon (Tilapia) mossambicus* in vitro. *General and Comparative Endocrinology* **50,** 105–115.

Kime, D. E. & Manning, N. J. (1982). Seasonal patterns of free and conjugated androgens in the brown trout *Salmo trutta. General and Comparative Endocrinology* **48,** 222–231.

Kindler, P. M., Bahr, J. M., Gross, M. R. & Philipp, D. P. (1991). Hormonal regulation of parental care behavior in nesting male bluegills: do the effects of bromocriptine suggest a role for prolactin? *Physiological Zoology* **64,** 310–332.

Lahnsteiner, F., Richtarski, U. & Patzner, R. A. (1990). Function of the testicular gland in two blenniid fishes, *Salaria* (=*Blennius*) *pavo* and *Lipophrys (=Blennius) dalmatinus* (Blenniidae, Teleostei) as revealed by electron microscopy and enzyme histochemistry. *Journal of Fish Biology* **37,** 85–97.

Laumen, J., Pern, U. & Blüm, V. (1974). Investigations on the function and hormonal regulations of the anal appendices in *Blennius pavo. Journal of Experimental Zoology* **190,** 47–56.

Leitz, T. (1987). Social control of testicular steroidogenic capacities in the Siamese fighting fish *Betta splendens* Regan. *Journal of Experimental Zoology* **244,** 473–478.

Liley, N. R. & Kroon, F. J. (1995). Male dominance, plasma hormone concentrations, and availability of milt in male rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Zoology* **73,** 826–836.

Liley, N. R. & Stacey, N. E. (1983). Hormones, pheromones and reproductive behaviour. In *Fish Physiology*, Vol. 9B (Hoar, W. S., Randall, D. J. & Donaldson, E. M., eds), pp. 1–63. New York: Academic Press.

Liley, N. R., Breton, B., Fostier, A. & Tan, E. S. P. (1986). Endocrine changes associated with spawning behaviour and social stimuli in a wild population of rainbow trout (*Salmo gairdneri*) 1. Males. *General and Comparative Endocrinology* **62,** 145–156.

Liley, N. R., Olsén, K. H., Foote, C. J. & van der Kraak, G. J. (1993). Endocrine changes cassociated with spawning behaviour in male kokanee salmon (*Oncorhynchus nerka*) and the effects of anosmia. *Hormones and Behavior* **27,** 470–487.

Manning, N. J. & Kime, D. E. (1985). The effect of temperature on testicular steroid production in the rainbow trout, *Salmo gairdneri*, in vivo and in vitro. *General and Comparative Endocrinology* **57,** 377–382.

Oliveira, R. F., Almada, V. C. & Canario, A. V. M. (1996). Social modulation of sex steroid concentrations in the urine of male cichlid fish *Oreochromis mossambicus. Hormones and Behaviour* **30,** 2–12.

Oliveira, R. F., Almada, V. C., Forsgren, E. & Gonçalves, E. J. (1999). Temporal variation in male traits, nesting aggregations and mating success in the peacock blenny. *Journal of Fish Biology* **54,** 499–512.

Olsen, K. H. & Liley, N. R. (1993). The significance of olfaction and social cues in milt availability, sexual hormone status, and spawning behavior of male rainbow trout (*Oncorhynchus mykiss*). *General and Comparative Endocrinology* **89,** 107–118.

Pankhurst, N. W. & Barnett, C. W. (1993). Relationship of population density, territorial interaction and plasma levels of gonadal steroids in spawning male demoiselles *Chromis dispilus* (Pisces, Pomacentridae). *General and Comparative Endocrinology* **90,** 168–176.

Papitsch, M., Patzner, R. A. & Adam, H. (1981). Effects of photoperiod and temperature on gonadal maturation of *Blennius (=Salaria) pavo* (Teleostei, Blenniidae). *Vie et Milieu* **31,** 215–219.

Patzner, R. A. & Lahnsteiner, F. (1999). The accessory organs of the male reproductive system in Mediterranean blennies (Blenniidae) in comparison with those of other blenniid fishes (tropical Blenniidae, Tripterygiidae, Labrisomidae, Clinidae, Chaenopsidae, Dactyloscopidae). In *Behaviour and Conservation of Littoral Fishes* (Almada, V. C., Oliveria, R. F. & Gonçalves, E. J., eds), pp. 179–228. Lisboa: ISPA.

Patzner, R. A. & Seiwald, M. (1987). The reproduction of *Blennius pavo*. VII. Secondary sexual organs and accessory glands of the testis during the reproductive cycle. In *Proceedings of the V Congress of European Ichthyologists* (Kullander, S. & Fernholm, B., eds), pp. 293–298. Stockholm: Swedish Museum of Natural History.

Patzner, R. A., Seiwald, M., Adlgasser, M. & Kaurin, G. (1986). The reproduction of *Blennius pavo*. V. Reproductive behaviour in the natural environment. *Zoologischer Anzeiger* **216,** 338–350.

Reinboth, R. & Becker, B. (1986). In vitro metabolization of [^{14}C] testosterone by spermatogenic tissue and accessory gland of *Blennius pavo* (Blenniiformes, teleostei). *General Endocrinology* **5,** 11–16.

Rouger, Y. & Liley, N. R. (1993). The effect of social environment on plasma hormones and availability of milt in spawning male rainbow trout (*Oncorhynchus mykiss* Walbaum). *Canadian Journal of Zoology* **71,** 280–285.

Scott, A. P. & Canário, A. V. M. (1992). 17a,20b-Dihydroxy-4-pregnen-3-one 20-sulphate; a major new metabolite of the teleost oocyte maturation-inducing steroid. *General and Comparative Endocrinology* **85,** 91–100.

Scott, A. P., Mackenzie, D. S. & Stacey, N. E. (1984). Endocrine changes during natural spawning in the white sucker, *Catostomus commersoni*. II. Steroid hormones. *General and Comparative Endocrinology* **56,** 349–359.

Scott, A. P., Liley, N. R. & Vermeirssen, E. V. M. (1994). Urine of reproductively mature female rainbow trout, *Oncorhynchus mykiss* (Walbaum), contains a priming pheromone which enhances plasma levels of sex steroids and gonadotrophin II in males. *Journal of Fish Biology* **44,** 131–147.

Silverman, H. I. (1978). Changes in male courting frequency in pairs of the cichlid fish, *Sarotherodon (Tilapia) mossambicus*, with unlimited or with only visual contact. *Behavioural Biology* **23,** 189–196.

Winer, B., Brown, D. R. & Michels, K. M. (1991). *Statistical Principles in Experimental Design* 3rd edn. New York: McGraw-Hill.

Wingfield, J. C. (1984). Environmental and endocrine control of reproduction in the song sparrow, *Melospiza melodia*. II. Agonistic interactions as environmental information stimulating secretion of testosterone. *General and Comparative Endocrinology* **56,** 417–424.

Wingfield, J. C., Ball, G. F., Dufty, A. M. Jr, Hegner, R. E. & Ramenofsky, M. (1987). Testosterone and aggression in birds. *American Scientist* **75,** 602–608.

Wingfield, J. C., Hegner, R. E., Dufty, A. M. & Ball, G. F. (1990). The ‘ challenge hypothesis ’: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *American Naturalist* **136,** 829–846.