



Short report

Does sex influence intraspecific aggression and dominance in Nile tilapia juveniles?



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ABSTRACT

Although sex of mature fish is known to influence aggression, this issue has so far been neglected in juveniles. Here, we tested this sex effect and showed that it does not significantly affect intraspecific aggression in juveniles of the cichlid Nile tilapia. To reach this conclusion, we measured the latency period before onset of confrontation, the frequency and types of aggressive interactions, the duration of a dispute, and the probability of becoming dominant. This was done on pairs of Nile tilapia that varied by sex: females × females, males × males, and females × males. In a double blind approach, after pairing, the sex of each individual was histologically verified and contrasted with behavioral data.

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1. Introduction

In adult fish, sex differences influence several traits, such as feeding behavior (Abrahams and Dill, 1989; Bastos et al., 2011), compensatory growth (Barreto et al., 2003; Livingston et al., 2014), parental care (Annett et al., 1999; Teresa and Goncalves-de-Freitas, 2011; Sneker et al., 2011), antipredator responses (Giles and Huntingford, 1984; Johnsson et al., 2001), and aggressive behavior (Yamamoto et al., 1999; Kolm and Berglund, 2004; Arnott and Elwood, 2009; Archard and Braithwaite, 2011; Rosvall, 2013). A common type of aggressive competition occurs between males when disputing over a potential mate (Desjardins et al., 2012); males are usually more aggressive than females, and males tend to be the dominant animal in a group (Andersson, 1994).

Many studies have shown differences in aggressive behavior between adult males and females. The question of sex differences in aggression in juvenile fish is often raised. However, few studies have addressed the question of sex differences in aggression in juvenile fish. In nature, animals may actively compete for environmental resources, which are often limited, through aggressive disputes (Ridley, 1995). Even though they do not yet reproduce, immature fish of both sexes compete for resources

and marked differences in this competition can adversely affect their survival (Huntingford and Turner, 1987). In fact, both sexes of stickleback (*Gasterosteus aculeatus*) have similar frequencies of directed aggressive acts toward their opponents, and exhibit territoriality during the juvenile stage (Bakker, 1994). In streams, juvenile rainbow trout (*Oncorhynchus mykiss*) are territorial and form hierarchical groups (Jenkins, 1969). In laboratory experiments, researchers have clearly detected aggressive behavior and territorial defense in juvenile cichlids (Alvarenga and Volpato, 1995; Kadry and Barreto, 2010; Barreto et al., 2011; Miyai et al., 2011; Carvalho et al., 2012; Sanches et al., 2012; Torrezani et al., 2013). However, different age-specific traits of juveniles can be correlated to different life histories strategies between the sexes (Cheverud et al., 1983). High aggression in juvenile males could thereby occur as a result of expression of sex-linked genes that modulate aggressiveness in adult males and relate to male competitive tasks, such as competition for females and reproductive territory. Male juvenile rainbow trout (*O. mykiss*) are more aggressive than females, indicating that some differences in aggression may develop early in some fish species (Johnsson and Akerman, 1998), although juvenile males do not win more contests than females (Johnsson et al., 2001). Overall, differences in the aggressive behavior of juvenile fish are clearly species-specific.

Nile tilapia (*Oreochromis niloticus*) adult males defend territories for breeding and other resources (Baerends and Baerends van Roon, 1950), and adult males and females compete for different

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reproductive resources and have different aggressive behaviors (Carvalho and Gonçalves-de-Freitas, 2008). As such, a potential asymmetry between the sexes, regarding their aggressive behavior, may occur even when Nile tilapia are juveniles. In this study, we tested the hypothesis that juvenile males of Nile tilapia are more aggressive than juvenile females and have a greater probability of becoming the dominant animal in dyadic contests. Moreover, cichlids are ideal animal models for studying aggression (e.g., Barcellos et al., 1999; Oliveira et al., 2001, 2005) and evaluating the relevant implications of aggression in juvenile fish, such as in this study.

2. Materials and methods

2.1. Animal welfare statement

This research agrees with the Ethical Principles in Animal Research adopted by the National Council for the Control of Animal Experimentation – Brazil (CONCEA – Conselho Nacional de Controle de Experimentação Animal – Brazil) and was approved by the Ethical Committee for Animal Research from the Instituto de Biociências/UNESP (CEUA – Comissão de Ética no Uso de Animais), protocol 578.

2.2. Animals and stock condition

Sexually immature, male and female specimens of Nile tilapia, *O. niloticus* (L.), were kept for at least 7 days in 310-L, indoor storage, plastic tanks (holding density ~0.4 fish/L of water) that had a constant supply of de-chlorinated water for constant water renewal and prevention of organic matter accumulation. Tanks were also equipped with a biological/mechanical/chemical filter and kept at room temperature. The photoperiod was 12 h light, provided by an artificial light (daylight lamp type), and 12 h darkness. We fed the fish daily at approximately noon with extruded feed for tropical fish (32% crude protein; 5% of fish biomass).

2.3. Experimental design

Without knowing sex, we (1) randomly paired juvenile Nile tilapia, (2) evaluated their aggressive behavior, and (3) identified sex by histological analysis of the gonads. In doing this, we created 55 size-matched pairs (fish showed body length differences lower than 3% of total body lengths), and introduced two fish at the same time into a neutral arena (23-L tank; 40 cm × 23 cm × 25 cm). These procedures reduce both the asymmetric effect from differential resource holding potential and the effect from previous residency (Beaugrand and Zayan, 1985; Beaugrand and Beaugrand, 1991; Arnott and Elwood, 2009; Boscolo et al., 2011). The pairs were formed from individuals that each came from a separate stock tank, to guarantee unfamiliarity with each other, which could reduce the rate of encounters (Giaquinto and Volpato, 1997). The paired fish were video-recorded for 40 min for subsequent analysis of aggressive behavior. Random pairing resulted in 10 female × female pairs, 30 male × female pairs, and 15 male × male pairs. Prior to the experiment, sex determination, by inspecting genital papilla with the methylene blue contrast technique (Makino et al., 2009) was not possible, hence the inspection of gonads occurred by histological analyses.

2.4. Aggressive behavior

The aggressive behavior of the Nile tilapia was quantitatively evaluated with measures of (1) latency to onset of fighting, (2) frequency of each agonistic activity, and (3) duration of fighting. The latency to onset was the time between the introduction of animals in the experimental tank and the first attack. Aggressive

interactions were qualitatively identified based on an ethogram of aggressive behavior for this species, as described in Alvarenga and Volpato (1995) and Barki and Volpato (1998). We quantified the number of threats, lateral and frontal displays, and directed attacks by counting the following: bites on the anterior (head), tail fin, medial area, and ventral area; lateral fights (sudden slaps between fish bodies), with fish heads oriented in either the same or opposite directions, chases, and mouth wrestles. The initiator of an attack was identified as the fish that approached the opponent and directed the attack; the loser was the fish that left the place of attack or retreat (Oliveira and Almada, 1996; Gonçalves-de-Freitas et al., 2008). The moment in the interaction at which the contest is over and the outcome is decided Nile tilapia change their body and eye color clearly. Thus, social status was identifiable because subordinate tilapias have dark striped bodies and dark eyes, while dominants are pale (Falter, 1987; Volpato et al., 2003). Also, dominance was estimated with an index of dominance (ID), which is the number of attacks of one individual in the pair/total number of attacks in the pair (Bailey et al., 2000). Fighting duration is the interval between the start of the interaction and the initiation of chasing behavior and fish signaling of dominance (pale body) and fish signaling of subordination (dark stripe).

2.5. Fish sex confirmation

To confirm sex, we sacrificed fish with an overdose of the anesthetic benzocaine (Gontijo et al., 2003). The gonads were removed through an abdominal incision and fixed in Karnovsky's solution for 24 h. Next, the gonads were dehydrated in 70% solution of alcohol for 24 h, and subsequently in 90% solution for 4 h. Prior to permanently embedding gonads in resin (Historesin®, Leica, Germany), they were placed in a 1:1 solution of alcohol and resin for 12 h. After embedding gonads in resin, they were sliced to a thickness of 3 μm, stained with 1% borax-toluidine blue, and analyzed with optical microscopy.

2.6. Statistical analysis

Data from this study were assessed for normality and homoscedasticity and met the assumptions for parametric testing. We analyzed the data of latency for the onset of confrontation, fight duration and total frequency of aggressive interactions by one-way ANOVA among the conditions of pair composition (male × male, female × male, or female × female). We analyzed the frequency of each agonistic activity by one-way repeated measures ANOVA, where pair composition was the independent factor, social status (dominant × subordinate) was the repeated measure, and aggressive behavior was the response variable. Individual measures during either dyadic or grouped animal interactions must be considered as repeated measures (see discussion in Briffa and Elwood, 2010). A chi-square test was used to analyze the frequency of dominant males and females in the male × female condition. Statistical differences were considered significant at $P < 0.05$. Estimates of statistical power are provided in Table 1S (Supplementary material).

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.beproc.2014.02.003>.

3. Results

We found no significant influence of pair sex composition for fighting latency (mean ± SD; male × male = 84.0 s ± 94.2; female × male = 95.3 s ± 108.2; female × female = 58.9 s ± 81.3; one-way ANOVA; $F_{(2;52)} = 0.885$; $P = 0.419$) and duration (mean ± SD; male × male = 745.0 s ± 342.3; female × male = 679.2 s ± 342.9; female × female = 519.0 s ± 200.5; one-way ANOVA; $F_{(2;52)} = 1.508$;

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