Hormones and Behavior **39,** 157–166 (2001)
doi:10.1006/hbeh.2001.1643, available online at http://www.idealibrary.com on IDEAL®

Effects of Androgens on Social Behavior and Morphology of Alternative Reproductive Males of the Azorean Rock-Pool Blenny

R. F. Oliveira,*,[1] L. A. Carneiro,* A. V. M. Canario,†
and M. S. Grober‡

**Unidade de Investigação em Eco-Etologia, Instituto Superior de Psicologia Aplicada, Rua Jardim do Tabaco 34, 1100 Lisbon, Portugal; †Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8000 Faro, Portugal; and ‡Life Sciences, Arizona State University West, 4701 West Thunderbird Road, Phoenix, Arizona 85069-7100*

Received August 30, 2000; revised November 20, 2000; accepted January 2, 2001

In the Azorean rock-pool blenny *Parablennius sanguinolentus parvicornis* two sequential reproductive tactics occur. Larger and older males establish breeding territories, while some of the smaller males become attached to nest-holder territories, acting as satellites on these territories, which they help to defend while trying parasitic fertilizations when females go in the nests to spawn. In the present paper we tested the effects of the androgens 17α-methyltestosterone (MT) and 11-ketotestosterone (KT) in the expression of male secondary sex characters and bourgeois behavior in satellite males. One week after satellites were implanted with Silastic tubes containing MT, KT, or castor oil (control), androgen-treated satellites had developed male secondary sex traits such as longer and wider male-type genital papilla and anal glands that secrete a sex pheromone, both traits being less expressed or absent, respectively, in satellite males. Androgen treatment had no effect on the gonadosomatic index or on the development of the testicular gland. KT treatment had a positive effect on relative liver weight. In terms of behavior, androgen-implanted individuals were less aggressive both in a mirror test and toward females when these were introduced into their tanks. MT-treated individuals spend more time inside the provided nests. Only androgen-implanted satellites managed to have the females entering their nests. When given a chance in a group tank either to try to attract females to their own nest or to act as satellites of an already established nest-holder's nest, MT-implanted males spent significantly more time in their own nest than near the nest-holder nest. These data suggest that androgens, particularly testosterone, may be involved in mating tactic switching in this species. © 2001 Academic Press

Within some species more than one behavioral tactic may be adopted by members of the same sex, usually males, to obtain access to females (Gross, 1996). These alternative reproductive tactics (ART) translate in many cases into male sexual polymorphism, with individuals following different tactics looking very distinct. For example, in some species a subset of breeding males may lack male secondary sexual characters (SSC) and behave like females while those in another subset develop SSC and male reproductive behavior, such as courting and/or breeding territoriality (e.g., peacock blenny *Salaria pavo*, Gonçalves, Almada, Oliveira, and Santos, 1996). These different tactics have been classified into bourgeois and parasitic. In bourgeois reproductive tactics males compete for the monopolization of mates while in parasitic tactics males take advantage of the success of the bourgeois males in attracting females (Taborsky, 1997). The occurrence of ART offers the opportunity to study the underlying endocrine mechanisms of reproductive behaviors, since in the parasite tactic the expression of behavioral and morphological male traits is dissociated from gonadal activity.

Moore (1991) has proposed the "relative plasticity hypothesis" to explain the role of hormones on within-sex differentiation of ART based on the classic activational–organizational model of hormone action (Phoenix, Goy, Gerall, and Young, 1959; Arnold and Breedlove, 1985). This hypothesis makes the distinc-

---

[1] To whom correspondence should be addressed. E-mail: ruiol@ispa.pt.

0018-506X/01 $35.00

tion between ARTs in which the individuals adopt one of the alternative morphs for their entire life time (fixed alternative phenotypes) from those in which individuals may switch from one tactic to the other (flexible alternative phenotypes). According to this hypothesis hormones would have an activational role in plastic alternative phenotypes and an organizational role in fixed alternative phenotypes. A first prediction of this hypothesis is that hormone profiles would differ in plastic adult morphs but not in fixed ones (Moore, 1991; Moore, Hews, and Knapp, 1998). A second prediction is that in flexible ART, hormone manipulations should be effective in adults but not during early development (i.e., would have an activational effect), while in fixed ART hormone manipulations should be effective during early development but not in adults; that is, hormones would have an organizational effect (Moore, 1991; Moore *et al.*, 1998). These predictions have been confirmed in several species from different phylogenetic groups. As an example of fixed alternative phenotypes, tree lizard (*Urosaurus ornatus*) males have a fixed dewlap color polymorphism and males of different throat color morphs use different reproductive tactics (Moore and Thompson, 1990). Orange–blue males aggressively defend breeding territories while orange males are nomadic and less aggressive (Hover, 1985). As predicted, early hormone manipulations influenced the adopted phenotype but hormone manipulations in adults failed to induce a change in morph behavior or morphology (Hews, Knapp, and Moore, 1994). Moreover, there were no differences in the circulating levels of testosterone or corticosterone between the two male morphotypes (Thompson and Moore, 1992). As an example of a species with plastic alternative phenotypes, the stoplight parrotfish (*Sparisoma viride*) is a hermaphroditic protogynic species in which some females change directly into terminal phase (TP), territorial, and brightly colored males, while others change into initial phase (IP), sneaker, and female mimic, males. IP males may later become TP males, concomitant with an increase in circulating 11-ketotestosterone (KT) levels (Cardwell and Liley, 1991). Although no hormone manipulations were performed on initial phase males, females injected with KT become TP males, developing the typical male bright coloration and testes. Other examples can be found in the review by Moore (1991), who concluded that the majority of available data conform to the relative plasticity hypothesis, although most of the data available consisted of hormone levels measured in the field and there was a deficit of hormone manipulation studies.

The objective of the present study was to test the effect of androgen manipulations in satellite males of a species with flexible alternative phenotypes, the Azorean rock-pool blenny (*Parablennius sanguinolentus parvicornis*). In this species, larger and older males (total length (CT) $\approx$14 cm; age $\geq$2 years old) defend breeding territories, while some of the smaller and younger males (CT $\approx$10 cm; age = 1 year old) become attached to breeding territories acting as satellites (Santos, 1985; Santos and Almada, 1988; Santos, Nash, and Hawkins, 1995). Satellite males help the nest-holders in the active defense of the breeding territory and try to achieve sneaking fertilizations when females go inside the nests to spawn (Santos, 1985; Santos and Almada, 1988). The gonadosomatic indexes of satellites are higher than those of territorial males (Santos *et al.*, 1995), and they lack the SSC displayed by nest-holders, namely, a small head hump and an anal gland that produces a sex pheromone (Santos, Hawkins, and Nash, 1996). Satellite males may become nest-holders in a subsequent breeding season (Santos *et al.*, 1995) and thus it is a flexible ART. Satellite males were implanted with KT and 17α-methyltestosterone (MT) and changes in their morphology and behavior were investigated 1 week after treatment.

METHODS

A total of 42 satellite males were randomly assigned to each treatment and implanted with Silastic tubes (silicone type A, Dow Corning: inner diameter = 1.47 mm, outer diameter = 1.96 mm, length = 0.95 ± 0.06 cm) containing one of the three solutions: 5 μg of MT (Argent Chemicals) per microliter of castor oil as vehicle ($n < 26$ 10), 5 μg of KT (Sigma) per microliter of castor oil ($n = 17$), and castor oil only (Sigma) ($n = 15$). The concentration used in the implants was calculated according to the dosage (amount of administered KT per body weight) used by Kobayashi, Aida, and Stacey (1991). MT was used as a way to administer testosterone (T), which is a common practice in fish studies (Liley and Stacey, 1983). The implants were placed in the peritoneal cavity by a small anterior ventro-lateral incision in the abdomen, which was sutured afterward. There were no significant differences in body weight (Kruskall–Wallis ANOVA: $H_{2,42} = 0.8$, $P = 0.61$) or in standard length (Kruskall–Wallis ANOVA: $H_{2,42} = 0.7$, $P = 0.69$) between the subjects allocated to each experimental group. The overall mean weight and standard length of the implanted individuals were 10.6 ± 4.2 g and 8.6 ± 1.1 cm, respectively. The individuals were anesthetized for the surgical procedure with MS222 (Tricaine, Argent Chemicals, dilution of 1:10,000 in salt water) and recovered from anesthesia in a small compartment supplied with plentiful aeration. During the postsurgical period and until the behavioral tests were carried out, each individual was kept separated in isolation aquaria, to avoid any risk of infection and also to standardize previous experience among the experimental subjects. The isolation aquaria (15 × 20 × 30 cm) had an artificial nest placed in a central location and were kept at 19 ± 1°C with a photoperiod of 12L:12D. There was no mortality in the implanted fish during the postsurgical period.

Eight days after being implanted, the individuals were exposed to the following sequence of behavioral tests: a mirror test to assess aggressive behavior, a female presentation test to assess courtship behavior, and a mating tactic choice to assess which tactic (nest-holding vs satellite) would be adopted by the subject when given a choice. Due to time constraints not all the individuals were tested. Thus there are smaller sample sizes in the behavioral tests. The tests were videotaped using a Sony Hi-8 camera and the tapes were subsequently analyzed using the PC Software Observer multievent recorder (Noldus Information Technology, Wageningen, The Netherlands). The first two tests were conducted in the aquarium where the subject was being isolated after surgery. In the mirror test, a mirror was placed against one of the lateral walls of the aquarium and the reaction of the subject to the mirror was recorded (Fig. 1a). The observation period lasted 10 min and started when the subject showed the first reaction toward the mirror. The behavioral variables registered were the following: number of attacks toward the mirror, number of agonistic displays toward the mirror, and time spent inside the nest. In the female presentation test a gravid female was introduced into the subject's aquarium and the behavior of the subject toward the female was recorded (Fig. 1b). The behavioral variables registered were the following: number of agonistic attacks toward the females, number of courtship displays directed to the female, and time spent inside the nest. The observation period lasted 10 min and started when the first interaction between the pair occurred. To standardize the stimulus females, ovulation was induced by an intraperitoneal injection of 200 μl saline containing 5 μg des-Gly^{10} [D-Ala^{6}]-luteinizing hormone releasing hormone ethylamide (Sigma) 48 h before being presented to the males.

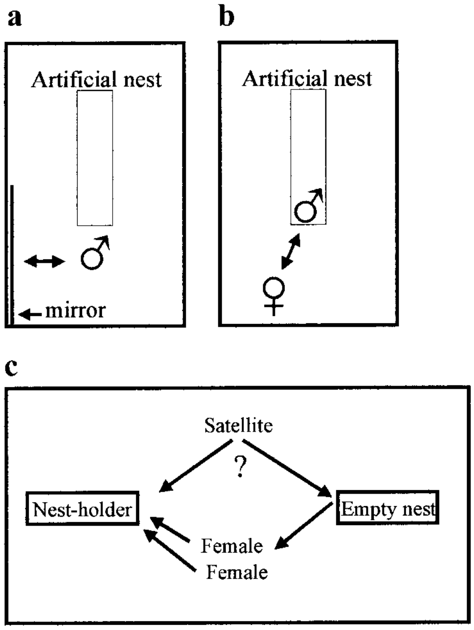

FIG. 1. Behavioral tests used: (a) mirror elicited aggression test; (b) female presentation test; (c) mating tactic choice test. For further details see text.

After these two tests, subjects were transferred to a larger tank (70 × 30 × 40 cm) in which two artificial nests were placed and a group composed of one nest-holder male and two receptive females was established. The goal of this test was to investigate which tactic the implanted male would follow when given the choice of associating with the nest-holder male and acting as a satellite or of occupying the empty artificial nest and trying to attract the females to it (mating tactic choice test, Fig. 1c). The behavioral variables measured were the following: time spent inside its own nest; time spent near the nest-holder's nest, as defined by a half-cone with its vertex centered on the nest-holder's nest and a height of 20 cm; agonistic behaviors directed to and received from conspecifics (separating the interactions with the females and the nest-holder); courtship behavior directed toward the females.

All individuals were tested in the same order: mirror test, female presentation test, and mating tactic choice test.

After the behavioral tests, the subjects were sacrificed with an overdose of the anesthetic MS222, morphometric measurements were taken, and gonads and liver were dissected out. The following morphometric measurements to the nearest 10 μm were taken on a ventral view using a calibrated micrometer on a stereomicroscope:

TABLE 1

Differences (Mean ± SEM) in Morphological Traits among the Three Treatments

Variable	C	KT	MT	$H_{2,N}$	P
Genital papillae width (μm)	−113 ± 181 (15)	209 ± 411 (17)*	309 ± 182 (10)*	15.5	0.0004
Genital papillae length (μm)	−163 ± 322 (15)	22 ± 249 (17)*	150 ± 194 (10)*	7.8	0.02
First ray anal gland width (μm)	0 ± 106 (15)	554 ± 168 (17)**	1022 ± 338 (10)**	32.9	<0.00001
First ray anal gland length (μm)	81 ± 187 (15)	237 ± 283 (17)**	600 ± 359 (10)**	15.2	0.0005
Second ray anal gland width (μm)	−138 ± 253 (15)	72 ± 236 (17)**	394 ± 176 (10)**	19.3	0.0001
Second ray anal gland length (μm)	63 ± 384 (15)	237 ± 356 (17)**	497 ± 446 (10)**	7.8	0.02
GSI (% body weight)	2.6 ± 0.9 (15)	2.3 ± 2.0 (17)	1.5 ± 1.3 (9)	1.21	0.54
TG relative area (% gonad area)	17.4 ± 7.1 (10)	28.2 ± 20.2 (15)	24.9 ± 13.7 (10)	1.24	0.53
HSI (% body weight)	2.2 ± 0.4 (15)	2.9 ± 1.0 (17)*	2.5 ± 0.7 (9)	10.0	0.007

Note. GSI, gonadosomatic index; TG, testicular gland; HSI, hepatosomatic index. Values for genital papillae and anal gland variables refer to increments between pre- and posttreatment values. Sample sizes are given in parentheses. * codes a difference with the control group and ** codes differences between the treatment groups $P < 0.05$, Dunn's multiple comparisons test). Sample sizes for each treatment vary from variable to variable and are given in parentheses.

Genital papillae width: maximum distance between the left and the right contours of the genital papillae.

Genital papillae length: distance between the anterior insertion of the papillae and its posterior tip measured.

First ray anal gland width: maximum distance between the left and the right contours of the glandular tissue of the first ray of the anal fin measured.

First ray anal gland length: distance between the anterior insertion of the glandular tissue of the first anal fin ray and its posterior tip measured.

Second ray anal gland width: maximum distance between the left and the right contour of the glandular tissue of the second ray of the anal fin measured.

Second ray anal gland length: distance between the anterior insertion of the glandular tissue of the second anal fin ray and its posterior tip measured.

Testis width: maximum distance between the left and the right edges of the right testis.

Testis length: maximum distance between the anterior and the posterior edges of the right testis.

Testicular gland width: maximum distance between the left and the right edges of the right testicular gland.

Testicular gland length: maximum distance between the anterior and the posterior edges of the right testicular gland.

Testicular gland index (TGI) was calculated as testicular gland area / total testis area. TG and testis areas were calculated from ventral view measurements of maximum width and length, assuming testis and TG to have an ellipsoidal shape.

Body, gonads, and liver were weighed (nearest milligram) with an electronic balance and used to calculate a gonadosomatic index (GSI = gonad weight / body weight × 100) and a hepatosomatic index (HIS = liver weight / body weight × 100). The gonadosomatic index was used as an indicator of gonadal maturation and the hepatosomatic index as a bioenergetic indicator, since it has been shown that the liver plays a major role in energy storage in this species (Santos *et al.*, 1995).

The choice of 8 days for the duration of the treatment was based on the results of experiments with similar implants in other species in which measurable effects were detected after 5–6 days (e.g., color change from the female to the male pattern in treated female *Thalassoma bifasciatum* 6 days posttreatment; Grober, Jackson, and Bass, 1991; sex-change as assessed by the differentiation of a male-like genital papilla in treated female *Lythrypnus dalli* 5 days after the treatment, Carlisle, Marxer-Miller, Canario, Oliveira, Carneiro, and Grober, 2000).

RESULTS

Morphology

Both androgen treatments significantly increased both the length and the width of the genital papillae (Table 1). Androgen-implanted males also showed a larger development of the anal gland, with MT being more effective than KT (Table 1). Androgen treatment had no effect on the GSI or on the TGI (Table 1). KT treatment had a significant positive effect on the HSI (Table 1). Considering all individuals together there

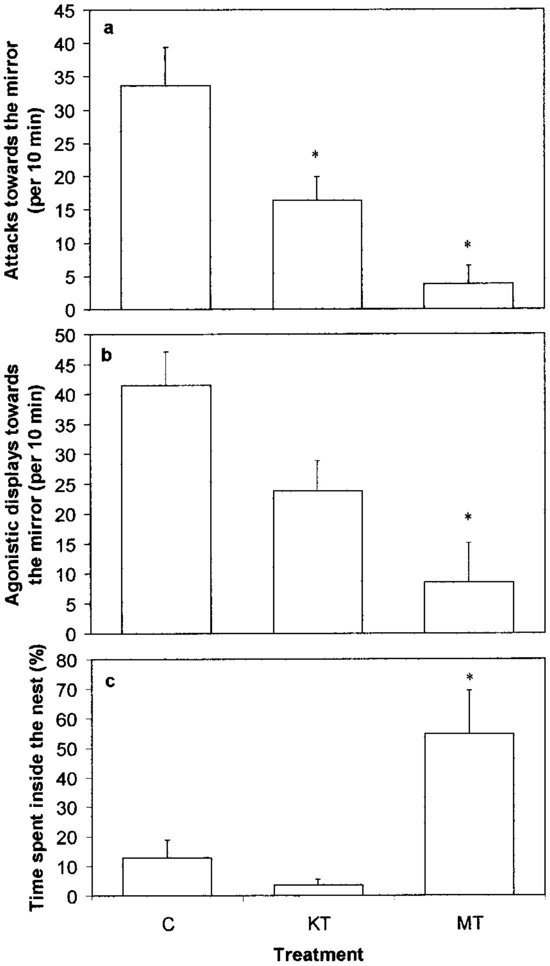

FIG. 2. Differences (mean ± SEM) among treatments (C, control; KT, 11-ketotestosterone; MT, 17-α-methyltestosterone) in the mirror elicited aggression test: (a) attacks directed toward the own image in the mirror; (b) agonistic displays toward the mirror; (c) time spent inside the artificial nest. * codes a difference with the control group and ** codes differences between the treatment groups ($P < 0.05$, Dunn's multiple comparisons test). Sample sizes for each treatment were as follows: $N_C = 14$, $N_{KT} = 16$, $N_{MT} = 10$.

was a significant negative correlation between GSI and TGI ($r_s = -0.85$, $P < 0.00001$, $N = 34$), suggesting a trade-off between gonadal investment and the development of the testicular gland.

Behavioral Tests

Mirror test. Androgen-implanted individuals attacked less (Kruskall–Wallis ANOVA: $H_{2,40} = 15.9$, $P = 0.0004$) and displayed less (Kruskall–Wallis ANOVA: $H_{2,40} = 15.5$, $P = 0.0004$) toward the mirror than controls (Fig. 2). The frequency of attacks directed toward the mirror was positively correlated with the frequency of displays, indicating that each individual was consistently using both behaviors (the attacks usually followed the displays when the subjects escalated their fighting behavior toward the mirror). MT-implanted individuals spent significantly more time inside the nest than both KT-implanted and control individuals (Kruskall–Wallis ANOVA: $H_{2,40} = 11.1$, $P = 0.004$, Fig. 2).

Female presentation test. MT-implanted individuals spent significantly more time inside the nest (Kruskall–Wallis ANOVA: $H_{2,40} = 9.6$, $P = 0.008$) and attacked the females less (Kruskall–Wallis ANOVA: $H_{2,40} = 12.7$, $P = 0.002$) than both KT-implanted and control individuals (Fig. 3). Although there were no significant differences in courtship frequency between the three treatments (Kruskall–Wallis ANOVA: $H_{2,40} = 2.5$, $P = 0.29$; see Fig. 3), only androgen-implanted males were able to attract females inside their nests (18.8% of the KT-implanted males and 20% of the MT-implanted males vs 0% of the sham-implanted males).

Mating tactic choice test. There was a marginal nonsignificant trend for MT-implanted males to spend less time near the nest occupied by the nest-holder and for androgen-implanted (both MT and KT) individuals to attack the females less (Table 2). There was a significant effect of androgen treatment on the courtship directed toward the females with MT-implanted satellites courting less than the other two groups (Table 2). However, MT-implanted males spent significantly more time in their own nest than near the nest-holder nest while for the other two groups (control and KT-implanted, see Fig. 4) there were no significant differences in the time spent inside their own nest and the time spent near the nest-holder nests. Finally, there were no other significant differences among treatments for the measured behavioral traits (Table 2).

DISCUSSION

Androgen's Effects on Male Reproductive Biology and SSC

Androgen-treated *P.s. parvicornis* satellite males developed male secondary characters within a week after implantation. Both MT- and KT-treated individuals had significantly higher increments both in genital papilla width and length and in anal gland development. This is indicative that androgen is mediating the

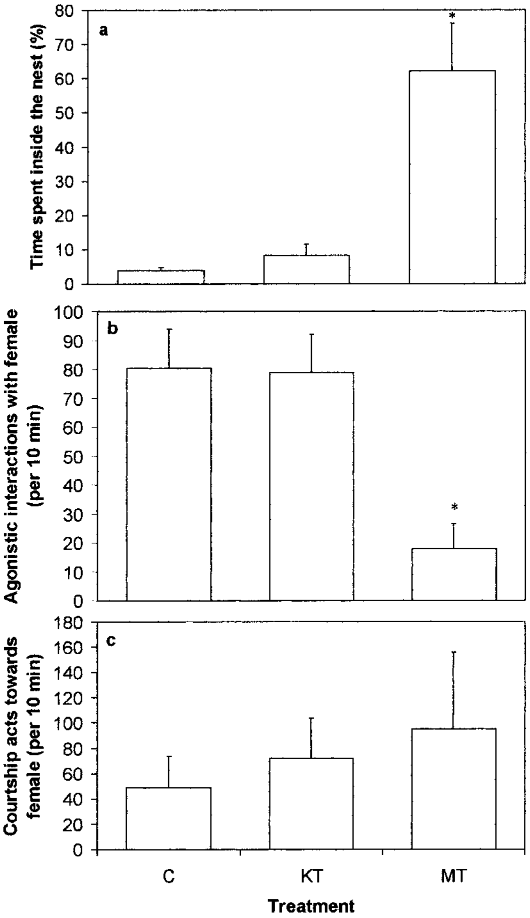

FIG. 3. Differences (mean ± SEM) among treatments (C, control; KT, 11-ketotestosterone; MT, 17-α-methyltestosterone) in the female presentation test: (a) time spent inside the artificial nest; (b) attacks directed toward the female; (c) courtship displays direct to the female. * codes a difference with the control group and ** codes differences between the treatment groups $P < 0.05$, Dunn's multiple comparisons test). Sample sizes for each treatment were as follows: $N_{\mathrm{C}} = 14$, $N_{\mathrm{KT}} = 16$, $N_{\mathrm{MT}} = 10$.

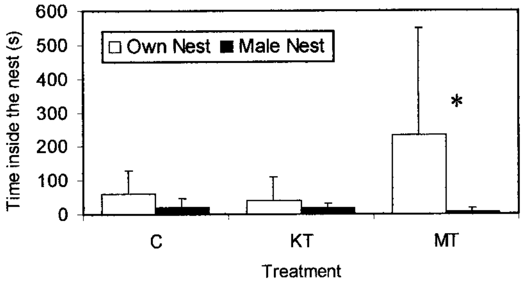

FIG. 4. Comparison between the time spent near the nest-holder's nest (satellite tactic) and the time spent inside the available nest (nesting tactic) (mean ± SEM) for the three treatment groups: control (C), 11-ketotestosterone-treated (KT), and 17-α-methyltestosterone-treated (MT). * Indicates a significant difference at $P < 0.05$. Sample sizes for each treatment were as follows: $N_{\mathrm{C}} = 9$, $N_{\mathrm{KT}} = 10$, $N_{\mathrm{MT}} = 10$.

morphological transition from satellites to bourgeois males. Since in nature bourgeois males have higher androgen levels than satellites (Oliveira, Canario, Grober, and Santos, in press b), satellite males also have larger GSI but lower TGI than nest-holders and the two indices are negatively correlated (this study and Oliveira *et al.*, in press, b), it is likely that the testicular gland is the main producer of androgens in this species, as it has been found for the accessory glands of other teleosts (e.g., urohaze-goby, *Glossogobius olivaceus;* Asahina, Suzuki, Aida, Hibya, and Tamaoki, 1985). In contrast to the positive effects of androgen treatment on SSC, no effect was found either in the relative gonad size (i.e., gonadosomatic index) or in the development of the testicular accessory gland.

In the present study KT treatment had a positive effect on relative liver weight (i.e., hepatosomatic index) when compared to the control group. Liver mass

TABLE 2

Differences (Mean ± SEM) in Behavioral Patterns among the Three Treatments in the Mating Choice Test

Variable	C ($N = 9$)	KT ($N = 10$)	MT ($N = 10$)	$H_{2,29}$	P
Time inside own nest(s)	59.1 ± 23.1	41.3 ± 21.2	232.8 ± 99.5	3.5	0.17
Time near nest-holder nest(s)	18.8 ± 8.7	17.7 ± 4.8	6.5 ± 3.5	5.2	0.07
Agonistic behavior toward female	9.7 ± 4.6	2.8 ± 1.3	0.6 ± 0.4	5.1	0.08
Courtship behavior toward female	7.6 ± 3.5*	6.4 ± 1.4*	0.8 ± 0.4	6.8	0.03
Agonistic behavior received from female	14.9 ± 3.2	19.5 ± 5.0	59.8 ± 16.9	4.4	0.11
Agonistic behavior toward nest-holder	0.2 ± 0.2	0.6 ± 0.3	0 ± 0	3.7	0.15
Agonistic behavior received from nest-holder	6.2 ± 2.1	6.7 ± 2.6	6.3 ± 2.0	0.1	0.94

Note. Frequencies of the different behaviors are expressed as acts per 20 min. * codes a difference with the control group and ** codes differences between the treatment groups ($P < 0.05$, Dunn's multiple comparisons test). Sample sizes for each treatment are given in parentheses.

variation in wild populations of teleosts has been linked to storage and utilization of lipids and glycogen. During the breeding season in temperate species, individuals rely on lipids stored in the liver after the winter. Energy stored in the liver is then redirected to the muscles for general breeding activity (e.g., courtship) and to the gonads for reproductive effort (Podroschko, Patzner, and Adam, 1985). This is particularly the case in species with male parental care where males rely on fat reserves to survive the breeding season since feeding during this period is highly reduced or even suspended (e.g., *S. pavo;* Gonçalves and Almada, 1997). Thus, an additional bourgeois trait may be the storage of energy reserves in the liver and this too is regulated by KT.

Differential Role of MT and KT on the Activation of Bourgeois Male Behavior

Of the behaviors typically displayed by *P.s. parvicornis* nest-holders, MT promoted nesting behavior in satellites, and only androgen-treated satellite individuals were able to attract females to enter their nests. This result was somewhat surprising since available evidence from androgen levels between alternative reproductive morphotypes (i.e., bourgeois vs parasitic tactic) showed a marked difference in KT levels between courting and noncourting morphotypes, but no clear pattern for T (Brantley, Wingfield, and Bass, 1993). And, while in some species there were no differences in T levels among morphotypes, in others T levels were higher in parasitic males than in bourgeois males. Furthermore, in *P.s. parvicornis* nest-holders have significantly higher levels of KT than satellites and the two phenotypes do not differ in T levels (Oliveira *et al.*, in press, b). Thus we would have predicted KT to be the more, or even the only, effective treatment on the differentiation of the bourgeois tactic in satellite males. However, although the outcome of the test could have been influenced by differences in behavior of the nest holders used as stimuli toward the different satellites in the mating tactic choice test, this possibility is very unlikely since satellites received similar rates of agonistic behavior (see Table 1).

Other possible explanations for the apparent higher effectiveness of MT in promoting SSC and nesting behavior include the following:

(a) Inappropriate hormone dosage, considering that SSC is dose-dependent (this appears to be the case in most species), and the dosage used could have been at the peak of effectiveness for MT but was either below or above the optimum for KT. Although we cannot rule out this possibility, without replicating the present study with different dosages of the two hormones, this is not a very likely reason. The same KT dosage has been used with positive results in other studies, including various gobies and blennies (e.g., McIntyre, 1998; Carlisle *et al.*, 2000; R. F. Oliveira, unpublished data for the peacock blenny, *S. pavo*). Another possibility is that differences in effectiveness could be due to metabolization of MT (a synthetic steroid) to a more potent androgen. For example, in rainbow trout several 6-, 7-, and 11-oxo-androsten metabolites of MT have been identified including 17α-methyl-17β-hydroxy-4-androsten-3,11-dione (Cravedi, Delous, Debrauwer, and Prome, 1993). This would also explain why MT has proved effective even in the masculinization of adult female fish (e.g., Mormyridae; Herfeld and Moller, 1998). However, recent experiments with the protogynous blackeye goby, *Coryphoterus nicholsii*, showed that MT (at doses that were two orders of magnitude higher than the doses than we used) was completely ineffective in inducing masculinization of mature females, in contrast to KT and adrenosterone, which were very effective (Kroon and Liley, 2000). Alternatively, the possibility that nesting by parental males may be induced by brain aromatization of T cannot be answered at this stage, but which could explain the positive effect of MT on nesting. In support for this hypothesis some studies have reported higher levels of T in males with empty nests or nests with eggs (Knapp, Wingfield, and Bass, 1999) and MT promoting nesting behavior in paradise fish (*Macropodus opercularis;* Machemer, 1971).

(b) Neither T nor KT is the active androgen in *P.s. parvicornis.* The most active androgens in fishes are 11-oxo-4-androstens (Borg, 1994) with 11-ketoandrostenedione, 11β-hydroxyandrostenedione, and 11β-hydroxytestosterone as alternative candidates to KT in some teleosts, where they appear to be the most active androgens stimulating SSC and reproductive behaviors (see reviews by Kime, 1993; Borg, 1994). This possibility will require determining whether these steroids (if any) are present in *P.s. parvicornis* and to analyze their effectiveness in promoting SSC.

The Relative Plasticity Hypothesis

The data presented here suggest an activational role of androgens on the expression of traits typical of the bourgeois tactic, which supports the relative plasticity hypothesis proposed by Moore (1991), according to which the transition between alternative phenotypes in plastic ART is accomplished by activation effects of sex steroids (see Introduction for more information on this hypothesis). Interestingly it has been recently shown that satellite males and nest-holder males have significantly different androgen plasma profiles (Oliveira *et al.*, in press, b), which together with the present results support the two predictions generated by the relative plasticity hypothesis for plastic ART in *P.s. parvicornis:* (a) that exogenous administration of androgens has an activational role on the alternative tactic expression and (b) that circulating levels of androgens in adult phenotypes differ between individuals following the alternative tactics (Moore, 1991).

More recently, Moore *et al.* (1998) developed a second generation of the relative plasticity hypothesis. While the original formulation of this hypothesis considered only one distinction between the ART, fixed vs plastic, in the upgraded version, further distinctions are made between reversible and irreversible plastic ART and between conditional and unconditional fixed ART. In the case of the plastic ART this further distinction generates another prediction at the endocrine level: that in reversible tactics the effects of hormones should be more activational while in irreversible tactics the effects should be organizational, since the transition to the “final” morphotype is permanent (like the original predictions, but now at a second hierarchical level) (Moore *et al.*, 1998). In other words, the hormone differences that cause the differentiation of the alternative morphotypes are only needed, and thus should only be present, during the transition phase. After the differentiation of the final irreversible phenotype there is no need for further hormone differences to maintain it. *P.s. parvicornis* is an example of a species with plastic irreversible ART. Thus, the effects of the androgen treatment can be viewed as effective in the transition period but should be proved ineffective afterward (e.g., castration or the administration of an androgen inhibitor should not revert nest-holders to satellites). Moreover, the above-mentioned differences in circulating levels of androgens between satellites and nest-holders (Oliveira *et al.*, in press, b) should be viewed, according to this new hypothesis, as the result of different social experiences of the two alternative phenotypes than as needed to maintain them. Interestingly, in a closely related species, the peacock blenny *S. pavo,* it was recently shown that the circulating levels of KT in breeding males were associated with their social environment (Oliveira, Almada, Gonçalves, Forsgren, and Canario, in press, a), which supports this view.

In our experimental situation the tactic switching, or at least its beginning, could be rapidly induced by exogenous steroid treatment, suggesting that in nature satellites may switch tactics within the same breeding season if the social context allows. In fact, we have observed some small nest-holder males with body lengths overlapping with those of satellites. It may be that these individuals took over the nests in which they were acting as satellites, after these became vacant (e.g., nest-holder death). These small nest-holders already had functional anal glands and larger genital papilla than satellites, but smaller than those of the average nest-holder (R. F. Oliveira, unpublished data). In the common goby, *Pomatochistus microps,* a short-lived species with a single breeding season, larger males build and defend nests while smaller males act as sneakers. The decision to breed as a sneaker or as a nest-holder depends on the relative size of the individual compared with the average male size in the local population and as it may change as the breeding season progresses, so is the breeding tactic susceptible to change (Magnhagen, 1992, 1994). Although most young/small *P.s. parvicornis* males will function as satellites (in the presence of larger/older males, R. F. Oliveira, unpublished data), social regulation allows males acting as satellites to increment their breeding success to nest-holding if the opportunity arises. Thus, mate tactic switching may be a rapid response to changes in social context, which involves the induction of a variety of SCC by sex steroids, especially androgens, since androgens are known to respond to social challenges and since elevated status is linked with a rise in androgen levels (Wingfield, Ball, Dufty, Hegner, and Ramenofsky, 1987).

Androgens and Aggressive Behavior

One puzzling result presented here is the apparent depressive effect of both androgens on aggressive behavior expressed in the mirror test. Androgens have been shown to be involved in the expression of aggressive behavior in a large number of teleost species (Liley and Stacey, 1983; Borg, 1994; Oliveira and Canario, 2000), which raises a strong prediction for enhanced aggressiveness in androgen-treated individuals. Why did we fail to detect such an effect in our experiment? One possible explanation is that we have used the wrong approach. Although mirror image stimulation (MIS) is a widely used technique in the study of fish visual communication, including the assessment of aggressive behaviors (Rowland, 1999), there has been some criticism based on some inconsistencies in the relationship between social status and the aggressive score of an individual in a MIS test (Ruzzante, 1992). However, in the present case the androgen-treated individuals not only achieved lower aggression scores in the MIS test but also displayed less aggressive behavior toward conspecifics in the mating tactic choice test (see Table 2). Thus, the MIS test seems to honestly reflect the aggressiveness of the tested males. One potential alternative explanation to the above-mentioned results is that androgen treatment, mainly MT, promotes nest establishment/defense, which is supported by the large amounts of time spent in the nest in MT-treated satellites.

Future research is needed to further explore the role of different androgens in the differentiation of alternative reproductive tactics in teleosts.

ACKNOWLEDGMENTS

The authors thank the Department of Oceanography and Fisheries of the University of Azores for logistic support, especially its director, Ricardo Serrão Santos. Thanks are also extended to Norberto Serpa, Paula Lourinho, André Silva, Natacha Carvalho, and Jason Miranda for their help collecting fish in the field and to Elsa Couto for steroid analysis. R.F.O. is indebted to Vitor Almada for stimulating discussions on this topic. Katharina Hirschenhauser, Albert Ros, David Gonçalves, and two anonymous referees provided valuable comments on early drafts of the manuscript. This study is part of a project funded by Fundação para a Ciência e a Tecnologia (FCT) and FEDER (PRAXIS/PCNA/BIA/94/96 to R.F.O.). M.S.G. was supported by NSF Grant IBN-9723817.

REFERENCES

Arnold, A. P., and Breedlove, S. M. (1985). Organizational and activational effects of sex steroids on brain and behavior: A reanalysis. *Horm. Behav.* **19,** 469–498.

Asahina, K., Suzuki, K., Aida, K., Hibya, T., and Tamaoki, B-I. (1985). Relationship between the structures and steroidogenic functions of the testes of the urohaze-goby (*Glossogobius olivaceus*). *Gen. Comp. Endocrinol.* **57,** 281–292.

Borg, B. (1994). Androgens in teleost fishes. *Comp. Biochem. Physiol. C* **109,** 219–245.

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

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

Carlisle, S. L., Marxer-Miller, S. K., Canário, A. V. M., Oliveira, R. F., Carneiro, L. A., and Grober, M. S. (2000). Effects of 11-ketotestosterone on genital papilla morphology in the sex changing fish, *Lythrypnus dalli* (Teleost, Gobiidae). *J. Fish Biol.* **57,** 445–456.

Cravedi, J. P., Delous, G., Debrauwer, L., and Prome, D. (1993). Biotransformation and branchial excretion of 17-α-methyltestosterone in trout. *Drug Metab. Dispos.* **21,** 377–385.

Gonçalves, E. J., and Almada, V. C. (1997). Sex differences in resource utilization by the peacock blenny. *J. Fish Biol.* **51,** 624–633.

Gonçalves, E. J., Almada, V. C., Oliveira, R. F., and Santos, A. J. (1996). Female mimicry as a mating tactic in males of the blenniid fish *Salaria pavo. J. Mar. Biol. Ass. U.K.* **76,** 529–538.

Grober, M. S., Jackson, I. M. D., and Bass, A. H. (1991). Gonadal steroids affect LHRH preoptic cell number in a sex/role changing fish. *J. Neurobiol.* **22,** 734–741.

Gross, M. R. (1996). Alternative reproductive strategies and tactics: Diversity within sexes. *TREE* **11,** 92–98.

Herfeld, S., and Moller, P. (1998). Effects of 17-α-methyltestosterone on sexually dimorphic characters in the weakly discharging electric fish, *Brienomyrus niger* (Gunther, 1866) (Mormyridae): Electric organ discharge, ventral body wall indentation, and anal-fin ray bone expansion. *Horm. Behav.* **34,** 303–319.

Hews, D. K., Knapp, R., and Moore, M. C. (1994). Early exposure to androgens affects adult expression of alternative male types in tree lizards. *Horm. Behav.* **28,** 96–115.

Hover, E. L. (1985). Differences in aggressive behavior between two thorat color morphs in a lizard, *Urosaurus ornatus. Copeia* **1985,** 933–940.

Kime, D. E. (1993). 'Classical' and 'non-classical' reproductive steroids in fish. *Rev. Fish Biol. Fish* **3,** 160–180.

Knapp, R., Wingfield, J. C., and Bass, A. H. (1999). Steroid hormones and paternal care in the plainfin midshipman fish (*Porichthys notatus*). *Horm. Behav.* **35,** 81–89.

Kobayashi, M., Aida, K., and Stacey, N. E. (1991). Induction of testis development by implantation of 11-ketotestosterone in female goldfish. *Zool. Sci.* **8,** 389–393.

Kroon, F. J., and Liley, N. R. (2000). The role of steroid hormones in protogynous sex change in the blackeye goby, *Coryphopterus nicholsii* (Teleostei: Gobiidae). *Gen. Comp. Endocrinol.* **118,** 273–283.

Liley, N. R., and Stacey, N. E. (1983). Hormones, pheromones, and reproductive behavior in fish. *In* W. S. Hoar, D. J. Randall, and E. M. Donaldson (Eds.), *Reproduction: Behavior and Fertility Control,* Vol. 9B, pp. 1–63. Academic Press, New York.

Machemer, L. (1971). Synergistische Wirkung von Sauger-Prolaktin und Androgen beim Schaumnestbau des Paradiesfisches, *Macropodus opercularis* L. (Anabantidae). *Z Tierpsychol.* **28,** 33–53.

Magnhagen, C. (1992). Alternative reproductive behavior in the common goby, *Pomatochistus microps:* An ontogenetic gradient? *Anim. Behav.* **44,** 182–184.

Magnhagen, C. (1994). Sneak or challenge: Alternative spawning tactics in non-territorial male common gobies. *Anim. Behav.* **47,** 1212–1215.

McIntyre, K. K. (1998). *Arginine Vasotocin in the Preoptic Area of the Bluehead Wrasse and the Effects of 11-Ketotestosterone.* M.Sc. Thesis, Arizona State University.

Moore, M. C. (1991). Application of organization–activation theory to alternative male reproductive strategies: A review. *Horm. Behav.* **25,** 154–179.

Moore, M. C., Hews, D. K., and Knapp, R. (1998). Hormonal control and evolution of alternative male phenotypes: Generalizations of models for sexual differentiation. *Am. Zool.* **38,** 133–151.

Moore, M. C., and Thompson, C. W. (1990). Field endocrinology of reptiles: Hormonal control of alternative reproductive tactics. *In* A. Epple, C. G. Scanes, and M. H. Stetson (Eds.), *Progress in Comparative Endocrinology,* pp. 685–690. A. R. Liss, New York.

Oliveira, R. F., Almada, V. C., Gonçalves, E. J., Forsgren, E., and Canario, A. V. M. (in press a). Androgen levels and social interactions in breeding males of the peacock blenny (*Salaria pavo*). *J. Fish Biol.*

Oliveira, R. F., and Canario, A. V. M. (2000). Androgens and social behavior in cichlid fishes: A case study in the Mozambique tilapia. *In* R. M. Coleman and W. Leibel (Eds.), Cichlid Research: State of the Art. Journal of Aquaria and Aquatic Science, Special Issue, Vol. 9, 109–129.

Oliveira, R. F., Canario, A. V. M., Grober, M. S., and Santos, R. S. (in press, b). Endocrine correlates of alternative reproductive tactics and male sexual polymorphism in the Azorean rock-pool blenny. *Gen. Comp. Endocrinol.*

Phoenix, C., Goy, R., Gerall, A., and Young, W. (1959). Organizing action of prenatally administered testosterone proprionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology* **65,** 369–382.

Podroschko, S., Patzner, R. A., and Adam, H. (1985). The reproduction of *Blennius pavo* (Teleostei, Blenniidae). IV. Seasonal variation in HSI, the liver glycogen value and histological aspects of the liver. *Zool. Anz.* **215,** 265–273.

Rowland, W. J. (1999). Studying visual cues in fish behavior: A review of ethological techniques. *Environ. Biol. Fish.* **56,** 285–305.

Ruzzante, D. E. (1992). Mirror image stimulation, social hierarchies, and population differences in agonistic behavior: A reappraisal. *Can. J. Fish. Aquat. Sci.* **49,** 1966–1968.

Santos, R. S. (1985). Parentais e satélites: tácticas alternativas de acasalamento nos machos de *Blennius sanguinolentus* Pallas (Pisces: Blenniidae). *Arquipélago Sér Ciênc. Nat.* **6,** 119–146.

Santos, R. S., and Almada, V. C. (1988). Intraspecific variations in reproductive tactics in males of the rocky intertidal fish *Blennius sanguinolentus,* in Azores. *In* G. Chellazi and M. Vannini, (Eds.), *Behavioral Adaptations to Intertidal Life,* pp. 421–447. Plenum, New York.

Santos, R. S., Hawkins, S. J., and Nash, R. D. M. (1996). Reproductive phenology of the Azorean rock pool blenny, a fish with alternative mating tactics. *J. Fish Biol.* **48,** 842–858.

Santos, R. S., Nash, R. D. M., and Hawkins, S. J. (1995). Age, growth and sex ratio of the Azorean rock-pool blenny, *Parablennius sanguinolentus parvicornis. J. Mar. Biol. Ass. U.K.* **75,** 751–754.

Taborsky, M. (1997). Bourgeois and parasitic tactics: Do we need collective, functional terms for alternative reproductive behaviors? *Behav. Ecol. Sociobiol.* **41,** 361–362.

Thompson, C. W., and Moore, M. C. (1992). Behavioral and hormonal correlates of alternative reproductive strategies in a polygynous lizard: Tests of the relative plasticity and challenge hypotheses. *Horm. Behav.* **26,** 568–585.

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