

Social Modulation of Sex Steroid Concentrations in the Urine of Male Cichlid Fish *Oreochromis mossambicus*

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The relationship between urinary concentrations (free + sulfates + glucuronides) of the steroids testosterone (T), 11-ketotestosterone (11KT), 17 α , 20 β -dihydroxy-4-pregnen-3-one (17,20 β -P) and 17 α ,20 α -dihydroxy-4-pregnen-3-one (17,20 α -P), and the social behavior of males of the cichlid fish *Oreochromis mossambicus* was investigated. After 8 days of isolation none of the steroids were good predictors of social dominance developed after subsequent formation of all-male groups. One day after group formation dominance indexes were good predictors of the urine concentrations of all sex steroids. Dominance indexes and androgen concentrations measured after all-male group formation were positively correlated with territoriality, courtship rate, and nest size. Similar relationships were found for progestins with the exception that they were not correlated with courtship rate. All-male group formation was also accompanied by an increase in urinary sex steroid concentrations in fish that became territorial and a decrease in non-territorial fish with the exception of T, which increased in both groups. Addition of ovulating females caused steroid concentrations to return to levels near isolation, except for 17,20 α -P in territorials, which underwent a large increase. Thus, social interactions may have an important modulatory effect on sex steroid concentrations in *O. mossambicus*.

The interrelationship between sex steroids and behavior in teleosts has been studied mainly from the point of view of the effect of androgens, testosterone (T), or 11-ketotestosterone (11KT). Cichlid fishes are one

of the teleost families in which there is a larger body of evidence in the role of androgens on the control of male social behavior, both in terms of organization (Billy and Liley, 1985; Groothuis and Ros, 1993) and activation (Kramer, Molenda, and Fiedler, 1969; Reinboth and Rixner, 1972; Wapler-Leong and Reinboth, 1974; Fernald, 1976). In *Oreochromis mossambicus* and other tilapiine species androgen treatment at an early sensitive period in the ontogeny may even reverse the sex of the individual (Clemens and Inslee, 1968; Hunter and Donaldson, 1983).

Castration and administration of androgens to castrated and intact fish have been the primary tools to study the influence of steroids on social behavior. Castration has been shown to lower both agonistic and sexual behaviors, while androgen administration restored those behaviors in castrates and had an enhancing effect on behavior in intact males (Aronson, Scharf, and Silverman, 1960; Reinboth and Rixner, 1972; Wapler-Leong and Reinboth, 1974; Fernald, 1976; Francis, Jacobson, Wingfield, and Fernald, 1992).

Androgen concentrations may also be affected by the interactions between conspecifics, suggesting a two-way type of interaction between androgens and behavior (Villars, 1983). For example, in a natural population of stoplight parrotfish (*Sparisoma viride*) territorial males had higher concentrations of androgens than non-territorial males and during territory acquisition androgen concentrations reached values above those found in already established territorial males. The peaks of androgens could also be induced by experimental intrusions of other males indicating that the agonistic interactions in themselves could affect the hormonal state of the fishes (Cardwell and Liley, 1991a).

Socially induced changes in plasma concentra-

tions of the progestin 17 α ,20 β -dihydroxy-4-pregnen-3-one (17,20 β -P) have been shown in spawning male rainbow trout (Liley, Breton, Fostier, and Tan, 1986). This hormone has been demonstrated to be involved in sperm maturation (Miura, Yamauchi, Takahashi, and Nagahama, 1992) and to function as a primer pheromone released by females (Sorensen, Stacey, and Chamberlain, 1989; Liley, Olsen, and Foote, 1991) but a direct role on male behavior has not yet been found.

The primary objective of the present study was to investigate the extent to which social interactions in *O. mossambicus* could be predicted by prior concentrations of androgens (T and 11KT) or whether the outcome of agonistic interactions could modulate androgen concentrations. In order to do this, parameters of social status and agonistic and sexual behaviors were determined in groups of sexually mature males, which were first isolated to minimize prior social experience and correlated to concentrations of androgens measured in the urine, assuming that urinary steroid profiles provide indications on the patterns found in the plasma. In the absence of data on the dynamics of excretion in *Tilapia* it may be argued that urinary concentrations do not reflect accurately what is going on in the plasma. Despite this problem (see Discussion for a more detailed consideration of this issue) it was decided to use urinary concentrations because they involve much less manipulation of the individuals, which can be a major source of bias in behavioral studies in which the same individual is sequentially sampled. In addition, a possible involvement of 17,20 β -P and 17,20 α -P in social behavior was also assessed using the same methodology as that used with the androgens.

METHODS

Synopsis of the Oreochromis mossambicus Mating System

O. mossambicus is an African mouthbrooding cichlid in which males form dense nest aggregations (arenas) in shallow water during the breeding season. Males establish territories within these aggregations where they dig pits (nests), to which they attract a succession of females. Spawning takes place in the nest. The female quickly takes eggs and sperm into her mouth where fertilization takes place and leaves the arena (Bruton and Boltt, 1975). In the wild, the embryos and fry are brooded in the female's mouth during 20 – 22 days (Bruton and Boltt, 1975). Thus parental care is restricted to females.

Subjects and Maintenance

Fish were obtained from a stock bred since the early 'seventies at Aquario Vasco da Gama (Lisboa, Portugal). The original stock was obtained from the Incomati River, Mozambique. In this work adult males, about 1½ years old, ranging from 7.9 to 10.4 cm standard length, and females ranging from 6.0 to 7.0 cm standard length were used. Fish were kept at 24 / 2°C on a 12D:12L light regime. They were fed daily with commercial fish food commonly used for tropical aquarium fish.

Procedures

Prior to being tested fish were stocked in mixed sex groups of 30 – 50 individuals in 500-l tanks for 2 months. At the start of the experiment 16 males were individually isolated in 46 x 36 x 40-cm³ standard aquaria for 8 days in order to minimize possible effects of prior social experience. At the end of the isolation period, groups of four individuals were formed and placed in similar aquaria (93 x 36 x 40 cm³) with a layer of sand in the bottom. Care was taken to minimize the within-group variance of size. The standard length coefficients of variation (CV = standard deviation/arithmetic mean) for each group were CV1 = 0.07, CV2 = 0.04, CV3 = 0.01, and CV4 = 0.10. After 5 days of group formation two ovulated females were added to each group. Ovulation was induced by an ip injection of 200 μ l saline containing 5 μ g des-Gly10, [D-Ala6]-luteinizing hormone releasing hormone ethylamide (Sigma) 48 h prior to their introduction in the experimental groups.

Urine samples were collected for determination of steroid concentrations at the end of the social isolation (day 0), after 5 days of all-male group formation (day 5) and 2 days after the addition of the females to the groups (day 7). The urine was collected into a 1.5-ml Eppendorf tube by applying a slight pressure on the lower part of the fish flanks behind the genital papilla. The procedure took less than a minute for each fish. Urine samples were stored at -20° C until they were processed for radioimmunoassay (RIA). It must be emphasized that steroid measurements were obtained without prior knowledge of the social status of each individual at any time.

In our study we make the assumption that the pattern of variation of the total concentration of each steroid in the urine reflects the pattern of variation of the same steroid in the plasma. Although there are no data on the dynamics of steroid excretion for *Tilapia*, Scott and Liley (1994) found that in rainbow trout (*Oncorhynchus*

mykiss) temporal variation in several steroids present in the urine correspond to similar patterns of variation in the plasma. In a similar vein Creel, Wildt, and Monfort (1993) found that urinary concentrations of androgens reflect adequately short-term variations of plasma concentrations in wild dwarf mongooses (*Helogale parvula*). Despite this problem it was decided to use the urinary concentrations since urine sampling involves only a brief manipulation of the fish, while plasma sampling may have more disruptive effects on the behavior of the fish and on their subsequent steroid profiles due to manipulative stress.

Steroid Radioimmunoassays

Urine samples (50 μ l) were extracted for steroid sulfates and glucuronides as described by Scott and Canario (1992) and the extracts were dissolved in phosphate buffer for RIA.

The RIA characteristics, including the cross-reactivity of the antibodies used, have been reported before: testosterone and 17,20 β -P by Scott, MacKenzie, and Stacey (1984); 11-ketotestosterone by Scott and Sumpter (1988); and 17,20 α -P by Canario and Scott (1989). The intra- and inter-assay variability for urine samples of *O. mossambicus* is as follows: (1) testosterone; CVintra-assay - 0.075, CVinter-assay - 0.124, n - 8; (2) 11-ketotestosterone: CVintra-assay - 0.082, CVinter-assay = 0.116, n - 9; 17,20 α -P: CVintra-assay - 0.089, CVinter-assay - 0.121, n - 7; 17,20 β -P: CVintra-assay - 0.085, CVinter-assay - 0.107, n - 11.

Behavioral Observations

Observations were conducted for 20 min per group every day between 11 AM and 6 PM. Every social interaction that occurred during this period was recorded and its behavior elements were noted together with the identity of the actor and the receiver of the acts. A detailed description of agonistic and courtship behavior can be found in Baerends and Baerends von Roon (1950) and in Neil (1964). Fish were individually identified by a small clip in the dorsal fin membrane. The following elements of behavior were recorded:

(a) Agonistic interactions — those that involved one or more of the following behavioral patterns: approach, charge, butting, biting, chasing, lateral and frontal display, circling, mouthfighting, pendelling, fleeing, and submission posture. An agonistic interaction was considered terminated when one of the opponents moved away from the other and no further agonistic exchanges

were observed in the next 3 s or more. A fish was classified as the loser of an agonistic interaction when it adopted a submissive posture or moved away, either by retreating or escaping, from the opponent, that was then classified as the winner. A dominance index, previously used by other authors (e.g., Barlow and Ballin, 1976; Winberg, Nilsson, and Olsen, 1991; Gomez-Laplaza and Morgan, 1993), was computed as the number of victories over the total number of interactions in which the individual participated. In *O. mossambicus* the rank attained by each fish using this index is highly correlated with the rank order obtained by the analysis of the sociometric matrices, and so it is a good indicator of dominance status (Oliveira and Almada, submitted for publication).

(b) Courtship episodes — when one or more of the following acts were detected: tilting, jerking, leading, quivering, and pit circling.

(c) Territoriality — a fish was classified as being territorial if it dug and actively defended a pit in the substratum and exhibited the characteristic black coloration (*sensu* Neil, 1964).

(d) Nest volume — estimated from measurements of the diameter and depth at the center of the pits, assuming the approximate shape of a cone. In order to reduce inter-group variability in pit depths each aquarium was provided with approximately the same amount of sand (7-cm layer). This measure was taken since nest size is known to be an important correlate of male reproductive success and male social status in other lek-breeding cichlids (McKaye, Louda, and Stauffer Jr., 1990; McKaye, 1991; Rossiter, 1992).

Statistical Analysis

Steroid concentrations in territorial and non-territorial fish along the three phases of the experiment (isolation, all-male, and mixed sex groups) were compared through a univariate two-way repeated measures analysis of variance (ANOVA) followed by contrasts analysis according to Winer, Brown and Michels (1991). When needed, to match the assumptions of ANOVA, the variables were log-transformed (i.e., T, 11KT). Dominance indexes of territorial and non-territorial fish were compared with the Mann – Whitney *U*-test. The Spearman Rank correlation was used to access the relationships among behavioral variables and between them and hormonal concentrations. Due to the large number of correlations computed we used a significance level of 1% to avoid obtaining significant correlations by chance. The Kendall *W* coefficient of concordance was used to access

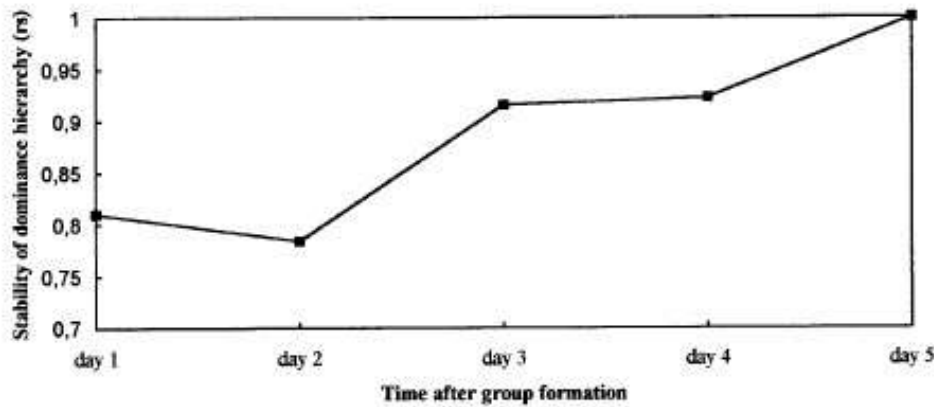


FIG. 1. Correlation coefficients between the dominance index computed daily, from day 1 to day 4, and the same index computed at day 5.

the consistency of behavioral data along the consecutive days of the study. The significance levels of the Kendall W coefficient were determined according to the procedure described by Siegel and Castellan (1988). The level of significance considered was 5%. All statistical treatments were performed using the software package STATISTICA for Windows v. 4.0 (copyright Statsoft, Inc., 1993).

RESULTS

Social Interactions after Group Formation

Isolated individuals had reduced locomotor activity and a neutral silvery coloration. They did not dig nests or show any pattern of social behavior. At day 0 during the first social interactions some individuals began to acquire a dark coloration. At the same time a substantial number of symmetric interactions (i.e., circling fights, frontal and lateral displays, mouth fighting) were observed, but these declined rapidly and from day 1 onwards the interactions observed were mostly asymmetrical (e.g., charging/fleeing). At day 1 the first signs of pit digging by the fish that became territorial were visible. By this time a rank order was already established within each group and persisted with little or no modification throughout the observation period. This conclusion was supported by the high correlation coefficient between the dominance index computed for each fish at day 1 and the same index computed at day 5 (Fig. 1). This index was consistent along the entire observation period (Kendall's coefficient of concordance for the dominance index computed for each day - 0.881, average rank R - 0.852, $p < 0.001$).

After the introduction of the females a reduction in the number of agonistic encounters was observed and

courtship behavior (already present in all-male groups) is almost exclusively directed towards females. The dominance index computed at day 1 was also a good predictor of courtship rate, nest dimensions at day 5 (see Table 1) and effective defence of a territory at the bottom (Mann - Whitney U test, $N = 16$, $U = 0$, territorial mean rank - 13.5, non-territorial mean rank - 5.5, $p < 0.01$). Thus, in the first day after group formation the social status of each individual was basically established, and it strongly influenced the likelihood of becoming territorial, the size of its nest and frequency of courtship after the introduction of females.

Urinary Steroids and Social Status

Urinary concentrations of free steroids were very low, and glucuronide and sulfate concentrations showed a high variability in relation to behavioral indexes. However, total steroid (free + glucuronide + sulfate) concentrations showed high correlations with behavior and those are the results presented hereafter. The use of total steroid concentrations also avoids the possible effects of differential excretion of the different steroid metabolites.

Androgens — As one of the main aims of this paper was to evaluate to what extent individual androgen concentrations prior to group formation were a good predictor of social status attained by each fish, or if social status itself could modulate individual androgen concentrations, we analyzed the correlations between androgen concentrations and behavioral variates in different social contexts.

As can be seen in Table 1, at the end of social isolation (day 0) neither T nor 11KT urinary concentrations were correlated with the dominance indexes obtained from day 1 to day 5. In contrast concentrations of both andro-

TABLE 1
Spearman Rank Correlation Coefficients (r_s) among Behavioral Variables and between Them and Sex Steroid Concentrations in the Urine

	V/V / D1	V/V / D5	V/V / D7	N _o Inter.	Court. rate	Nest volume
V/V + D1	—					
V/V + D5	0.81***	—				
V/V + D7	0.81***	0.98***	—			
N _o Inter.	0.40	0.55	0.56	—		
Court. rate	0.74**	0.92***	0.88***	0.58	—	
Nest volume	0.75***	0.91***	0.90***	0.61	0.88***	—
T ₀	0.23	0.003	-0.04	-0.17	-0.01	-0.13
T ₅	0.71**	0.80**	0.80**	0.47	0.79**	0.85***
T ₇	0.53	0.61	0.64	0.67**	0.60	0.75**
11KT ₀	0.18	0.27	0.19	-0.15	0.34	0.17
11KT ₅	0.7**	0.80**	0.83***	0.37	0.70**	0.91***
11KT ₇	0.49	0.55	0.53	0.39	0.62	0.68**
17,20 α P ₀	0.13	0.09	0.02	0.09	0.15	-0.11
17,20 α P ₅	0.81***	0.73**	0.71**	0.37	0.64	0.78**
17,20 α P ₇	0.45	0.66**	0.70**	0.57	0.60	0.68**
17,20 β P ₀	-0.23	-0.44	-0.42	-0.21	-0.41	-0.53
17,20 β P ₅	0.69**	0.54	0.59	0.61	0.51	0.71**
17,20 β P ₇	0.37	0.36	0.49	0.12	0.26	0.47

Note. $N = 16$ except for the pairs of variables that involve T₅, 11KT₅, 17,20 α P₅, or 17,20 β P₅ on which case $N = 13$, and those that involve T₇, 11KT₇, 17,20 α P₇, or 17,20 β P₇ on which case $N = 14$. Significance levels are: $P < 0.01$ (**) and $P < 0.001$ (***). V/V + D_{1, 5, 7} are the dominance indexes respectively in Days 0, 5, and 7 of the experiment. N_o Inter is the total number of interactions on which each fish participated during the all-male groups period. Court. rate is the total number of courtship episodes after the addition of the females to the groups. Nest volume was measured at Day 5. T_{0, 5, 7}, 11KT_{0, 5, 7}, 17,20 α P_{0, 5, 7}, and 17,20 β P_{0, 5, 7} are the concentrations of the sex steroids in the urine, respectively, at Day 0, 5, and 7 of the experimental procedure.

gens at day 5 were highly correlated to the dominance index at day 1. However, no significant correlation was detected between androgen concentrations and the total number of interactions, except for T in day 7. Concentrations of both androgens measured at day 5 were also correlated with the courtship rate and the volume of the nest.

Since fish that established territories showed consistently higher dominance indexes than non-territorials, we compared the androgen variations of the two categories in the different social contexts.

Social isolation fish, which after group formation became territorial, did not differ significantly both in T and 11KT from those that later failed to become territorial (Figs. 2 and 3). In this section when we designate the individuals as territorials or non-territorials we are referring to these two classes. T concentrations increased significantly in territorial and non-territorial fish when male groups were formed followed by a significant decrease when ovulating females were introduced. However, statistically significant differences ($0.02 < p < 0.03$) were only found between territorial all-male groups and non-territorials in isolation or in mixed sex groups. When male groups were formed

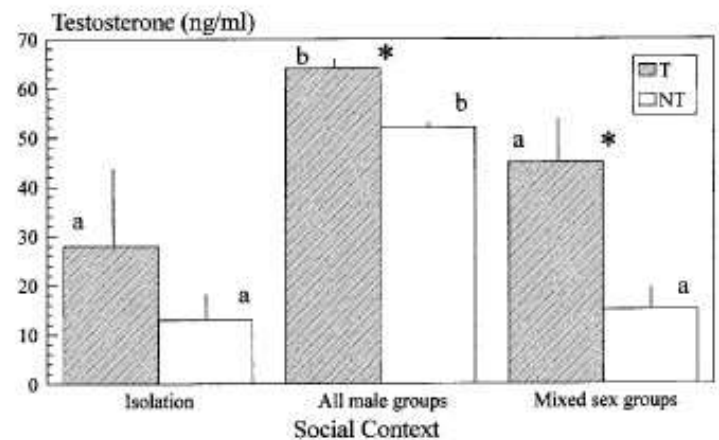


FIG. 2. Differences in testosterone concentrations in the urine (mean + SE) between territorial (T) and non-territorial fish (NT) ($F = 6.11$, $p < 0.05$), along the three phases of the experiment ($F = 8.62$, $p < 0.01$). The effect of the interaction between territoriality and the 3 different social contexts was not significant ($F = 0.95$, $p > 0.05$). Sample sizes are as follows: (1) territorials: $n_{isolation} - n_{all\ male} - n_{mixed\ sex}$ - 6; (2) non-territorials: $n_{isolation} - 10$, $n_{all\ male} - 7$, $n_{mixed\ sex} - 8$. Results of the contrast analysis are noted as follows: (i) significant differences between territorial and non-territorial fish at each phase of the experiment are marked with an *; (ii) for the comparison of the same sample (i.e., territorials or non-territorials) along the three phases of the experiment, homogeneous groups are marked with the same letter.

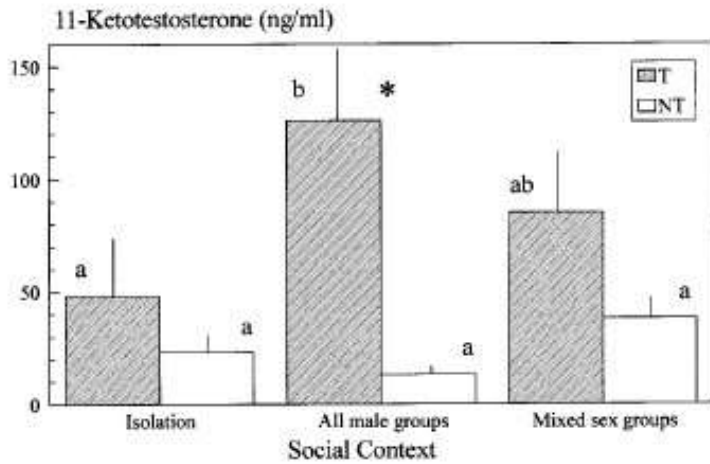


FIG. 3. Differences in 11-ketotestosterone concentrations in the urine (mean / SE) between territorial (T) and non-territorial fish (NT) ($F = 13.98$, $p < 0.01$), along the three phases of the experiment ($F = 3.08$, $p > 0.05$). There was a significant effect of the interaction between territoriality and the three different social contexts ($F = 5.47$, $p < 0.05$). Sample sizes are as follows: (1) territorials: $n_{isolation} = n_{all\ male} = n_{mixed\ sex} = 6$; (2) non-territorials: $n_{isolation} = 10$, $n_{all\ male} = 7$, $n_{mixed\ sex} = 8$. Results of the contrast analysis are noted as follows: (i) significant differences between territorial and non-territorial fish at each phase of the experiment are marked with an *; (ii) for the comparison of the same sample (i.e., territorials or non-territorials) along the three phases of the experiment, homogeneous groups are marked with the same letter.

11KT concentrations increased dramatically in territorial fish ($p < 0.001$) followed by a slight non-significant decrease when females were added. Non-territorial fish showed no significant variations in 11KT concentrations.

Progestins — In order to evaluate to what extent the concentrations of the different steroids could reflect common patterns of variation, the correlations among them were also investigated. At the end of the isolation period correlations among all steroids were not statistically significant with the exception of the correlation between T and 17,20 α -P ($r^2 = 0.625$, $n = 15$, $p < 0.05$). At this stage no significant differences in 17,20 α -P or 17,20 β -P concentrations were found between territorial and non-territorial fish (Figs. 4 and 5). When all-male groups were formed progestin concentrations increased in territorial fish and decreased in non-territorial fish. However, owing to the high degree of variability only the reduction in 17,20 β -P was found to be statistically significant ($p < 0.05$). When females were added urinary concentrations of both progestins increased slightly in non-territorials, but in territorials there was a further significant rise in 17,20 α -P concentrations ($p = 0.03$) and little change in 17,20 β -P. Like in the case of the androgens, the concentrations of both progestins

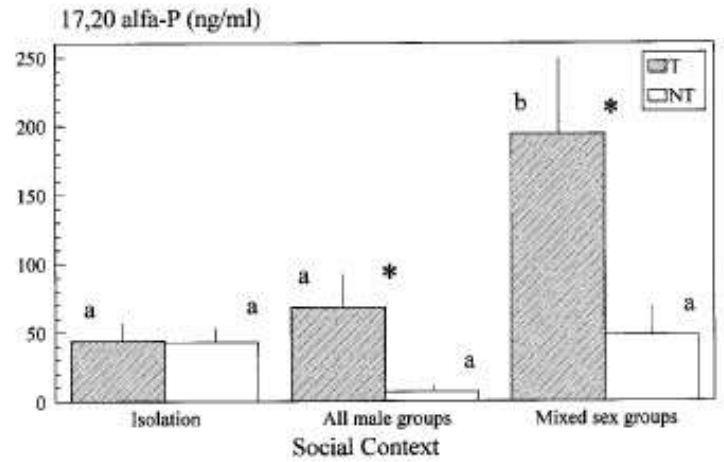


FIG. 4. Differences in 17,20 α -P concentrations in the urine (mean / SE) between territorial (T) and non-territorial fish (NT) ($F = 10.24$, $p < 0.01$), along the three phases of the experiment ($F = 6.96$, $p < 0.01$). There was a significant effect of the interaction between territoriality and the three different social contexts ($F = 3.48$, $p < 0.05$). Sample sizes are as follows: (1) territorials: $n_{isolation} = n_{all\ male} = n_{mixed\ sex} = 6$; (2) non-territorials: $n_{isolation} = 9$, $n_{all\ male} = 7$, $n_{mixed\ sex} = 8$. Results of the contrast analysis are noted as follows: (i) significant differences between territorial and non-territorial fish at each phase of the experiment are marked with an *; (ii) for the comparison of the same sample (i.e., territorials or non-territorials) along the three phases of the experiment, homogeneous groups are marked with the same letter.

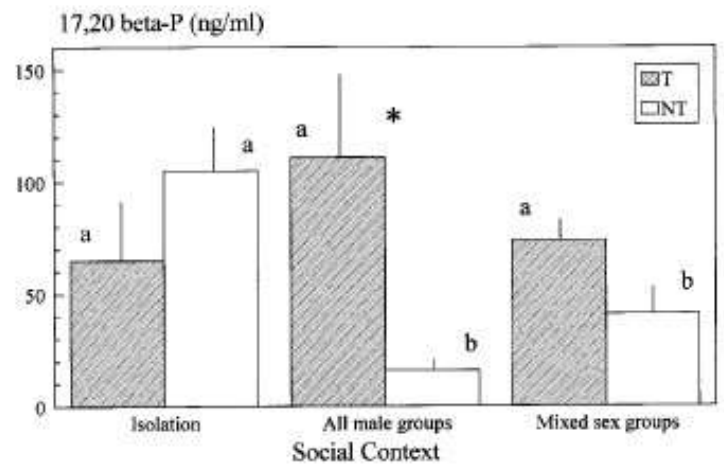


FIG. 5. Differences in 17,20 β -P concentrations in the urine (mean + SE) between territorial (T) and non-territorial fish (NT) ($F = 1.73$, $p > 0.05$), along the three phases of the experiment ($F = 1.23$, $p > 0.01$). There was a significant effect of the interaction between territoriality and the three different social contexts ($F = 5.53$, $p < 0.05$). Sample sizes are as follows: (1) territorials: $n_{isolation} = n_{all\ male} = n_{mixed\ sex} = 6$; (2) non-territorials: $n_{isolation} = 10$, $n_{all\ male} = 7$, $n_{mixed\ sex} = 8$. Results of the contrast analysis are noted as follows: (i) significant differences between territorial and non-territorial fish at each phase of the experiment are marked with an *; (ii) for the comparison of the same sample (i.e., territorials or non-territorials) along the three phases of the experiment, homogeneous groups are marked with the same letter.

measured at the end of the isolation period were not correlated with the dominance indexes after group formation. The dominance index 1 day after all-male group formation was highly correlated to the concentrations of both 17,20 α -P and 17,20 β -P 5 days after group formation (Table 1), when the concentrations of the four steroids measured were also highly correlated (Table 2). When ovulated females were introduced 17,20 α -P and 17,20 β -P concentrations were correlated to nest size (Table 1).

DISCUSSION

One basic assumption of the present paper that needs to be considered with caution is that urinary steroid concentrations provide indications about their concentrations in the plasma, and the validity of the following discussion is contingent on the validity of this assumption. Different steroids may show different temporal patterns of excretion and it may even be possible that some metabolic pathways lead to the formation of steroids that have higher excretion rates and thus a high concentration of a given steroid in the urine may reflect a physiological means for its removal from the blood. In this case, the steroid profile as measured in the urine would not be a good indicator of the profile that actually occurs in the plasma. However, there are no indications in the literature that the production of T, 11KT, 17,20 α -P, or 17,20 β -P are involved in processes of inactivation of other steroids. On the contrary, they are known to play important physiological roles in teleost fish (Kime, 1987, 1993). Moreover, the conclusions of the present study do not require that the concentration of each steroid in the urine reflect precisely its concentration in the plasma. Indeed, our results are not dependent on absolute values and moment-to-moment variations of each steroid concentration but rather in contrasting steroid profiles obtained in different social contexts after some days in each social situation. Thus,

it is likely that the errors resulting from the temporal dynamics of the excretion that would be critical in the assessment of short-term fluctuations are minimized in our experimental context. Finally, as stated in the Methods section, the available evidence for other teleost species points to the existence of good correlations between the blood and urinary levels of several steroids (Scott and Liley, 1994). Thus, in the absence of data on the dynamics of excretion in *Tilapia*, in the following discussion we assume that the contrast in steroid profiles found in the urine reflect similar contrasts in circulating steroids.

In the present study, the individuals with higher androgen levels were also higher in the social hierarchy; they were more effective in defending territories and had larger nests and a higher frequency of courtship behavior when the females were introduced. Males with a high social status also had larger gonadosomatic indexes and showed longer dorsal fin and a larger genital papilla, both being morphological displaying characters in this species (Oliveira and Almada, in preparation). Similar observations have been done in *Astatotilapia (Haplochromis) burtoni* where, in comparison with subordinates and isolation-reared fish, dominant males had higher gonadosomatic indexes, with larger Leydig cell nuclei, more spermatozoa in the testes (Fralei and Fernald, 1982), and larger cell bodies in a neuronal population in the preoptic area of the brain involved in gonadotropin-releasing hormone secretion (Davis and Fernald, 1990; Francis, Soma, and Fernald, 1993). In contrast, both subordinate and isolation-reared males *A. burtoni* showed slower development of several androgen dependent traits (Fralei and Fernald, 1982). In *O. mossambicus* social isolation also affects negatively sexual development of males (Silverman, 1978). Dominant male *Tilapia mariae* also had larger genital papillae (Schwanck, 1980), a feature that has been demonstrated to be under androgen control (Levy and Aronson, 1955).

Progesterin, 17,20 α -P and 17,20 β -P, concentrations also showed very high correlations with sexual and agonistic behavior. This is probably partly because of an acceleration of gonadal development in dominant fish in connection with a putative role for these steroids in male reproduction. 17,20 β -P is a hormone largely associated with acquisition of sperm mobility and spermiation in male salmonids (Miura *et al.*, 1992) and cyprinids (Asahina, Barry, Aida, Fusetani, and Hanyu, 1990; Asahini, Aida, and Higashi, 1993), where it also has a pheromonal role (Dulka, Stacey, Sorensen, and Van Der Kraak, 1987).

Concentrations of 17,20 β -P were higher in territorial

TABLE 2
Spearman Rank Correlation Coefficient for the Concentrations in the Urine of the Four Measured Steroids at Day 5 after Group Formation

	17,20 α -P	17,20 β -P	T	11KT
17,20 α -P	—	0.831	0.803	0.867
17,20 β -P		—	0.786	0.808
T			—	0.846

Note. All the correlation coefficients have $P < 0.01$ with $n = 13$.

fish (dominant) than in non-territorial fish in all-male groups. The addition of females brought concentrations of 17,20 β -P to similar levels in territorial and non-territorial fish. This is rather different from what happened in male kokanee salmon (*Oncorhynchus nerka*) where, despite considerable agonistic interaction in an all-male group, no clear correlations were found between androgens and 17,20 β -P and agonistic activity. When females were present dominant kokanee salmon had similar levels of steroids to subordinate fish but had more gonadotrophin and milt (Liley, Olsen, Foote, and Van Der Kraak, 1993). Finally, rainbow trout and kokanee salmon males allowed to interact with nesting females had higher steroid 17,20 β -P levels than males in the absence of females (Olsen and Liley, 1993; Rouger and Liley, 1993).

Both progestins correlated positively with nest size, but only the androgens correlated positively with courtship rate. Also, the changes observed with 17,20 α -P in *O. mossambicus* are more in line with the changes associated with 17,20 β -P in male salmonids and cyprinids, i.e., a large increase in response to the presence of ovulating females, a change that would mediate social induced increases in milt volume (Liley et al., 1993). This increase is particularly high in dominant fish and we propose that 17,20 α -P may play a role in sperm function and/or spawning behavior in male *O. mossambicus* and possibly in most male non-salmonid fish.

The question of whether or not the increase in 17,20 α -P is dependent on visual or chemical cues is still to be clarified, although evidence points towards the existence of a pheromonal component in *O. mossambicus*. Males with an unlimited access to females show an increased courtship rate during the ovulatory period in comparison to males, which have only visual contact with females (Silverman, 1978). Males also have preference to water where ovulated females were kept as opposed to water from unovulated females or pure water (Falter and Dolisy, 1989).

It has been demonstrated that androgens, in particular testosterone, have a primary role in the control of agonistic and sexual behavior of male vertebrates, including fish (see Becker, Breedlove, and Crews, 1992 for references). However, in recent years an increasing body of evidence has shown that short-term fluctuations of androgen concentrations in male vertebrates can be modulated by social interactions (Harding and Follett, 1979; Eberhart, Keverne, and Meller, 1980; Harding, 1981; Hannes, Franck, and Liemann, 1984; Sachser and Prove, 1984; Hannes, 1986; Sapolsky, 1987; Wingfield and Moore, 1987; Booth, Shelley, Mazur, Tharp, and Kittok, 1989; Greenberg and Crews, 1990; Cardwell

and Liley, 1991a). This has been interpreted as an adaptation to adjust the readiness and intensity of aggressive behavior to changes in the social environment, and to the capabilities of the animals ("challenge hypothesis," Wingfield, 1984). Such a mechanism would explain the higher concentrations of T and 11KT in territorial males than in non-territorial males of stoplight parrotfish (*Sparisoma viride*). The androgens would be responsible to maintain a higher level of aggressiveness required by territorial status. Particular high increases above the androgen levels of established territorial males during territorial disputes could also stimulate increased aggressiveness and territory vigilance, therefore maximizing the chances of territory defence (Cardwell and Liley, 1991a).

We propose that the present study provides further evidence to the "challenge hypothesis." *O. mossambicus* males were initially placed in isolation in order to provide a baseline condition in which the endocrine system would be free of social stimuli. In this situation sex steroid concentrations were presumably an indicator of the state of gonadal development. They were not correlated with the dominance index or steroid concentrations that developed when groups of males were formed. When male groups were formed there were drastic changes in steroid concentrations, highly correlated to the social hierarchy that was immediately established. The dominance index after only one day of group formation was a good predictor of the androgen concentrations measured 4 days later. This increase in androgen levels during territorial acquisition and the establishment of social hierarchies could be explained by the need to maintain increased aggressiveness during agonistic encounters. In *A. burtoni*, a cichlid fish with a breeding system similar to that of *O. mossambicus*, males kept in isolation show both low levels of aggression (Heiligenberg and Kramer, 1972) and plasma androgen concentrations and these increase very rapidly after a "challenge" stimulation by visual exposure to a territorial male (Hannes and Franck, 1983). Elevated androgen concentrations in territorial male demoiselles (*Chromis dispilus*) seemed also to be primarily involved in maintaining a high level of aggressive response demanded by status. This was particularly evident during the establishment of territorial boundaries and was a function of population density and frequency of territorial interactions (Pankhurst and Barnett, 1993; Barnett and Pankhurst, 1994).

In lekking cichlids, with a breeding system similar to that of *O. mossambicus*, it was shown that dominant fish occupy more central positions in the leks, dig larger nests, and have a higher breeding success than subordi-

nate males that occupy peripheral territories (Mckaye et al., 1990; Mckaye, 1991; Rossiter, 1992). Although field data on reproductive success for males of *O. mossambicus* are missing, similar results were found in aquarium observations (Falter and Foucart, 1991). In such social systems it may be of great adaptive value to respond to the presence of other males by a rapid increase in readiness to fight that may be maintained by increased androgen concentrations. Since larger fish tend to be dominant, smaller ones should adjust their agonistic behavior to a level that maximizes their breeding success without excessive confrontation with larger dominant fish. Social modulation of androgen concentrations as a mediating agent of agonistic regulation may be an efficient way to accomplish this compromise.

Territorial individuals had generally higher steroid concentrations than non-territorial individuals. However, the pattern of change of individual concentrations of sex steroids according to social context (isolated to all male to presence of females) was different for the various steroids measured.

Among the androgens, T had a larger increase in territorial fish when the groups of males were created, whilst 11KT increased in fish that became territorial but have no change in non-territorials. A possible explanation for this difference could be that one of the physiological consequences of subordination would be to block 11 β -hydroxylase leading to a reduction in 11KT production and an accumulation of T. This is supported by studies of steroid metabolism in male Siamese fighting fish (*Betta splendens*), where keeping fish in high densities inhibits territorial, aggressive, and reproductive behavior, and also blocks the conversion of testosterone to 11-oxy-androgens (Leitz, 1987). We suggest that this is consistent with different roles for the two androgens. T may be involved in keeping the fish in a state of behavioral readiness allowing a rapid shift to a more aggressive behavior if favorable opportunities are presented, and 11KT may be more directly involved in gamete development and maintenance of male secondary sexual characteristics, as it is more commonly accepted for male fish (Fostier, Le Gac, and Loir, 1987). The increased concentrations of T in non-territorial fish can be explained by stimulation of the social environment to which they were exposed, including territorial disputes, which they observed but restrained from participating. A differentiation of the role of the two androgens has also been proposed by Cardwell and Liley (1991b), which suggested that in the stoplight parrotfish high levels of T are associated with male status and behavior and that 11KT would play

a role in initiating sex change, the production of secondary sex characteristics, and some aspects of spawning behavior. In a review of the hormonal bases for male alternative reproductive tactics in teleosts, Brantley, Wingfield, and Bass (1993) found that the male morphotype that actively courts and shows the full set of secondary sex characteristics has higher concentrations of 11KT than the non-courting morphotype (e.g., sneakers, satellites). In contrast for T there is no consistent difference between these two male morphotypes. A characteristic peak of 11KT was associated with the onset of spawning in bluegill *Lepomis macrochirus* (Kindler, Philipp, Gross, and Bahr, 1989) and in three-spined stickleback *Gasterosteus aculeatus* (Mayer, Borg, and Schulz, 1990), and 11KT implants were more effective than T implants in increasing spawning behavior in these species (Borg, 1987; Kindler, Bahr, and Philipp, 1991). Elevated plasma concentrations of T and 11KT have been found in garibaldi, *Hypsypops rubicundus*, to be associated with courtship behavior and spawning activity (Sikkel, 1993).

In conclusion, we have shown that steroid concentrations prior to group formation were not a good predictor of social status attained by each fish and that social status itself could modulate sex steroid concentrations after group formation. We have further suggested that there is a differential role for androgens and progestins. T is more likely involved in agonistic behavior while 17,20 α -P changes are consistent with T being perhaps a sex specific (male) hormone. However, despite the high correlations obtained these results need to be consubstantiated by experiments designed to establish the roles of the individual steroids.

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