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Social Stability Related to Sex Group Composition in the Cichlid Nile Tilapia

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ABSTRACT

Aquaculture practices usually put the Nile tilapia in an artificial social environment, which males predominate due to their faster growth desirable for aquaculture purposes. Such a situation can increase male-male fighting because males are generally more aggressive than females, and also because fighting ability is similar within the same sex, leading to longer contests. As behavior has been used to infer welfare in several fish species, the aim of this study was to investigate whether sex composition affects agonistic interactions, social hierarchy and energetic demand in groups of Nile tilapia (*Oreochromis niloticus*; L.). Size-matched adult fish were divided in two treatments: MM = four males and MF = two males and two females (10 repetitions for each treatment). The experiment lasted for 11 days and social interactions (aggressiveness and rank order) were recorded at the 2nd, 6th and 10th days (15 min per day). Fish were food deprived and body weight loss was used to infer energetic cost. A higher frequency of lateral threat (Student's t independent test; t = 2.55; p = 0.02) and total interactions (Student's t independent test; t = -2.81; p = 0.01) was observed in the MF treatment. MM group showed unstable hierarchy (Binomial test, p = 0.04), which is considered a social stressor. However, mean weight loss was not affected by treatments (Student's t independent test; t = -0.74; p = 0.47). These results support the idea that sexual composition affects aggressive interactions and destabilizes social hierarchy, but not energy cost.

Keywords: aggressiveness, growth rate, social hierarchy, welfare

INTRODUCTION

Monosexual groups are used for fish production in several species in aquaculture systems (Beardmore *et al.* 2001). This practice is useful for Nile tilapia culture because early reproduction is one of the main biological problems in this species, which leads to crowding and subsequent growth depensation (Longalong *et al.* 1999; Biswas *et al.* 2005). As the growth rate is significantly higher for males than for females, the preferred sex for aquaculture purposes is the male one (Paiva *et al.* 1988; Beardmore *et al.* 2001).

Several commercial techniques are used to produce populations with a high percentage of males, but some disadvantages emerge from such techniques (for review see Kubitza 2000; Beardmore *et al.* 2001). Although high growth rate is desirable in aquaculture, it does not mean that animals growing faster are in good welfare condition. As pointed out by FSBI (2002) and Huntingford *et al.* (2006), growth rate is only one indicator of welfare in fish. However, aspects such as health status, physiology and behavior should be considered, and these three variables should be taken into account when assessing fish welfare (Huntingford *et al.* 2006).

Considering fights usually are more intense when competitive abilities are similar among competitors (Maynard-Smith and Parker 1976), agonistic interaction should be higher among animals within the same sex (Tinbergen 1972). According to Wooton (1998), reproductive resources differ between sexes in fish, and males generally are more aggressive than females (e.g. Oliveira and Almada 1996a). In this way, monosex male culture conditions could produce a higher competitive environment than when females are present. On the other hand, fights also can be more intense when limited resources are present in a group (Grant *et al.* 1995). For poliginic fish, females are a reproductive resource that males will fight for (Gonçalves-de-Freitas and Nishida 1998). In such a situation, presence of females could increase fights between males.

Another point to be considered is that enhanced agonistic encounters increase energetic demands in fish (Haller and Wittenberger 1988; Alvarenga and Volpato 1995; Volestad and Quinn 2003; Ros *et al.* 2006). Although fights cost energy, this is attenuated when social hierarchy is stable (Haller and Wittenberger 1988; Johnsson 1997). When dominance hierarchies are settled, dominant fish can access limited resources, such as food, shelter, space and mates (Huntingford and Turner 1987; Ridley 1995). According to Zayan (1991), changes in dominance hierarchy are a psychological stressor that may impair welfare. Thus, we predicted that group sexual composition affects agonistic interactions and group energy demands in Nile tilapia, with condition of only male being more aggressive and more costly for such individuals. The Nile tilapia (*Oreochromis niloticus*; L.) was the chosen species because these cichlid fish maintain social hierarchies and defend territories by fighting opponents (Volpato *et al.* 1989; Carvalho and Gonçalves-de-Freitas 2008; Gonçalves-de-Freitas *et al.* 2008).

MATERIALS AND METHODS

Animals and holding conditions

Adult Nile tilapia, *Oreochromis niloticus*, coming from fish farm tanks, were housed in indoor tanks (500 L, 1 fish.5L^{-1}) by 15 days before experimentation. Water temperature was set at 27°C, light at 12 h light: 12 h dark (from 0700 to 1900 h) and constant aera-

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tion was supplied. Fish were fed with 28% protein pellets for tropical fish (Guabi/Pirá-Campinas, Brazil) to apparent satiation 1 h after starting light period (0800 h) and 1 h before ending light period (1800 h).

Experimental design

For the study we used glass aquaria (60 × 60 × 40 cm; 140 L) with blue opaque coverings on the lateral and the posterior walls to provide good contrast for video-recording, and to avoid visual contact with neighbor fish. Blue color was chosen because it is known to reduce stress in Nile tilapia (Volpato and Barreto 2001). Water mean temperature was 27 ± 1°C in all groups and photoperiod was 12L: 12D (starting at 0700 h). Individual biological filters and constant aeration were provided for each aquarium, pH was set at ~7.5, and ammonia at 0.00 ppm. During the study, fish were kept in groups composed of animals of similar size to avoid effect of size on social rank (Beeching 1992). The standard length (cm) of fish at the beginning of the experiment was: MM = 8.95 ± 0.53 cm and MF = 9.08 ± 0.73 cm (n = 40 for each group; Student's t independent test, p = 0.89). Fish were weighed before grouping and at the 11th day of grouping to evaluate growth decreasing. To avoid effect of replacing energy by food intake, fish were not fed during the 11 days of observation, following Barreto *et al.* (2003). Single animals were identified by small cuts in the tail fin, as used by Fernandes and Volpato (1993) and by Höglund *et al.* (2005). Fish were anesthetized with benzocaine (12.8 mg/L) and sex was determined by inspection of the genital papillae. Genital papillae were stained with methylene blue to stand out the oviduct opening in females, as described in Carvalho and Gonçalves-de-Freitas (2008). Sex and developmental gonad stage were confirmed at the end of the experiments by macroscopic inspection of the gonads, according to Paiva *et al.* (1988). At the end of the study fish were killed by a lethal dose of benzocaine (25.6 mg/L). Size-matched adult fish were divided in two treatments: MM = four males, and MF = two males and two females (10 repetitions for each treatment). Groups were maintained for 11 days and they were video-recorded (15 min) at the 2nd, 6th, and 10th days after grouping to quantify social interactions and rank.

Social interactions

Agonistic interaction was quantified by following an ethogram adapted from Falter (1983) and Alvarenga and Volpato (1995) as described below:

Chase: one fish follows the opponent that swims away.

Lateral fight: the fish remain alongside each other facing the same or opposite direction and beat their tails sideways.

Lateral threat: one fish with their fins spread and mouth opened approaches laterally to the opponent, which keeps away. This behavior is like a lateral fight, but there is no physical touching and even body undulation.

Mouth fight: both fish approach frontally each other with their mouths opened and bite the opponent's mouth. Their mouths are kept tightly together while one fish displaces the opponent backward.

Nipping: the aggressor swims towards the opponent and bites its body.

Undulation: only one fish beats its tail sideways (undulating the body), without spreading its fins. The opponent may flee or attack back.

Total fighting: sum of all agonistic events.

Social rank

Rank was defined by a dominance index, as used by Gómez-Laplaza and Morgan (1993), Oliveira and Almada (1996a), and Gonçalves-de-Freitas *et al.* (2008):

$$\text{Dominance index (DI)} = \frac{\text{number of given fights}}{(\text{number of given fights} + \text{number of received fights})}$$

DI varies from 0 to 1.0; dominant DI is expected to be higher than subordinate DI. The correlations of DI between the 2nd and the 6th days, and also the 6th and the 10th days were tested by Spearman correlation test. Based on these tests, a hierarchy was considered stable when Spearman r was ≥ 0.7 in both correlation test for the same sex condition, a criteria used by Oliveira and Almada (1996b) for *Oreochromis mossambicus*.

Energetic cost

The energetic cost of each treatment was estimated from the Specific Growth Rate by considering the difference in biomass of entire group instead of individual body weight.

$$\text{Specific Growth Rate (SGR)} = \frac{(\text{Ln final biomass} - \text{Ln initial biomass}) \times 100}{11 \text{ days}}$$

Statistical analysis

Data normality was assessed by Shapiro Wilk's test (Zar 1999) and homocedasticity by Fmax (Lehner 1996). Fight frequency showed non-normal distribution and thus it was normalized by $(x + 0.5)^{1/2}$, according to Lehner (1996). These data and SGR mean values were then analyzed by Student's t independent test (Zar 1999). Frequency of socially stable groups was tested between sexes (treatments) by Binomial test (Ayres *et al.* 2003). Statistical significance was established at $\alpha = 0.05$.

RESULTS

Total frequency of aggressive interactions and lateral threat were significantly lower in MM than in MF condition (t= -2.81; p< 0.02; **Fig. 1**). Sex composition of the groups did not affect frequency of chase, lateral fight, mouth fight, nipping and undulation (p > 0.05). Social hierarchies emerged in groups, irrespective of sex composition. However, significant higher frequency of stable hierarchy occurred in the MF than in the MM treatment (p = 0.04; **Table 1**). Initial and final weights of fish (g) were similar between MM and MF groups (p > 0.32), and thus, there was no effect of treatment on body weight loss (t = -0.74; p = 0.47; **Fig. 2**).

Table 1 Frequency of stable and unstable groups in Nile tilapia according to sex composition.

Sex composition	Hierarchical stability	
	Stable	Unstable
Only males[a]	3	7
Males and females[b]	7	3

[a] four males
[b] two males and two females
Binomial test; p = 0.04

DISCUSSION

Higher social interaction has been reported in monosex fish groups. Grant *et al.* (1995) and Kvarnemo *et al.* (1995) previously showed that the frequency of intra-sexual interaction is increased in groups with higher number of individuals of one sex, thus being a consequence of intra-sexual competition. Moreover, males and females of the cichlid *Neolamprologus multifasciatus* are more aggressive in intra-sexual contests (Schradin and Lamprecht 2000). Thus, we expected an increasing in fights in monosexual groups in Nile tilapia. However, in the present study monosex groups showed fewer lateral threats and total agonistic interactions than the sex-mixed groups. This may be explained because Nile tilapia males fight for females and thus absence of females may decrease motivation for social disputes. In fact, female affects the behavior of the resident male, increasing both undulation (Carvalho and Gonçalves-de-Freitas 2008) and total fights in Nile tilapia (unpublished data).

Unstable hierarchies are energetic costly to individuals in a group because of the social stress (Haller and Witten-

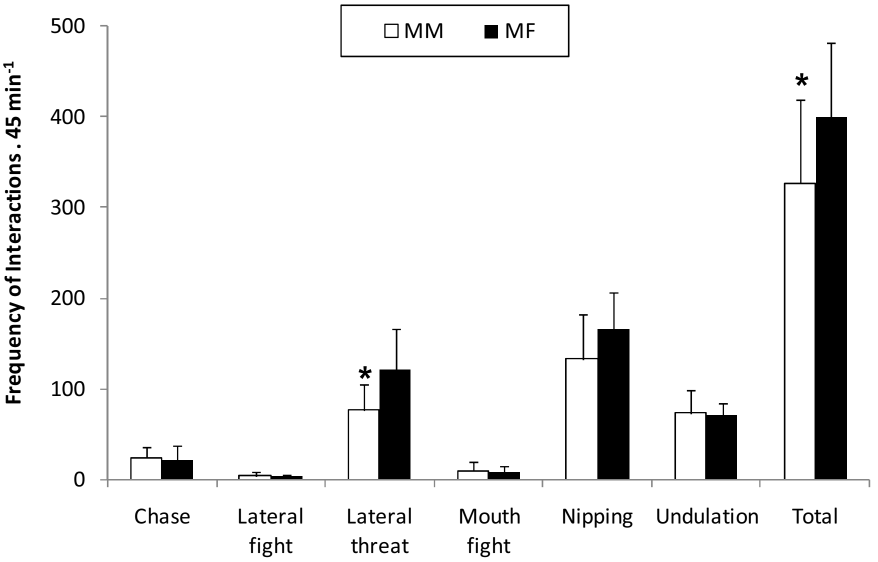

Fig. 1 Mean (± sd) frequency of social interaction in adult Nile tilapia groups on different sex composition (MM= only males and MF= two males and two females). Asterisk indicates significant difference (Student's t independent test, $p < 0.05$) after data transformation by $(x + 0.5)^{1/2}$.

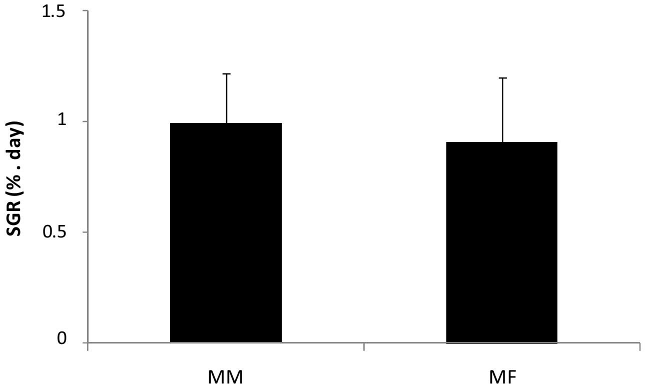

Fig. 2 Mean (± sd) specific growth rate (SGR) in different sex composition groups (MM= only males and MF= two males and two females). n = 10 for each group; Student's t independent test; t = -0.74; p = 0.47.

berger 1988; Zayan 1991; Fox *et al.* 1997). According to Alvarenga and Volpato (1995), metabolic demand in a group depends also on the history of agonistic interactions in the group, even within stable hierarchies. In this study, although the sex-mixed group had shown higher agonistic interaction, the higher frequency of stable hierarchies was observed in such treatment. Moreover, the social interactions increased mainly because of lateral threat in sex-mixed group, which are low-energy-cost displays (Haller and Wittenberger 1988; Alvarenga and Volpato 1995). This result suggests that energetic cost from fights could be similar to energetic cost from stress on social instability in monosexual groups.

Specific growth rate (SGR) is a variable widely used to study energy cost in fish (e.g. Fernandes and Volpato 1993; Volestad and Quinn 2003). In the present study, mean SGR was - 0.918% per day during the 11 days without feeding, which is similar to SGR described by Barreto *et al.* (2003) for *Oreochromis niloticus*, and by Uchida *et al.* (2003) for *O. mossambicus* during fasting periods. Considering the effect of sex composition on energy demands, we expected effect of the treatment on SGR because energy consumed could not be replaced by feeding. However, no treatment effect was detected. This result is similar to the observed by Carvalho and Gonçalves-de-Freitas (2008) where energy cost (in terms of O_2 consumption) was not affected by sex composition in pairs of Nile tilapia, thus showing that long time interaction does not prejudice growing in Nile tilapia monosexual groups.

In cichlid fish, males grow faster and reach higher body size and weight than females (Lowe-McConnell 1958; Paiva *et al.* 1988). Such sex-linked differences have been attributed to genetic (Fryer and Iles 1972) and hormonal (Toguyeni *et al.* 1996) factors. However, Barreto *et al.* (2003) and Uchida *et al.* (2003) observed respectively in *O. niloticus* and *O. mossambicus* that SGR is not affected by sex during feed-deprived periods. In fact, fish were not fed during this study and a similar rate of body weight loss was observed between treatments.

Our data show some link between sex group composition and social behavior. There was effect of the sexual composition on the social behavior of Nile tilapia, but it did not affect the energy cost. Although agonistic interaction was lower in the monosex group, the higher frequency of unstable hierarchy was also observed in this treatment. According to FSBI (2002) and Huntingford *et al.* (2006), behavioral studies have been an important issue in welfare research. Thus, in this study, different group sexual composition does not indicate differences in welfare in terms of growth parameters. However, aquaculture practices affect the environment and animal welfare (Conte 2004; Huntingford *et al.* 2006). In this way, the use of monosex condition in aquaculture must be considered with caution and it is necessary to evaluate other variables that also indicate fish welfare (such as health status, physiology and other behaviors). Although high growth rate is the main goal of aquaculture, this aspect could not be the only variable to access welfare state in fish. In this way, preference tests should provide additional and useful information (Volpato *et al.* 2007, 2009) and more long-term studies are necessary.

CONCLUSIONS

This study showed that group sexual composition affects social interaction in the Nile tilapia. Under the experiment settings of this study, while agonistic interaction were higher in the sex-mixed group, social stability was lower in male monosexual groups. However, sex group composition did not cause energy impairment in Nile tilapia.

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