Hormones and Behavior 71 (2015) 75–82

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Intra- and interspecific social challenges modulate the levels of an androgen precursor in a seasonally territorial tropical damselfish

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ARTICLE INFO

Article history:
Received 22 November 2014
Revised 8 April 2015
Accepted 10 April 2015
Available online 24 April 2015

Keywords:
Testosterone
Cortisol
11-Ketotestosterone
11-Ketoandrostenedione
Aggression
Challenge Hypothesis
Simulated territorial intrusion
Intra- and interspecific interactions

ABSTRACT

Studies on different vertebrate groups have provided evidence that androgen levels in males increase after competitive social interactions during the breeding season, as postulated by the Challenge Hypothesis. However, social modulation of androgen levels may vary with latitude and may differ between species holding seasonal versus year-round territories. Here, we tested the Challenge Hypothesis on a seasonal tropical damselfish, *Abudefduf sexfasciatus*, where males temporarily defend territory and eggs against both intra- and interspecific individuals. Carrying out simulated territorial intrusions (STIs) in the laboratory, we document for the first time a consistent increase in the plasma level of the androgen precursor 11-ketoandrostenedione (11KA) in fish confronted to either intra- or interspecific challenges. Collecting samples in the field also revealed higher 11KA levels in fish facing frequent territorial interactions than in non-territorial individuals. Levels of 11-ketotestosterone (11KT) were high in territorial males in the field, but were not incremented after simulated territorial intrusions in the laboratory. Plasma levels of cortisol and testosterone were not affected by challenges but were different in wild and captive specimens. Although the endocrine responses to STIs did not differ between intra- and interspecific challenges, agonistic displays expressed by resident fish were more intense towards intraspecific intruders. Taken together, our study emphasizes the need to incorporate androgen precursor concentrations to advance our understanding on the physiology of territorial interactions.

Introduction

Among the wide range of behaviors displayed by animals, aggression plays an essential role in social interactions. It serves central functions that increase survival and reproductive success, such as maintaining territories, gaining access to partners, guarding offspring, as well as dominance and cooperative behavior (Soma et al., 2008; Thresher, 1976; Wingfield et al., 2006). While emphasis is often put on discussing causes and consequences of aggression in intraspecific competition (Myrberg and Thresher, 1974), aggression is also an important component of relationships between species (Thresher, 1976). For example, interspecific aggression may stabilize mutualistic interactions (Bshary and Grutter, 2005), while territorial animals use aggression to defend their valuable resources against specific species that are potentially harmful (Myrberg and Thresher, 1974; Thresher, 1976). Individuals have to acquire the skills to dose aggression contingent on the threat posed by the challenges from their social environment and on their own physiological capacities (Carre et al., 2014; Di Paola et al., 2012). By targeting aggressive responses only to species that pose a threat, territory-holders save energy and may minimize costs of aggression by decreasing the number of interactions (Di Paola et al., 2012; Haley and Müller, 2002).

In male vertebrates, androgens and stress hormones play an important modulatory role in the frequency and form of aggressive responses to social challenges (Adkins-Regan, 2005). Direct evidence that androgens promote aggressive and territorial behavior has been provided by experiments in which hormones are manipulated (Goymann, 2009; Oliveira, 2004; Trainor et al., 2009). While hormones facilitate behavioral changes, this relationship can be reciprocal as well (Oliveira, 2009; Soares et al., 2010). Famously, Wingfield et al. (1990) proposed an influential concept attempting to elucidate how social factors impact on testosterone secretion (Goymann, 2009; Wingfield et al., 1990). In this concept, known as the "Challenge Hypothesis", they postulate that a small increase in androgen levels from the low baseline present outside of the breeding season suffices for males to develop secondary sexual characters and to be able to reproduce. Increases to high levels of androgens are avoided unless their physiological costs, such as augmented energy demands and reduced immune function (Wingfield et al., 2001), are outweighed by the benefits of increased competitiveness. The latter condition is apparently fulfilled in species with high male–male competition over access to females, and allows males to possess high androgen levels throughout the reproductive season

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http://dx.doi.org/10.1016/j.yhbeh.2015.04.011
0018-506X/

(Wingfield et al., 1990). Alternatively, in species with less male–male competition, only periods of social instability, for example when a territory owner is challenged by intruders, lead to a temporary surge in androgen levels. Such phenomena have been observed in taxa as diverse as birds, fish or mammals, including humans (reviewed in Archer, 2006; Goymann et al., 2007; Oliveira, 2004).

Data collected on birds living in tropical and temperate areas show that species with seasonal territoriality and short breeding seasons have higher circulating testosterone levels during these periods than species maintaining a year-round territory (reviewed in Goymann et al., 2004; Hau et al., 2008). Since elevated testosterone concentrations are sustainable only during a limited amount of time, peaks in testosterone levels may be linked to intermale aggression in seasonal birds, while year-round territoriality may be regulated by other mechanisms. Recently, these patterns have been studied in teleosts in damselfishes (*Pomacentridae*), which are a relevant family to investigate as they are among the most abundant inhabitants of coral reef fish communities and greatly contribute to benthic ecology (Ceccarelli, 2007; Frédérich et al., 2008). These fishes are easily accessible, since they usually live in shallow waters and spawn on dedicated sites. Importantly, they exhibit high degrees and various types of territoriality. Simulated territorial intrusions (STIs) were conducted on the tropical and year-round territorial species *Stegastes nigricans* outside of the reproductive season. While androgen levels remained consistently low (Ros et al., 2014b; Vullioud et al., 2013), social challenges induced elevations in cortisol levels (Ros et al., 2014b). In another tropical and year-round territorial species, *Abudefduf septemfasciatus*, standardized challenges were organized during the breeding period. Low androgen and cortisol levels were measured and did not increase after STIs (Ros et al., 2014a).

The low androgen responsiveness to experimental intruder exposure in damselfishes might be due to their tropical distribution, or to the fact that the tested species were year-round territorial. To understand under which circumstances androgen levels respond or not to social challenges, more studies on species with different life history patterns are necessary. Therefore, we aimed to test the effect of agonistic challenges on hormone levels in a tropical damselfish species with seasonal territoriality. We observed and sampled wild *Abudefduf sexfasciatus* males to describe their natural behavior and hormone profile, taking advantage of the co-occurrence of territorial and non-territorial individuals to obtain the physiological range of steroid circulating values. During the same period in which we found reproductive males in the field, we tested the effects of intra- and interspecific STIs on the aggressive and endocrine responses in captured individuals kept in aquaria.

We used a sensitive ultra-high pressure liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method that allowed us to measure several steroids from a small amount of blood plasma and to analyze which steroid pathways could be modulated by social challenges: cortisol (C) as recent studies on damselfish showed that stress hormones might be involved in territorial defense (Ros et al., 2014b; Van Duyse et al., 2004); 11-ketotestosterone (11KT) and testosterone (T), which are the most abundant androgens in fish with 11KT being biologically more active than T (Borg, 1994); and 11-ketoandrostenedione (11KA), which is an important precursor of 11KT. The precursor was included as studies on tropical species maintaining year-round territories indicate low social modulation of androgen levels (Goymann and Landys, 2011; Soma et al., 2008; Wingfield et al., 2006), while a study on a neotropical bird suggests that year-round territorial species may reduce the cost of androgen responses by increasing the production of an androgen precursor rather than of the androgen itself (Hau et al., 2004). We therefore measured the precursor 11KA, which can be readily metabolized to 11KT in fishes.

Based on the premises of the Challenge Hypothesis and on the current results reported for teleosts and birds, we predicted to measure surges in androgen levels in territorial *A. sexfasciatus* following standardized challenges (STI experiments) in the laboratory. Moreover, we expected that breeding fish in the field, which guard temporary territories during the reproductive period, would present higher androgen levels than non-territorial fish. Finally, as animals are able to tune their aggressive reactions to the identity of a potential intruder, we expected that intraspecific territorial intrusions might generate distinct behavioral responses compared to interspecific ones.

Material and methods

The study was conducted from November 2013 to February 2014, which overlapped with the reproductive period of the study species. We were hosted by the UC Berkeley's Richard B. Gump South Pacific Research Station, in Mo'orea, French Polynesia.

Study species and behavioral observations in the field

For the purpose of this study, we selected the scissortail sergeant *A. sexfasciatus* so that the endocrine responses to social challenges could be assessed in a seasonally territorial and tropical damselfish, and compared to the data already reported for year-round species (Ros et al., 2014a,b; Vullioud et al., 2013). *A. sexfasciatus* can be found on coral reefs in our study region of the Pacific (Randall, 2005). Only males become territorial during the breeding season and take care of the eggs (Shadrin and Emel'yanova, 2007). Their territories are small (50–60 cm) and clustered in colonies on coral structures, where spawning occurs synchronously (Manica, 2010).

We located two colonies of breeding *A. sexfasciatus* on exposed parts of the back reef (17°28′57.3″S, 149°49′29.2″W and 17°28′29.5″S, 149°47′59.6″W, 3.5 m depth). The complex topology and high population density of *A. sexfasciatus* at these sites were not suited for controlled experiments. Instead, we regularly visited the colonies (with intervals of at least 4 days). On each dive, the breeding stage was recorded, and two specimens were observed during 15 min. Routine activities, as well as reactions to trespassing fishes were noted using underwater slates (see supplements). After the observation several males were captured using a barrier net, enclosed in a zip lock bag, and within 5 min after capture a blood sample (300 μl) was drawn underwater. The sampled individuals were tagged with colored elastomer under a scale to avoid repeated sampling of the same fish and immediately released close to the nests.

During the reproductive season, *A. sexfasciatus* undergo brood cycling, alternating between periods of territory establishment, mating and egg-caring (Manica, 2010). To correct hormonal measurements for these different breeding stages, specimens in the field were assigned to one of the following groups: (a) *A. sexfasciatus* that defended eggs were classified as “breeding” males; (b) *A. sexfasciatus* devoid of eggs but guarding a territory were classified as “territorial” males; and (c) non-territorial *A. sexfasciatus* living in schools in the open water were termed “shoaling” males. The latter were caught for blood sampling, but their behavior was not recorded. Altogether, we successfully collected blood samples from 10 breeding, 10 territorial and 10 shoaling *A. sexfasciatus*.

Simulated territorial intrusions in the laboratory

To avoid interrupting breeding colonies, 30 *A. sexfasciatus* were sampled from a non-territorial shoaling population (17°28′48.5″S, 149°49′10.0″W). After determining the sex of captured individuals by inspecting the urogenital opening, males were enclosed in a bucket and transferred to laboratory facilities. Animals were immediately isolated in opaque cylindrical tanks (125 cm diameter, 850 l), which were shaded and supplied with continuously flowing seawater. Four tanks were at disposal, each containing an artificial shelter. Larger holding tanks were used to house fishes serving as intraspecific or interspecific intruders. Lined bristletooth *Ctenochaetus striatus* were selected as interspecific intruders because they were frequently attacked by nesting *A. sexfasciatus* when approaching guarded territories (see supplements). Before conducting the