

Intra- and interspecific aggression do not modulate androgen levels in dusky gregories, yet male aggression is reduced by an androgen blocker

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A B S T R A C T

Discussions about social behavior are generally limited to fitness effects of interactions occurring between conspecifics. However, many fitness relevant interactions take place between individuals belonging to different species. Our detailed knowledge about the role of hormones in intraspecific interactions provides a starting point to investigate how far interspecific interactions are governed by the same physiological mechanisms. Here, we carried out standardized resident–intruder (sRI) tests in the laboratory to investigate the relationship between androgens and both intra- and interspecific aggression in a year-round territorial coral reef fish, the dusky gregory, *Stegastes nigricans*. This damselfish species fiercely defend cultivated algal crops, used as a food source, against a broad array of species, mainly food competitors, and thus represent an ideal model system for comparisons of intra- and interspecific territorial aggression. In a first experiment, resident *S. nigricans* showed elevated territorial aggression against intra- and interspecific intruders, yet neither elicited a significant increase in androgen levels. However, in a second experiment where we treated residents with flutamide, an androgen receptor blocker, males but not females showed decreased aggression, both towards intra- and interspecific intruders. Thus androgens appear to affect aggression in a broader territorial context where species identity of the intruder appears to play no role. This supports the idea that the same hormonal mechanism may be relevant in intra- and interspecific interactions. We further propose that in such a case, where physiological mechanisms of behavioral responses are found to be context dependent, interspecific territorial aggression should be considered a social behavior.

Keywords:

Testosterone
11-ketotestosterone
Flutamide
Teleost
Simulated resident–intruder test
Year-round territoriality
Aggression

Introduction

A behavior is considered social if it has fitness effects on both its actor and receiver (West et al., 2007). Scientists discussing social behavior typically refer to intraspecific interactions (Blumstein et al., 2010), and as an example, a recent textbook on the topic did not mention a single case of interspecific interactions (Székely et al., 2010). This omission may look surprising since organisms are sometimes embedded in a heterogeneous network where interspecific interactions are frequent and exert profound effects on fitness (Bshary, 2001; Peiman and Robinson, 2007). In addition, intra- and interspecific behaviors can be classified largely along the same line depending on their effect on the direct reproductive fitness of the different partners. At the exception of altruism which is not expected in interspecific interactions, behaviors directed towards con- or heterospecific individuals can be mutually beneficial, selfish or spiteful. From a modeling perspective, the main difference is that interaction partners belong to different gene pools in interspecific interactions, which causes mainly quantitative adjustments (Bergström et al., 2003; Doebeli and Knowlton, 1998). Thus, the question arises

whether a distinction between intra- and interspecific interactions is useful or whether it hinders a better integration of concepts.

A classical argument for a distinction between intra- and interspecific behaviors originates from studies of aggression. Intraspecific aggression shown during escalated fighting is generally restricted to the reproductive season, sex specific and facilitated by androgen hormones such as testosterone (T) (Borg, 1994; Liley and Stacey, 1983; Wingfield et al., 2006). In contrast, aggression directed against heterospecifics, like killing of prey, may be shown by both sexes and unrelated to T (Bernard, 1976; Gammie et al., 2003; Giammanco et al., 2005). This argument takes a general behavior (aggression) as the starting point for the distinction. At the proximal level though, it was postulated early on that it would be more appropriate to explore the causation of intra- and interspecific behaviors in a specific context, rather than as a function of against whom it is directed (Huntingford, 1976). In order to do so we studied the relationship between hormonal correlates with intra- and interspecific aggression in the context of territoriality. In this context, the immediate consequences of an aggressive act by the territory holder on intra- or interspecific intruders are comparable, and according to Huntingford (1976) in such a situation the aggressive response would be best brought about by a shared physiological factor. We selected androgens as a candidate physiological factor, because castration and

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replacement studies have demonstrated a central role of these steroid hormones in the causation of aggressive behavior (Adkins-Regan, 2005). For intraspecific territorial aggression a two-way causal relationship has been described, where social challenges stimulate the release of androgens, which in turn facilitate territorial aggression towards intruders (Hau, 2007; Oliveira, 2004; Ros et al., 2002, 2004; Wingfield et al., 1990). This relationship has been mostly investigated during the reproductive period, and has generally been shown to vary with the degree of social instability associated with parenting style, and mating system (Goymann, 2009; Hirschenhauser and Oliveira, 2006; Wingfield et al., 1990, 2006). Indeed, it has been shown that socially induced increases in androgen levels, in particular in testosterone (T), can be induced experimentally by simulating a territorial intrusion using decoys (Desjardins et al., 2006; Gleason et al., 2009; Goymann, 2009; Hay and Pankhurst, 2005; Hirschenhauser et al., 2004; Ramenofsky, 1984).

In coral reefs, niche overlaps between species may lead to both intra- and interspecific competition for shelter or food (Holbrook and Schmitt, 2002; Muñoz and Motta, 2000; Myrberg and Thresher, 1974). This is particularly evident in some species of damselfish that are called 'gardeners' as they grow and harvest algae as a food source (Ceccarelli, 2007; Karino and Nakazono, 1993), and defend these in a permanent territory against a broad array of potential competitors throughout the year (Di Paola et al., 2012; Ebersole, 1977; Hata and Kato, 2002, 2003, 2006; Hata et al., 2010). Our study species, the dusky gregory, *Stegastes nigricans*, forms colonies where each individual solitary defends its algal crop (Karino and Kuwamura, 1997). Here we address two main issues. We first ask whether the relationship between androgens and intraspecific territorial aggression, as observed in reproductively active territorial fish (Desjardins et al., 2006; Hirschenhauser et al., 2004; Ros et al., 2004), hold outside of the reproductive period. We then question whether this relationship found for intraspecific territoriality would be similar when territorial aggression is directed against heterospecific intruders.

In our first experiment, we measured the effects of both intra- and interspecific territorial aggression on plasma levels of androgens, focusing on T and 11-ketotestosterone (KT). These two androgens are behaviorally the most relevant in teleosts (Borg, 1994; Kime, 1993). In a second experiment, we investigated any causal effect of androgens on territorial aggression by treating the residents with slow-release implants of flutamide, an androgen receptor blocker (Sebire et al., 2008), or castor oil as a control. We compared territorial aggression towards conspecific and heterospecific intruders to test whether androgens might act as a common causal factor for both types of responses. As a heterospecific intruder we selected another year round territorial damselfish, the herbivore *Plectroglyphidodon lacrymatus*. Under natural conditions, *P. lacrymatus* are often found on the edges of *S. nigricans* colonies and competition might occur over vacant territories which also represent a food resource (Hata and Kato, 2006). Occasional aggressive interactions have indeed been observed between free living individuals of the two species (Vulllioud, personal observation). Because the endocrine system varies with sex, we tested for possible sex-specific effects of androgens on territorial behaviors by including both males and females in our study.

Materials and methods

Animals, handling and housing

S. nigricans is a monomorphic damselfish species, whose small colonies are found on shallow coral reef patches (Karino and Kuwamura, 1997). Its territory has multiple purposes as it supplies algae to feed on, shelter between the coral, and substrate for breeding. During the reproductive period, which is restricted to summer months (see Galzin, 1987; Jan et al., 2003; Karino and Nakazono, 1993), females temporarily leave their territory to spawn in the territories

of males (Karino, 1999; Karino and Nakazono, 1993). Gonads are regressed in non-reproductively active *S. nigricans* (Karino, 1999), and seasonal variation in gonad size has been described at low to intermediate levels in the winter period (Galzin, 1987). The experiment measuring the behavioral and androgen responses of residents *S. nigricans* exposed to standardized resident-intruder (sRI) tests (experiment 1) was conducted from June to September 2010 at the Lizard Island Research Station (Southern hemisphere: -14.696, 145.455). The experiment determining the flutamide effects on behavioral response towards intra- and interspecific sRI (experiment 2) was carried out from September to November 2011 at the Dahab Marine Research Centre (DMRC, DiveIn) in Dahab, Egypt (Northern hemisphere: 28.470, 34.508).

In both experiments individuals were collected (at 2–10 m depth) by spraying a small amount of clove oil (20% clove oil diluted in 80% ethanol) in the territories, and capturing the slightly sedated individuals using a hand net. Subsequently, fish were placed in a sealed plastic bag with fresh seawater and quickly transported to laboratory facilities where they were sexed by microscopic examination of genital papillae (e.g. Fishelson, 1998; Thresher, 1984).

Fish were housed individually and prevented from any visual and chemical contact with other fish. Each tank was provided with continuously flowing seawater and shaded from direct sunlight to keep water temperature and light conditions similar to those on the reef. To provide shelter and provoke natural behavior, a PVC pipe was placed at the back of the tank as a substitute for the coral rubble with algae that *S. nigricans* defends in nature (see Glickman and Caldwell, 1994). Pilot experiments with *S. nigricans*, and experiments with the related damselfish *P. lacrymatus* (Di Paola et al., 2012), have shown that residents in a tank with a pipe display vigorous aggressive behavior during sRI tests, resembling territorial aggression in the field. Fish were daily fed with commercial flakes for tropical fishes. Before beginning the experiments, all individuals were allowed to recover and acclimatize to the new environment for at least one week. This is a standard procedure in studies on fish endocrinology to minimize the impact of possible variation in prior social experiences.

Experiment 1: androgen modulation and territorial aggression

The measurements were based on a common design to test modulation of steroid hormones in territorial animals, the simulated resident-intruder (sRI) test (Trainor et al., 2009; Wingfield et al., 1990): the territory holder or resident is temporarily exposed to an unknown opponent, the intruder, and subsequently captured for blood sampling. The effect of sRI tests on steroid hormone levels is expressed as the level measured after sRI test (challenge level) compared to the level measured after a control period. Our sRI test was carried out by placing a conspecific or a heterospecific (*P. lacrymatus*) intruder in a transparent tube (Plexiglas 13 cm × 10 cm × 35 cm, filled with sea water) opposite to the shelter of the resident. The tube was left in the aquarium for 15 min during which all the behaviors of the resident were recorded using a Dictaphone. After the test the tube was taken out of the aquarium, and after a small delay a blood sample was drawn (see below). Controls for sRI comprised all of the sRI procedures but in the absence of the intruder in the transparent tube: i.e. standardized resident-intruder control (sRI-c) test. sRI and sRI-c tests took place in the afternoon between 12:30 h and 16:50 h.

A total of 69 *S. nigricans* individuals (Fork Length, FL, mean = 11.01 cm; SE = 0.54 cm) were captured. Of these individuals 46 were assigned to be "residents" and the remaining 23 individuals were assigned to be the conspecific "intruders". Additionally, 21 *P. lacrymatus* (FL mean = 8.02 cm; SE = 0.53 cm) were captured to be used as heterospecific "intruders". This resulted in the following 11 male-male and 12 female-female resident-intruder dyads per intra- and interspecific sRI test. The allocation of individuals to each group was semi-random as we balanced each group for size differences. Each resident was tested

in a sRI-c and in a sRI test, with the order counterbalanced across subjects. Intraspecific intruders were tested once only. Owing to limitations in capture time, two *P. lacrymatus* were used twice as interspecific intruders, with a delay of at least 24 h between these tests. sRI-c and sRI tests were interspaced by at least 14 days to allow the fish to recover from blood sampling. For each resident–intruder dyad, we matched the individuals' size in order to minimize asymmetries in fighting ability between contestants. For inter-specific sRI tests, owing to the smaller average size of *P. lacrymatus* than *S. nigricans*, we used assortative matching of resident–intruder dyads, i.e. bigger *P. lacrymatus* were paired with bigger *S. nigricans*.

Experiment 2: effects of flutamide on territorial aggression

A total of 31 *S. nigricans* and 9 *P. lacrymatus* were captured. As described for experiment 1, *S. nigricans* were assigned to the “resident” or the “intruder” group, whereas *P. lacrymatus* were exclusively used as intruders. Residents were semi-randomly allocated to a control or a flutamide treatment. Two days after capture, the residents were removed from their home tank and placed in a bucket containing 2-phenoxy-ethanol (0.5 ml/l, Koimed) for anesthesia. They then received a 1 cm intra peritoneal silastic implant (20 µl volume) filled with either crystallized flutamide (flutamide group) or castor oil (control group), and were then returned to their home tank where they were observed to rapidly resume normal activities. One of the fish with a flutamide implant lost its implant during the experiment, and was excluded of the analysis. This resulted in the following groups: control = 6 males and 9 females, flutamide = 8 males and 8 females.

Each resident was challenged in one intraspecific and one interspecific sRI test on day 5 or 6 post-surgery between 11.00 h and 14.00 h. The order of sRI was balanced within each treatment group. The same intruders were used repeatedly for sRI tests. sRI tests followed a similar protocol as in experiment 1.

Behavioral observations

The protocol for recording behavior was described for a related damselfish (Di Paola et al., 2012). Behavior was recorded by a single observer using a Dictaphone. We analyzed the behavior using the following classification: (1) all occurrences of mouth contact of the resident with the tube in which the intruder was placed was called “aggression” (2) the sum of the frequency of “threat display” and “tail beat” was called “agonistic display”; (3) as a measurement of activity we calculated the amount of time the resident was in the PVC pipe and called it “hiding”; (4) the latency in seconds to express the first agonistic display or aggression was called the “Response latency”. Hereafter we refer to territorial aggression as including both agonistic display and aggression.

Blood sampling

Prior to sampling, residents were hand netted and placed in a bucket containing MS222 (concentration 1:10,000; experiment 1) or 2-phenoxy-ethanol (0.5 ml/l, Koimed; experiment 2) for anesthesia. Blood samples were then collected from the caudal vasculature with a heparinized syringe. In experiment 1, blood samples were collected 5–6 min after the end of the sRI(-c) tests in order to further establish the androgen responsiveness to sRI. In experiment 2, blood samples were collected on day seven post-surgery (i.e. one day after the last sRI test) in order to assess the effect of the treatment (i.e. flutamide or control) on basal plasma levels of androgen. Samples were then centrifuged for 5 min at 7000 rpm and plasma was stored in a freezer until further analyses in the laboratory. In experiment 1, we allowed a three minutes delay after the sRI(-c) tests because evidence exists that positive information on a contest outcome (winning) is required to trigger an increase in androgen levels (Oliveira et al., 2005). We assumed that

residents would interpret the disappearance of the intruder as such a winning experience.

Hormonal analysis

Experiment 1: Plasma levels of T and KT were measured using two commercial EIA kits (Cayman Chemical, Ann Arbor, MI, ref. nr. 582701 and 582751). Values of T and KT level were expected to fall in the range of data that could be found in the literature for other damselfish species (Pankhurst, 1990; Sikkel, 1993). The procedure included two serial extractions of 20 µl of blood plasma in 2 ml of diethyl ether. Extracts were vacuum dried and suspended in ELISA buffer. The rest of the analysis was carried out following the protocol provided by the manufacturer and plates were read under 412 nm (Microplate Reader Synergy HT).

Experiment 2: Levels of androgens were low in the EIA results of experiment 1. This raised the question whether a metabolite of T with some affinity for the antibodies used would influence the measurements. In order to develop a method in which we would be more sensitive in detecting the metabolite of choice we applied a HPLC method to measure androgen plasma levels. The extraction of the sample was carried out as in experiment 1 with the exception that we used a mixture of 25% hexane, 75% diethyl ether (HPLC grade) for collecting the steroid fraction from 80 µl of plasma.

The analyses were optimized by ultra-high pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS), (Ultimate 3000 RS-Dionex, Thermo Fisher Scientific coupled to 4000 QTrap-ABSciex, MA, USA). Using the multiple reaction monitoring (MRM) mode, transitions specific for testosterone, 11-ketotestosterone and adrenosterone (also known as 11-ketoandrostenedione) were monitored. This MRM method greatly increased the sensitivity of the mass spectrometer analysis by decreasing the background noise. The steroid fraction was reconstituted in 80 µl 50% methanol 50% miliQ H₂O and 10 µl was injected in a Acquity BEH C18 column (1.7 µm particle size, 2.1 × 50 mm, Waters, Milford, MA, USA), in a gradient mode with as eluent acetonitrile–H₂O, the flow rate at 0.4 mL/min.

Spiking the samples with the steroids of interest gave a 93% recovery. Results for the different hormones were variable. We did not consider the cases where the LC/MS results were having a high noise to signal level. This was especially the case for many KT measurements (34 out of 79, other steroids 1 out of 79).

Ethical commitment

The reefs around Lizard Island in Australia and Dahab in Egypt are protected areas with strict regulations issued not to cause damage to the fragile reefs. We took special care to select procedures that allowed us to collect our samples with minimal impact on these reef ecosystems. We adhere to the Animal Care and Protection Act Qld (2002), the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th edition 2004), and the regulations set by the Egyptian Environmental Affairs Agency (EEAA, Cairo). We used anesthesia to reduce stress of treatment, handling and sampling the fish. After anesthesia, fish were allowed to recover in an isolated and well-aerated container. After the experiments, all fish were released at the reef flat from which they were captured.

Research permits for Lizard Island were granted by the GBMPA and the Animal Ethics Committee of the University of Queensland, and for Dahab by the Suez Canal University (affiliated with NCS/EEAA, Egypt).

Data analysis

First variables were checked for violations of the normality assumption using the Lilliefors adaption of the Kolmogorov–Smirnov tests. To

meet this assumption we reduced the influence of outliers by transforming data using the rankit transformation (Conover and Iman, 1981) in case of behavior (agonistic display, aggression, hiding, and response latencies) and using $\log_{10}(x + 1)$ in case of steroid hormone levels. Second, in order to correct for the collection of multiple behaviors we calculated multivariate statistics (MANOVA) to test the effect of sex, species (intra- or interspecific sRI), treatment (flutamide or control), as well as their interaction effects. In these analyses, the individual was used as error term to assign sRI-c and sRI tests as repeated measures. In case the resulting Pillai's tests were significant we further calculated univariate statistics. The correlation between aggression and androgen levels was established with Spearman correlations. In all the graphs presented in the Results section, arithmetic means are shown as well as standard errors. Statistical analyses were performed in R version 2.15 (R Core Team, 2012).

Results

Experiment 1: effects of sRI on behavior and androgen levels

The multivariate test (Table 1) showed a significant effect of the intruder (sRI) on behavioral response (effect sRI vs sRI-c: Pillai's Trace [1,42] = 0.79, $p < 0.001$). No significant effect of the intruder species, or of the gender of the resident was found (main and interaction effects: Pillai's Trace [1,42] < 0.095, $p > 0.13$). Post-hoc univariate tests showed a significant increase due to the intruder in agonistic displays and in aggression (Fig. 1; agonistic display, $F[1,45] = 105.4$, $p < 0.001$; aggression, $F(1,45) = 85.2$, $p < 0.001$) and a decrease in hiding duration (Fig. 1; $F[1,45] = 29.85$, $p < 0.001$). Levels of aggression, agonistic display and hiding duration during sRI tests were significantly related to each other (Spearman correlation, $|r_{\text{rho}}| > 0.47$, $n = 46$, $p < 0.001$).

Response latencies of residents were significantly longer in interspecific than in intraspecific sRI tests (see Table 1; $F[1,42] = 14.4$, $p < 0.001$). The median response time of residents in intraspecific sRI tests was 17 s while in interspecific sRI tests it was 29 s. No significant effect of gender was detected ($F[1,42] = 3.02$, $p = 0.089$; interaction with intruder species: $F[1,42] = 0.27$, $p = 0.61$).

In contrast to the effects on aggression and territorial behaviors, sRI tests did not cause a significant increase in T or KT levels (Fig. 2; sRI vs sRI-c: T, $F[1,38] = 2.45$, $p = 0.13$; KT, $F[1,38] = 2.52$, $p = 0.12$).

Also no differences in androgen levels were found between males and females or between intra- and interspecific sRI tests (Fig. 2; within subject effects for sex and intruder species: T, $F[1,38] < 2.45$, $p > 0.13$; KT, $F[1,38] < 2.21$, $p > 0.15$). T levels after sRI were positively related with KT levels (Spearman correlation, $\rho = 0.36$, $p = 0.020$). No significant relationships were found between the territorial behaviors and androgen levels (Spearman correlation, $|r_{\text{rho}}| < 0.09$, $p > 0.57$).

Experiment 2: effects of flutamide on behavioral response to sRI

The effects of flutamide manipulation are summarized in Table 1. Different from experiment 1, interspecific and intraspecific sRI tests resulted in significant different behavioral responses (intruder species: Pillai's Trace [1,26] = 17.3, $p < 0.001$, interaction effects with treatment and sex: Pillai's Trace [1,26] < 0.26, $p > 0.057$). This effect could be attributed to a higher frequency of agonistic display in intra-specific than in inter-specific sRI tests ($F[1,29] = 33.07$, $p < 0.001$). The values of aggression and hiding did not show a difference due to type of sRI ($F[1,29] < 2.44$, $p > 0.13$). No significant differences in response latency were found based on any of the factors of interest ($F[1,26] < 4.06$, $p > 0.054$).

The overall effects of flutamide treatment on the different behaviors were not significant (Pillai's Trace [1,26] < 0.13, $p > 0.32$). However the effect of flutamide treatment significantly differed between males and females (Fig. 3; Pillai's Trace [1,26] = 0.34, $p = 0.018$) and this was not dependent on the identity of the intruder species (interaction effect treatment and intruder species: Pillai's Trace [1,26] = 0.02, $p = 0.92$). Post-hoc analyses indicated that flutamide treatment blocked territorial behaviors and increased the time hiding in males (Fig. 3; aggression: $t[12] = -3.07$, $p = 0.0098$; agonistic display: $t[12] = -2.23$, $p = 0.046$; hiding: $t[12] = 3.08$, $p = 0.0096$), whereas it had no significant effect on these behaviors in females ($|t[12]| < 2.04$, $p > 0.060$).

In the flutamide treatment experiment, T and KT levels (drawn one day after sRI test) were close to the lower quantitation levels of the HPLC. Our estimation of these levels was 0.07 ng/ml (sem = 0.02 ng/ml, $n = 16$) for T and 0.23 ng/ml (sem = 0.20 ng/ml, $n = 10$) for KT. We found high levels of adrenosterone (a prohormone for KT). However, no significant effects of sex or flutamide treatment on any of these levels could be detected (all $p > 0.08$).

Table 1

Median values with 25% and 75% quartiles between brackets and the results of the main statistical models.

| Treatment | Gender | sRI-type | N | Aggression | Agonistic display | Hiding | Response latency |
|---|--------|---------------|----|---------------------|--------------------|---------------------|--------------------|
| <i>Experiment 1, sRI = standardized resident-intruder test, sRI-c = similar as sRI but with empty tube as "intruder"</i> | | | | | | | |
| sRI-c | Female | Intraspecific | 12 | 2.0 (0.8–39.8) | 1.0 (0.0–3.0) | 403.5 (184.3–774.0) | 191.5 (30.8–432.8) |
| sRI | Female | Intraspecific | | 94.5 (45.0–139.8) | 24.0 (11.5–36.0) | 284.5 (8.3–494.8) | 20.0 (5.8–40.8) |
| sRI-c | Male | Intraspecific | 11 | 0.0 (0.0–17.5) | 0.0 (0.0–3.5) | 845.0 (411.5–883.5) | 380.0 (64.5–900.0) |
| sRI | Male | Intraspecific | | 49.0 (18.5–107.5) | 25.0 (17.5–42.5) | 485.0 (126.0–623.5) | 17.0 (7.0–29.5) |
| sRI-c | Female | Interspecific | 12 | 0.0 (0.0–7.0) | 0.0 (0.0–0.8) | 838.5 (409.5–900.0) | 782.0 (61.3–900.0) |
| sRI | Female | Interspecific | | 34.0 (2.3–105.3) | 11.5 (1.5–27.8) | 692.5 (149.8–891.8) | 106.5 (10.0–774.0) |
| sRI-c | Male | Interspecific | 11 | 0.0 (0.0–18.5) | 0.0 (0.0–4.0) | 678.0 (461.0–890.5) | 365.0 (95.5–900.0) |
| sRI | Male | Interspecific | | 123.0 (75.5–216.5) | 22.0 (15.5–26.0) | 330.0 (53.5–626.0) | 24.0 (4.5–161.5) |
| Manova Pillai's trace: effect treatment (repeated factor) = 0.79, $p < 0.001$; effect gender = 0.10, n.s.; effect sRI-type = 0.11, n.s. | | | | | | | |
| <i>Experiment2, standardized resident-intruder (sRI) tests with animals treated with oil (as control) or flutamide implants</i> | | | | | | | |
| Oil | Female | Intraspecific | 8 | 95.0 (22.5–138.8) | 23.5 (4.5–41.5) | 165.5 (54.0–843.8) | 7.5 (3.8–43.0) |
| Oil | Female | Interspecific | | 13.5 (6.5–50.3) | 28.5 (5.3–71.8) | 408.0 (170.3–861.0) | 12.5 (3.0–346.5) |
| Oil | Male | Intraspecific | 6 | 144.0 (80.3–224.3) | 43.5 (17.3–57.0) | 171.5 (59.8–312.5) | 8.5 (4.5–23.0) |
| Oil | Male | Interspecific | | 178.0 (73.3–296.3) | 103.5 (68.5–139.3) | 42.0 (5.0–201.3) | 3.5 (3.0–10.0) |
| Flutamide | Female | Intraspecific | 8 | 207.0 (139.5–305.5) | 29.0 (19.5–36.8) | 53.0 (16.8–337.3) | 7.0 (3.0–12.8) |
| Flutamide | Female | Interspecific | | 138.5 (86.8–246.3) | 71.5 (55.3–117.5) | 94.0 (14.5–500.8) | 4.5 (2.8–7.7) |
| Flutamide | Male | Intraspecific | 8 | 25.0 (7.0–112.8) | 10.5 (0.0–24.8) | 560.0 (337.8–888.3) | 261.0 (10.7–900.0) |
| Flutamide | Male | Interspecific | | 13.5 (0.0–59.0) | 28.5 (0.0–67.0) | 671.5 (306.3–899.3) | 289.0 (10.0–652.5) |
| Manova Pillai's trace: effect treatment = 0.067, n.s.; effect gender = 0.13, n.s.; effect sRI-type (repeated factor) = 0.68, $p < 0.001$ significant interaction: effect treatment * effect gender = 0.34, $p = 0.018$; other interactions: n.s. | | | | | | | |

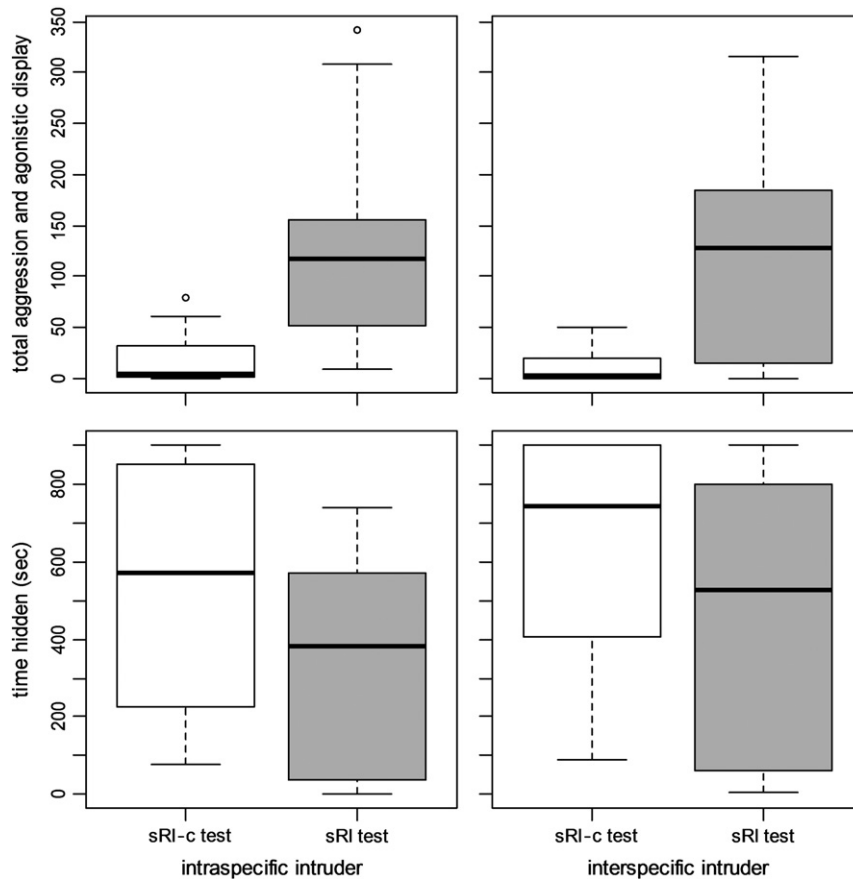


Fig. 1. Boxplots of territorial behaviors (aggression and agonistic display) and hiding during standardized tests. The tests consisted of 15 min exposure to an empty tube as control [i.e. simulated resident-control (sRI-c) test, white bars], or to a tube with a conspecific or heterospecific intruder [i.e. simulated resident-intruder (sRI) test, grey bars].

Discussion

We measured the behavioral and endocrine responses to sRI tests in a damselfish, *S. nigricans*, with the aim to assess i) the relationship between androgens and territorial behavior in a non-reproductively active teleost and ii) whether this relationship might differ across intra- and interspecific situations. sRI tests triggered a strong behavioral response, including a high frequency of agonistic displays and aggression, which was not significantly different between males and females. In contrast to our expectation, the marked increase in aggression did not translate into changes in the circulating levels of T and KT. Furthermore, there was no significant relationship between variation in androgen levels and behavioral responses to sRI tests. These findings corroborate for the first time in a teleost, the hypothesis that territorial aggression displayed outside of the reproductive period does not show a relationship with variation in peripheral levels of androgens. Still low levels of androgens may be necessary to maintain aggressive responsiveness, since we found that long term treatment with the androgen receptor blocker flutamide decreased the behavioral responses in males, but not in females. In addition, in a broader territorial context, these mediating effects of androgens on aggression might be shared across intra- and interspecific situations.

Year-round territorial aggression and androgens

Hormonal regulation of territorial behavior plays an important role in periods of social instability as occurs, among others, during intruder challenges on the territory (reviewed in Goymann, 2009; Oliveira, 2004; Wingfield et al., 1997; but see Apfelbeck et al., 2013). To our knowledge, androgen levels after sRI tests had never been measured in a year-round territorial fish species outside of the reproductive period. In

experiment 1, we found relatively low circulating levels of androgens, 1.0 ng/ml for T and 0.25 ng/ml for KT, that were not modulated by intruder challenges in both male and female residents. Social modulation of androgens in teleosts has mainly been studied in reproductively active species and elevated T or KT levels are generally associated with increased aggressive behavior. For example T but not KT levels increase in residents after a male sRI test in the related seasonally territorial species *Acanthochromis polyacanthus* (to about T = 2.2 ng/ml; KT = 1.0 ng/ml; Hay and Pankhurst, 2005). Further, T and KT levels are higher in males of *Chromis dispilus* that defend a territory in an area with high population density and that have more frequent social interactions in comparison to males that defend a territory at low population density (T = up to 7 ng/ml KT up to 38 ng/ml; Pankhurst and Barnett, 1993). The results we obtained for sRI in *S. nigricans* are consistent with those of bird species that maintain territorial aggression year-round. In these species, testosterone levels are relatively low and unrelated to sRI tests in the non-reproductive period (Canoine and Gwinner, 2002; Goymann and Landys, 2011; Levin and Wingfield, 1992; Soma et al., 2008; Wingfield, 1994). Whether in *S. nigricans* the relationship between androgens and sRI changes from the reproductive period to the non-reproductive period as found for bird species (e.g. Marasco et al., 2011) remains to be studied.

We found that circulating levels of androgens were low (experiment 1). This result is consistent with data of Galzin (1987) who showed that gonads of *S. nigricans* regress in the non-reproductive period. Still the low circulating levels might be necessary to maintain territoriality, and in addition androgens and estrogens might be locally produced in the brain (see Soma et al., 2008). Furthermore, in experiment 2 using an HPLC method we found elevated plasma levels of adrenosterone, a steroid that may be converted to KT in fishes (Páll et al., 2002). Thus, a

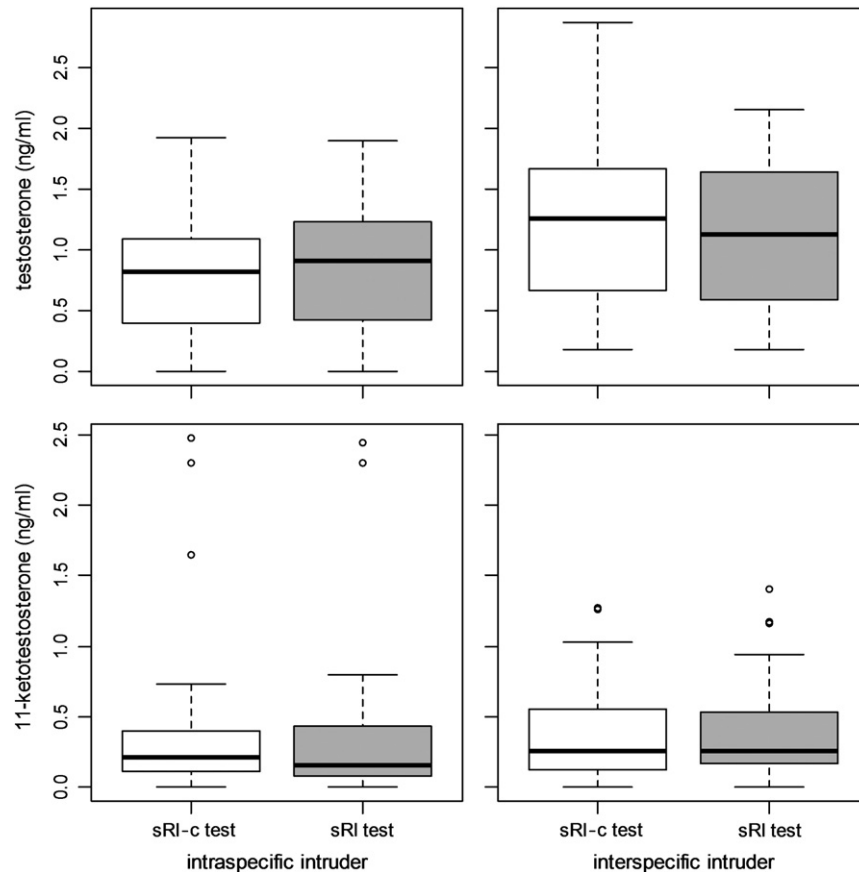


Fig. 2. Boxplots of androgen levels (testosterone and 11-ketotestosterone) measured 5 min after standardized tests. The tests consisted of 15 min exposure to an empty tube as control [i.e. simulated resident-control (sRI-c) test, white bars], or to a tube with a conspecific or heterospecific intruder [i.e. simulated resident-intruder (sRI) test, grey bars].

possible substrate for local androgen production was available in non-reproductive *S. nigricans*. In support of androgen control of territoriality, we found that treatment with the androgen receptor blocker flutamide disrupted the expression of aggressive responses in males. However, this was not the case in females where flutamide treatment even resulted in a non-significant increase in aggressive behavior. Interestingly, opposite neuro-modulatory effects of sex steroids on aggressive behavior of males and females have been proposed in a review on mammalian and avian literature by Simon (2002). In our opinion, it would be anticipated here to discuss how such opposite effects could match the year-round male and female territorial aggressiveness in *S. nigricans* and in damselfishes in general. Nevertheless there are some important differences in the territorial behavior of males and females during the reproductive period, namely, females frequently leave their territory to spawn in males territories, whereas males remain in their territory and guard the eggs or attempt to sneak sperm into neighboring nests (Karino and Nakazono, 1993). During these periods, higher levels of androgen are likely to be produced by the enlarged gonads and further hormonal manipulations might elucidate whether such changes in steroids lead to sex specific differences in territory defense, and the associated different behavioral tactics (e.g. Gonçalves et al., 2007).

The two populations sampled in this study were distant from each other. We cannot rule out that the mechanisms underlying territorial behavior in *S. nigricans* might have diverged across the two locations in response to different ecological pressures. However, an attempt to test the validity of our results on free-living animals in the Red Sea paralleled the results we obtained in Australia (experiment 1). In both locations, androgen baseline levels were low and remained unresponsive to sRI tests (Ros, Bruintjes and Vulloud, unpublished results). This is consistent with the assumption that the relationship between androgen and territorial aggression do not differ across our two study sites.

Even though we found no indication of social modulation of T or KT levels, our results indicate that low levels of these hormones may maintain some control over non-reproductive territorial aggression in males. Androgens like testosterone represent one among numerous causal factors that modulate aggression. Vasopressinergic neuropeptides like AVP/AVT, and the serotonergic systems have been identified as crucial determinants in the regulation of aggression (Trainor et al., 2009), including coral reef teleosts (Semsar et al., 2001). Indeed, AVT has been shown to increase aggression in a dose dependent manner in *Stegastes leucosticus*, a tropical year-round territorial damselfish (Santangelo and Bass, 2006). Fluoxetine, a serotonin reuptake inhibitor which enhances serotonergic neurotransmission, caused a decline in aggressive behavior in *Thalassoma bifasciatum* (Perreault et al., 2003). This is in line with the view that in general in vertebrates serotonergic activity negates the expression of aggression. Finally, stress hormones have also been shown to modulate aggressive behaviors also in an interspecific context. Androgens may act as second messengers on neurotransmitter systems and this may regulate aggressive behavior in a slow acting manner (Cologer-Clifford et al., 1998; Delville et al., 1996; Panzica et al., 2001). Such additional causal factors and their relation with androgens should be investigated to increase our knowledge on non-seasonal aggressive behavior.

Causation of interspecific social behavior

We postulated that causal mechanisms of intra- and interspecific aggressive behavior could converge when the ecological context in which the behaviors occur is similar. The territorial context in which *S. nigricans* displays intra- and interspecific aggression matches this condition as territory holders may reap similar benefits from successfully chasing conspecifics or heterospecific competitors such as *P. lacrymatus*.

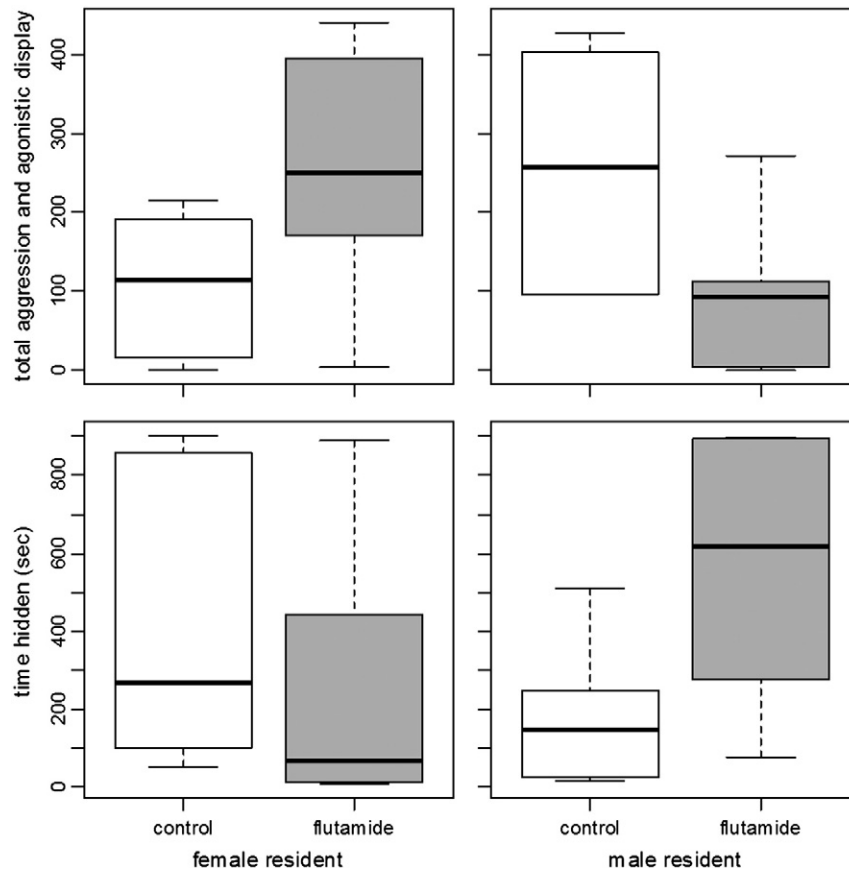


Fig. 3. Effect of slow release implant with flutamide in comparison to control treatment separated for males and females. Boxplots are of territorial behaviors (aggression and agonistic display) and hiding during a 15 min standardized resident-intruder test.

Failure or poorer ability to do so would, in both situations, translate into the loss of vital resources such as space and food. We now show that in males, flutamide blocks both intra- and interspecific territorial aggression in a similar fashion. Although this is an indication for only a modest role of androgens in modulating intra- and interspecific aggression, this result does suggest a physiological parallel across these two situations.

At the behavioral level, intra- and heterospecific sRI tests elicited territorial aggression that was very much alike in form and intensity. A single significant difference was detected between intra- and interspecific territorial response to sRI tests: the frequency of agonistic display was higher against conspecifics in experiment 2, though not in experiment 1. Individual recognition and past experience are mechanisms which have been acknowledged to mediate aggression (Oliveira, 2004) and might thus account for the differential expression of territorial aggression as a function of intruder's identity. Such factors have indeed been put forward to explain the variation of intraspecific aggression in *S. nigricans* (Jan et al., 2003) as well as differences between intra- and interspecific aggression in related species (Di Paola et al., 2012; Myrberg and Thresher, 1974). Further detailed observations and experiments on *S. nigricans*, especially on free-living individuals, are however required to clarify the similarities between intra- and interspecific territorial aggression towards *P. lacrymatus* as well as other intruding species. This, only, will help to determine the functional value of these differences and the mechanisms by which they arise.

Overall, our results suggest parallels between intra- and interspecific territorial aggression ranging from the behavioral to the physiological level, and we hypothesized that this might be brought about by convergent selection pressures. We propose that when the physiological processes mediating behaviors are context (i.e. territoriality) rather than

species (i.e. intra- or interspecific) dependent, such as occurs with aggression in non-reproductively active *S. nigricans*, those behaviors should be regarded as social.

In the last decades, research in behavioral endocrinology has changed from studying mainly species from temperate zones to include species from more tropical areas (e.g. see bird studies reviewed in Hau et al., 2008, and in Goymann and Landys, 2011, for amphibians and reptiles in Eikenaar et al., 2012, and for mammals in Cristóbal-Azkarate et al., 2006). The species-rich tropics and the coral reefs are examples of environments where many species live close together, often as competitors or mutualists. Consequently, interspecific interactions may have a large impact on the evolution of behavioral systems. We hope that integrative studies in animal behavior, such as the one presented here, would foster further research on evolutionary relevant interspecific interactions. This is necessary if we aim to increase our understanding of the dynamics and consequences of endocrine regulation of social behavior in complex social environments.

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References

- Adkins-Regan, E., 2005. *Hormones and Animal Social Behavior*. Princeton University Press.
- Apfelbeck, B., Mortega, K., Kiefer, S., Kipper, S., Vellema, M., Villavicencio, C.P., Gahr, M., Goymann, W., 2013. Associated and dissociated patterns in hormones, song, behavior and brain receptor expression between life-cycle stages in male black redstarts, *Phoenicurus ochruros*. *Gen. Comp. Endocrin.* 184, 93–102.
- Bergström, C.T., Bronstein, R.J., Bshary, R., Connor, R.C., Daly, M., Frank, S.A., Gintis, H., Keller, L., Leimar, O., Noë, R., Queller, D.C., 2003. Puzzles and predictions. In: Hammerstein, P. (Ed.), *Genetic and Cultural Evolution of Cooperation*. The MIT Press, pp. 241–256.
- Bernard, B.K., 1976. Testosterone manipulations: effects on ranacide aggression and brain monoamines in the adult female rat. *Pharmacol. Biochem. Be.* 4, 59–65.
- Blumstein, D.T., Ebensperger, L.A., Hayes, L.D., Vásquez, R.A., Ahern, T.H., Burger, J.R., Dolezal, A.G., Dosmann, A., González-Mariscal, G., Harris, B.N., Herrera, E.A., Lacey, E.A., Mateo, J., McGraw, L.A., Olazábal, D., Ramenofsky, M., Rubenstein, D.R., Sakhal, S.A., Saltzman, W., Sainz-Borgo, C., Soto-Gamboa, M., Stewart, M.L., Wey, T.W., Wingfield, J.C., Young, L.J., 2010. Toward an integrative understanding of social behavior: new models and new opportunities. *Front. Behav. Neurosci.* 4, 34.
- Borg, B., 1994. Androgens in teleost fishes. *Comp. Biochem. Phys. C* 109, 219–245.
- Bshary, R., 2001. The cleaner fish market. In: Noë, R., Van Hooff, J.A.R.A.M., Hammerstein, P. (Eds.), *Economics in Nature: Social Dilemmas. Mate Choice and Biological Markets*. Cambridge University Press, Cambridge, pp. 146–172.
- Canoine, V., Gwinner, E., 2002. Seasonal differences in the hormonal control of territorial aggression in free-living European stonechats. *Horm. Behav.* 41, 1–8.
- Ceccarelli, D.M., 2007. Modification of benthic communities by territorial damselfish: a multi-species comparison. *Coral Reefs* 26, 853–866.
- Cologer-Clifford, A., Simon, N.G., Richter, M.L., Smoluk, S.A., Lu, S., 1998. Androgens and estrogens modulate 5-HT 1A and 5-HT 1B agonist effects on aggression. *Physiol. Behav.* 65, 823–828.
- Conover, W.J., Iman, R.L., 1981. Rank transformations as a bridge between parametric and nonparametric statistics. *Am. Stat.* 35, 124–129.
- R Core Team, 2012. R: A language and environment for statistical computing.
- Cristóbal-Azkarate, J., Chavira, R., Boeck, L., Rodríguez-Luna, E., Veàl, J.J., 2006. Testosterone levels of free-ranging resident mantled howler monkey males in relation to the number and density of solitary males: a test of the challenge hypothesis. *Horm. Behav.* 49, 261–267.
- Delville, Y., Mansour, K.M., Ferris, C.F., 1996. Testosterone facilitates aggression by modulating vasopressin receptors in the hypothalamus. *Physiol. Behav.* 60, 25–29.
- Desjardins, J.K., Hazelden, M.R., Van der Kraak, G.J., Balshine, S., 2006. Male and female cooperatively breeding fish provide support for the “Challenge Hypothesis”. *Beh. Ecol.* 17, 149.
- Di Paola, V., Vulliod, P., Demarta, L., El-Alwany, M.A., Ros, A.F.H., 2012. Factors affecting interspecific aggression in a year-round territorial species, the jewel damselfish. *Ethology*.
- Doebeli, M., Knowlton, N., 1998. The evolution of interspecific mutualisms. *Proc. Natl. Acad. Sci. U. S. A.* 95, 8676–8680.
- Ebersole, J.P., 1977. The adaptive significance of interspecific territoriality in the reef fish *Eupomacentrus leucostictus*. *Ecology* 58, 914–920.
- Eikenaar, C., Husak, J., Escallón, C., Moore, I.T., 2012. Variation in testosterone and corticosterone in amphibians and reptiles: relationships with latitude, elevation, and breeding season length. *Am. Nat.* 180, 642–654.
- Fishelson, L., 1998. Behaviour, socio-ecology and sexuality in damselfishes (Pomacentridae). *Ital. J. Zool.* 65 (S1), 387–398.
- Galzin, R., 1987. Potential fisheries yield of a Moorea fringing reef (French Polynesia) by the analysis of three dominant fishes. *Atoll Res. Bull.* 305, 1–22.
- Gammie, S.C., Hasen, N.S., Rhodes, J.S., Girard, I., Garland, T., 2003. Predatory aggression, but not maternal or intermale aggression, is associated with high voluntary wheel-running behavior in mice. *Horm. Behav.* 44, 209–221.
- Giammanco, M., Tabacchi, G., Giammanco, S., Di Majo, D., La Guardia, M., 2005. Testosterone and aggressiveness. *Med. Sci. Monit.* 11, RA136–RA145.
- Gleason, E.D., Fuxjager, M.J., Oyegbile, T.O., Marler, C.A., 2009. Testosterone release and social context: when it occurs and why. *Front. Neuroendocrinol.* 30, 460–469.
- Glickman, S.E., Caldwell, G.S., 1994. Studying natural behavior in artificial environments: the problem of “salient elements”. In: Gibbons Jr., E.F. (Ed.), *In Naturalistic Environments in Captivity for Animal Behaviour Research*. Suny Press, Albany/NY, pp. 197–216.
- Gonçalves, D., Alpedrinha, J., Teles, M., Oliveira, R.F., 2007. Endocrine control of sexual behavior in sneaker males of the peacock blenny *Salaria pavo*: effects of castration, aromatase inhibition, testosterone and estradiol. *Horm. Behav.* 51, 534–541.
- Goymann, W., 2009. Social modulation of androgens in male birds. *Gen. Comp. Endocr.* 163, 149–157.
- Goymann, W., Landys, M.M., 2011. Testosterone and year-round territoriality in tropical and non-tropical songbirds. *J. Avian Biol.* 42, 485–489.
- Hata, H., Kato, M., 2002. Weeding by the herbivorous damselfish *Stegastes nigricans* in nearly monocultural algae farms. *Mar. Ecol. Prog. Ser.* 237, 227–231.
- Hata, H., Kato, M., 2003. Demise of monocultural algal farms by exclusion of territorial damselfish. *Mar. Ecol. Prog. Ser.* 263, 159–167.
- Hata, H., Kato, M., 2006. A novel obligate cultivation mutualism between damselfish and *Polysiphonia* algae. *Biol. Lett.* 2, 593.
- Hata, H., Watanabe, K., Kato, M., 2010. Geographic variation in the damselfish–red alga cultivation mutualism in the Indo-West Pacific. *BMC Evol. Biol.* 10, 185.
- Hau, M., 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *Bioessays* 29, 133–144.
- Hau, M., Gill, S.A., Goymann, W., 2008. Tropical field endocrinology: ecology and evolution of testosterone concentrations in male birds. *Gen. Comp. Endocr.* 157, 241–248.
- Hay, A.C., Pankhurst, N.W., 2005. Effect of paired encounters on plasma androgens and behaviour in males and females of the spiny damselfish *Acanthochromis polyacanthus*. *Mar. Freshw. Behav. Phy.* 38, 127–138.
- Hirschenhauser, K., Oliveira, R.F., 2006. Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. *Anim. Behav.* 71, 265–277.
- Hirschenhauser, K., Taborsky, M., Oliveira, T., Canário, A.V.M., Oliveira, R.F., 2004. A test of the “challenge hypothesis” in cichlid fish: simulated partner and territory intruder experiments. *Anim. Behav.* 68, 741–750.
- Holbrook, S.J., Schmitt, R.J., 2002. Competition for shelter space causes density-dependent predation mortality in damselfishes. *Ecology* 83, 2855–2868.
- Huntingford, F., 1976. The relationship between inter- and intra-specific aggression. *Anim. Behav.* 24, 485–497.
- Jan, R.Q., Ho, C.T., Shiah, F.K., 2003. Determinants of territory size of the dusky gregory. *J. Fish Biol.* 63, 1589–1597.
- Karino, K., 1999. Growth or reproduction: intrasexual competition in a colonial damselfish *Stegastes nigricans*. *J. Ethol.* 17, 57–62.
- Karino, K., Kuwamura, T., 1997. Plasticity in spawning visits of female damselfish, *Stegastes nigricans*: effect of distance to mates. *Beh. Ecol. Sociobiol.* 41, 55–59.
- Karino, K., Nakazono, A., 1993. Reproductive behavior of the territorial herbivore *Stegastes nigricans* (Pisces: Pomacentridae) in relation to colony formation. *J. Ethol.* 11, 99–110.
- Kime, D.E., 1993. “Classical” and “non-classical” reproductive steroids in fish. *Rev. Fish Biol. Fisher.* 3, 160–180.
- Levin, R.N., Wingfield, J.C., 1992. The hormonal-control of territorial aggression in tropical birds. *Ornis Scand.* 23, 284–291.
- Liley, N.R., Stacey, N.E., 1983. Hormones, pheromones, and reproductive behavior in fish. In: Hoar, W.S., Donaldson, E.M., Randall, D.J. (Eds.), *Fish Physiology: Reproduction, Part B: Behavior and Fertility Control*.
- Marasco, V., Fusani, L., Dessi-Fulgheri, F., Canoine, V., 2011. Non-migratory stonechats show seasonal changes in the hormonal regulation of non-seasonal territorial aggression. *Horm. Beh.* 60, 414–419.
- Muñoz, R.C., Motta, P.J., 2000. Interspecific aggression between two parrotfishes (*Sparisoma*, Scaridae) in the Florida Keys. *Copeia* 2000, 674–683.
- Myrberg, A.A., Thresher, R.E., 1974. Interspecific aggression and its relevance to the concept of territoriality in reef fishes. *Am. Zool.* 14, 81.
- Oliveira, R.F., 2004. Social modulation of androgens in vertebrates: mechanisms and function. *Adv. Stud. Behav.* 34, 165–239.
- Oliveira, R.F., Carneiro, L.A., Canário, A.V., 2005. Behavioural endocrinology: no hormonal response in tied fights. *Nature* 437, 207–208.
- Páll, M.K., Mayer, I., Borg, B., 2002. Androgen and behavior in the male three-spined stickleback, *Gasterosteus aculeatus*. II. Castration and 11-ketoandrostenedione effects on courtship and parental care during the nesting cycle. *Horm. Beh.* 42, 337–344.
- Pankhurst, N.W., 1990. Changes in plasma levels of gonadal steroids during spawning behaviour in territorial male demoiselles *Chromis dispilus* (Pisces: Pomacentridae) sampled underwater. *Gen. Comp. Endocr.* 79, 215–225.
- 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). *Gen. Comp. Endocr.* 90, 168–176.
- Panzica, G.C., Aste, N., Castagna, C., Viglietti-Panzica, C., Balthazart, J., 2001. Steroid-induced plasticity in the sexually dimorphic vasotocinergic innervation of the avian brain: behavioral implications. *Brain Res. Brain Res. Rev.* 37, 178–200.
- Peiman, K., Robinson, B., 2007. Heterospecific aggression and adaptive divergence in brook stickleback (*Culaea inconstans*). *Evol.* 61, 1327–1338.
- Perreault, H.A.N., Semsar, K., Godwin, J., 2003. Fluoxetine treatment decreases territorial aggression in a coral reef fish. *Physiol. Behav.* 79, 719–724.
- Ramenofsky, M., 1984. Agonistic behaviour and endogenous plasma hormones in male Japanese quail. *Anim. Behav.* 32, 698–708.
- Ros, A.F.H., Dieleman, S.J., Groothuis, T.G.G., 2002. Social stimuli, testosterone, and aggression in gull chicks: support for the challenge hypothesis. *Horm. Behav.* 41, 334–342.
- Ros, A.F.H., Bruintjes, R., Santos, R.S., Canário, A.V.M., Oliveira, R.F., 2004. The role of androgens in the trade-off between territorial and parental behavior in the Azorean rock-pool blenny, *Parablennius parvicornis*. *Horm. Behav.* 46, 491–497.
- Santangelo, N., Bass, A.H., 2006. New insights into neuropeptide modulation of aggression: field studies of arginine vasotocin in a territorial tropical damselfish. *Proc. Biol. Sci.* 273, 3085–3092.
- Sebire, M., Allen, Y., Bersuder, P., Katsiadaki, I., 2008. The model anti-androgen flutamide suppresses the expression of typical male stickleback reproductive behaviour. *Aquat. Toxicol.* 90, 37–47.
- Semsar, K., Kandel, F.L., Godwin, J., 2001. Manipulations of the AVT system shift social status and related courtship and aggressive behavior in the bluehead wrasse. *Horm. Behav.* 40, 21–31.

- Sikkel, P.C., 1993. Changes in plasma androgen levels associated with changes in male reproductive behavior in a brood cycling marine fish. *Gen. Comp. Endocr.* 89, 229–237.
- Simon, N.G., 2002. Hormonal processes in the development and expression of aggressive behavior. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Rubin, R.T. (Eds.), *Hormones Brain and Behavior*. Academic Press, New York, pp. 339–392.
- Soma, K.K., Scotti, M.A., Newman, A.E., Charlier, T.D., Demas, G.E., 2008. Novel mechanisms for neuroendocrine regulation of aggression. *Front. Neuroendocrinol.* 29, 476–489.
- Székely, T., Moore, A.J., Komdeur, J., 2010. *Social Behaviour: Genes*. Cambridge University Press, Ecology and Evolution.
- Thresher, R., 1984. *Reproduction in Reef Fishes*. Tfh Pubns Inc.
- Trainor, B.C., Sisk, C.L., Nelson, R.J., 2009. Hormones and the development and expression of aggressive behavior. In: Pfaff, D., Arnold, A., Etgen, A., Fahrbach, S., Rubin, R. (Eds.), *Hormones. Brain and Behavior*. Academic Press, San Diego, pp. 167–203.
- West, S.A., Griffin, A.S., Gardner, A., 2007. Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. *J. Evol. Biol.* 20, 415–432.
- Wingfield, J.C., 1994. Regulation of territorial behavior in the sedentary song sparrow, *Melospiza melodia morphna*. *Horm. Behav.* 28, 1–15.
- Wingfield, J.C., Hegner, R.E., Dufty, J., Ball, G.F., 1990. The “Challenge Hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am. Nat.* 136, 829–846.
- Wingfield, J.C., Jacobs, J., Hillgarth, N., 1997. Ecological constraints and the evolution of hormone–behavior interrelationships. In: Carter, C.S., Lederhendler, I.I. (Eds.), *The Integrative Neurobiology of Affiliation*. MIT Press, pp. 22–41.
- Wingfield, J.C., Moore, I.T., Goymann, W., Wacker, D.W., Sperry, T., 2006. Contexts and ethology of vertebrate aggression: implications for the evolution of hormone–behavior interactions. In: Nelson, R.J. (Ed.), *Biology of Aggression*, pp. 179–211.