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Photoperiod modulation of aggressive behavior is independent of androgens in a tropical cichlid fish

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ABSTRACT

Photoperiod is a major environmental cue that signals breeding conditions in animals living in temperate climates. Therefore, the activity of the reproductive (i.e. hypothalamic–pituitary–gonadal, HPG) axis and of the expression of reproductive behaviors, including territoriality, is responsive to changes in day length. However, at low latitudes the seasonal variation in day length decreases dramatically and photoperiod becomes less reliable as a breeding entraining cue in tropical species. In spite of this, some tropical mammals and birds have been found to still respond to small amplitude changes in photoperiod (e.g. 17 min). Here we tested the effect of 2 photoperiod regimes, referred to as long-day (LD: 16L:08D) and short-day (SD: 08L:16D), on the activity of the HPG axis, on aggressive behavior and in the androgen response to social challenges in males of the tropical cichlid fish *Tilapia rendalli*. For each treatment, fish were transferred from a pre-treatment photoperiod of 12L:12D to their treatment photoperiod (either LD or SD) in which they were kept for 20 days on stock tanks. Afterwards, males were isolated for 4 days in glass aquaria in order to establish territories and initial androgen levels (testosterone, T; 11-ketotestosterone, KT) were assessed. On the 4th day, territorial intrusions were promoted such that 1/3 of the isolated males acted as residents and another 1/3 as intruders. Territorial intrusions lasted for 1 h to test the effects of a social challenge under different photoperiod regimes. Photoperiod treatment (either SD or LD) failed to induce significant changes in the HPG activity, as measured by androgen levels and gonadosomatic index. However, SD increased the intensity of aggressive behaviors and shortened the time to settle a dominance hierarchy in an androgen-independent manner. The androgen responsiveness to the simulated territorial intrusion was only present in KT but not for T. The percent change in KT levels in response to the social challenge was different between treatments (SD > LD) and between male types (resident > intruder). The higher androgen response to a social challenge in residents under SD may be explained by the time course of the androgen response that due to the long time it takes to fight resolution under LD, might have been delayed. This result illustrates the importance of incorporating time response data in social endocrinology studies.

1. Introduction

Photoperiod plays a key role as an environmental cue signaling the breeding season in temperate species, including fish (Borg, 2010; Bromage et al., 2001; Pankhurst and Porter, 2003). Therefore, it has a profound effect on reproductive and aggressive behaviors, which is mediated by the hypothalamic–pituitary–gonadal (HPG) axis. In general, long days (LD) stimulate the activity of the HPG axis and the expression of reproductive and aggressive behaviors whereas short days (SD) depress them (see (Nelson et al., 2010) for recent reviews in different taxa). Thus, for animals living in high-latitudes aggressive interactions generally increase as the breeding season approaches and this seasonal effect is mediated by photoperiod. However, the amplitude of the photoperiod seasonal cycle decreases towards the tropics and close to the equator only slight variations are observed. Nevertheless, the tropical environment still presents seasonal variability with alternating dry and rainy seasons, but the year-to-year precision of these seasonal fluctuations is less precise (Hau, 2001). Therefore, tropical species

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are expected to rely on environmental cues other than photoperiod to entrain reproduction, such as temperature, rainfall, food availability or social factors (Hau, 2001; Pankhurst and Porter, 2003), and to have more flexible responses to changes in local environmental conditions than temperate species (Hau, 2001; Wingfield et al., 1992).

In contrast to the wealth of research on the effects of photoperiod on reproduction and behavior of temperate species, research on tropical species is much more scarce. However, responses to slight fluctuations in photoperiod, in the order of minutes and within the range of photoperiod variation between equinoxes and solstices at tropical latitudes, have been documented in different taxa (e.g. mammals: Heideman and Bronson, 1993; Wayne and Rissman, 1991); birds: (Gwinner and Dittami, 1985; Gwinner and Scheuerlein, 1999; Hau et al., 1998). For example, an increase of photoperiod as small as 17 min is enough to induce gonadal growth in equatorial Spotted Antbirds (*Hylophylax n. naevioides*) (Hau et al., 1998). Therefore, photoperiodic responsiveness may be more present among tropical species than initially expected, and photoperiod may still be used by tropical species as a seasonal cue. Alternatively, animals at equatorial latitudes may be using other solar cues, such as subtle periodic patterns of sunrise and sunset, rather than variation in day length to synchronize their circannual clock (e.g. Goymann et al., 2012).

The reproductive axis and HPG-dependent behavior are also known to respond to social factors. Cichlid fish have been widely studied in this respect (e.g. Fernald and Maruska, 2012; Oliveira, 2009). For example, in the African cichlid *Astatotilapia burtoni* GnRH-immunoreactive neurons in the POA respond to social opportunity with increased activity that drives an increase in circulating androgen levels and dominant behavior in subordinate males ascending in social status (Maruska and Fernald, 2011; Maruska et al., 2010). Thus, socially-driven changes in HPG activity and androgen levels in the breeding context lead to territorial males usually displaying higher androgen levels than non-territorials during the breeding season (Hirschenhauser and Oliveira, 2006; Oliveira et al., 2002). This androgen response to social challenges is moderated by the regime of male intra-sexual competition and by a trade-off with parental care as summarized by the "Challenge Hypothesis" proposed by Wingfield et al. (1990); see also (Goymann et al., 2007; Hirschenhauser et al., 2003). According to this hypothesis, environmental factors that signal the approach of the breeding season, such as photoperiod, are expected to act as anticipatory cues for breeding baseline levels of androgens needed for gonadal development. On the other hand social interactions, such as territorial intrusions or fights for dominance status, are expected to further increase androgen levels towards a physiological maximum, whose function is to adjust the expression of behavior to the local social environment (Oliveira, 2009; Wingfield et al., 1990). The dependence of androgen levels above the breeding baseline on relevant social cues would thus limit the cost associated with keeping androgen levels high for long periods of time (Wingfield et al., 2001). The "Challenge Hypothesis" has received ample evidence across vertebrates (Hirschenhauser and Oliveira, 2006) but see (Goymann et al., 2007), but most studies have been conducted in temperate species. Given the well known latitudinal gradients in biodiversity with maximum species richness in the tropics (Willig et al., 2003), and the contrast between temperate and tropical environments (see above) this variability may limit the universality of the "Challenge Hypothesis". For example, the costs of keeping elevated testosterone levels during the breeding season in tropical species with long breeding seasons may become unsustainable. Similarly, the higher reliance on local conditions rather than on global entraining cues, such as photoperiod, to signal breeding opportunities in tropical environments, may lead to asynchronous breeding, with reduced regimes of male–male competition and concomitantly low androgen levels. Studies on tropical birds indicate that they do have indeed lower peak plasma testosterone levels than temperate species (Goymann et al., 2004). For example, in the Spotted Antbird, a tropical species with year-round territoriality, testosterone levels are very low with brief peaks induced by social challenges (Hau et al., 2000), and these low androgen levels are compensated by increased sensitivity of target tissues (Canoine et al., 2006). However, when tropical environments approach the conditions of temperate ones, namely in terms of seasonal territoriality, short breeding seasons or low environmental temperatures, as found in high altitude habitats, higher peak androgen levels are also observed among tropical birds (Goymann et al., 2004). In tropical fish a comparative study on 5 cichlid species differing in mating system and parental care type showed a similar pattern of modulation of the androgen response to social challenges by mating system and parental care as the pattern observed previously described for temperate birds (Hirschenhauser et al., 2004). Finally, in amphibians and reptiles testosterone levels are negatively related to breeding-season length, which may be explained by the higher male–male competition regime expected in shorter breeding seasons (Eikenaar et al., 2012). More studies across different taxa on the response of tropical species to social challenges are still needed.

The redbreast tilapia, *Tilapia rendalli* (Boulenger, 1896), is a good species in which to investigate both the photoperiodic and the social androgen responsiveness of a tropical fish species. It is native to Africa occurring in a latitudinal range from approximately −2° to −25°, which corresponds to a maximum photoperiod amplitude of approximately 3 h, from ca. 10.5L:13.5D at the Winter solstice (June) to ca. 13.75L:10.25D at the Summer solstice (December) at its most southern limit. *T. rendalli* is a monogamous cichlid with substrate spawning breeding where male and female form strong pair bonds (Fryer and Iles, 1972; Ribbink et al., 1981). It has a long breeding season (5–7 months) with multiple spawnings throughout the rainy season (De Bont, 1950; Ribbink et al., 1981). Males are highly territorial during breeding and exhibit offspring defense (Baerends and Baerends-van Roon, 1950; Fryer and Iles, 1972), thus being a good model to test the effects of photoperiod both on the reproductive axis and on aggressive behavior.

We investigated the responsiveness of the reproductive axis and aggressive behavior to changes in photoperiod in *T. rendalli* males, as well as their androgen response to social challenges under short- and long-daylight cycles. If this species is responsive to photoperiod then an increase in androgen levels and aggressiveness is predicted under long-days. Moreover, as a monogamous species, a high amplitude androgen response to a social challenge is predicted by the Challenge Hypothesis, which should be more marked under short-days, when the scope for response (i.e. difference between initial level and maximum physiological response) would be higher, assuming that the maximum physiological level is kept constant across seasons (i.e. different photoperiods).

2. Methods

2.1. Animals and housing

T. rendalli males from Centro Nacional de Pesquisa e Conservação de Peixes Continentais (CEPTA, Pirassununga, SP, Brazil) were used. Fish was kept in an outdoor fish pond (185 m^3) at UNESP (São José do Rio Preto, SP, Brazil) until the beginning of the experiment. São José do Rio Preto is located at 20°49′ S which falls within the natural latitudinal range of the species, with a natural variation in day length between 10 h 40 min at the Winter