Sex group composition, social interaction, and metabolism in the fish Nile tilapia

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Abstract

We tested whether the fish Nile tilapia (*Oreochromis niloticus*) in a monosex male group spends more energy and displays an agonistic profile, differently from males in male-female groups. Such differences are expected because males and females should compete for different reproductive resources. An intruder male (MM) or female (MF) was paired with a resident male and agonistic interaction was quantified during 20 minutes, 10 minutes after pairing and another 10 minutes period 30 minutes later. Energetic cost was evaluated from O_2 consumption, determined by Winckler's Method after 40 minutes pairing. Latency for fighting (mean ± SD, MM = 27.40 ± 25.15 s; MF = 14.22 ± 21.19 s; Mann-Whitney test, U = 33.50, P = 0.21) and frequency of the all agonistic acts in the first 10 minutes session (mean ± SD, MM < 72.30 ± 25.29; MF < 73.50 ± 21.65.10/min; Mann-Whitney test, P > 0.10) were not affected by group composition, thus suggesting that each intruder was a potential competitor at the beginning of the agonistic interaction. However, frequency of undulation (a behavior displayed also during courtship) was higher in the MF than in the MM resident fish (mean ± SD, MM = 3.56 ± 5.89; MF = 8.56 ± 4.00.10/min; Mann-Whitney test, U = 15.50, P = 0.01) at the end of the 10 min session. Frequency of flight, however, was lower in MF than in MM intruder (mean ± SD, MM = 3.90 ± 4.33; MF = 0.44 ± 0.96.10/min; Mann-Whitney test, U = 23.50, P = 0.04). Moreover, the agonistic profile in MM groups was composed of more types than in MF groups (less fighting types were exhibited by both resident and intruder fish). Despite the behavioral differences, energy cost in terms of O_2 consumption was not affected by group composition (mean ± SD, MM = 1.93 ± 0.54; MF = 1.77 ± 0.46 mgO_2. gDW^{-1}.40/min; Student's t independent test, t = 0.71, P = 0.49).

Keywords: aggressive interaction, cichlid; courtship, O_2 consumption, sex recognition.

Composição sexual do grupo, interação social e metabolismo na tilápia-do-Nilo

Resumo

O objetivo deste trabalho foi testar se grupos monossexuais de machos gastam mais energia e exibem perfil agonístico diferente de grupos formados por um macho e uma fêmea na tilápia-do-Nilo (*Oreochromis niloticus*). Tais diferenças são esperadas, pois machos e fêmeas competem por diferentes recursos reprodutivos. Foram utilizadas duplas de machos (MM) e duplas de macho-fêmea (MF) que permaneceram pareadas por 40 minutos. Durante esse período foi feito o registro da interação agonística (10 minutos iniciais e 10 minutos finais do pareamento) e determinado o gasto energético (consumo de O_2) pelo Método de Winckler. A latência para o início dos confrontos (média ± DP, MM = 27,40 ± 25,15 s; MF = 14,22 ± 21,19 s; Mann-Whitney, U = 33,50, P = 0,21) e a freqüência de todas as unidades comportamentais (média ± DP, MM < 72,30 ± 25,29; MF < 73,50 ± 21,65.10/min; Mann-Whitney, P > 0,10) foram semelhantes entre os grupos MM e MF nos 10 minutos iniciais. Isso indica que cada intruso foi considerado um potencial competidor no início da interação. No entanto, a freqüência de ondulação (interação também exibida durante a corte) foi maior para o residente do grupo MF nos 10 minutos finais (média ± DP, MM = 3,56 ± 5,89; MF = 8,56 ± 4,00.10/min; Mann-Whitney, U = 15,50, P = 0,01). A freqüência de fuga, entretanto, foi menor para o intruso do mesmo grupo (média ± DP, MM = 3,90 ± 4,33; MF = 0,44 ± 0,96.10/min; Mann-Whitney, U = 23,50, P = 0,04). Além disso, o perfil agonístico no grupo MM foi composto por um maior número de itens comportamentais do que o MF (para residentes e intrusos). Apesar das diferenças comportamentais, o consumo de O_2 não foi afetado pela composição sexual do grupo (média ± DP, MM = 1,93 ± 0,54; MF = 1,77 ± 0,46 mgO_2.g peso $seco^{-1}$.40/min; *t*-teste de Student, t = 0,71, P = 0,49).

Palavras-chave: Cichlidae, consumo de O_2, corte, interação agressiva, reconhecimento de sexo.

1. Introduction

Aggressive fights are common in territorial fish. Territory owners are threatened by male or female intruders in a frequent competitive scenario. Such fights for environmental resources usually impose energy cost to the fish, which may result in growth depensation (Volpato and Fernandes, 1994), increased O_2 consumption and ventilatory rate (Alvarenga and Volpato, 1995), and metabolic disturbances (Haller and Wittenberger, 1988; Fernandes and Volpato, 1993). In the Nile tilapia, the intensity of such energy costs depends on the aggressiveness level of the interaction (Alvarenga and Volpato, 1995). In *Gasterosteus aculeatus* (Linnaeus, 1758), for example, hepathic glycogen is spent rapidly in more aggressive interactions (Chellappa and Huntingford, 1989), and in the coho salmon (*Oncorhynchus kisutch* Walbaum, 1792) growth rate is negatively correlated with aggression (Volestad and Quinn, 2003).

Fights become more intense in competition for scarce resources (Grant et al., 1995) and when competitors have similar fighting abilities (Maynard-Smith and Parker, 1976). Considering that the reproductive resources differ between sexes in fish (Gonçalves and Nishida, 1994; Wooton, 1998), we expect that fights are more intense in monosex groups than in male-female groups. In fact, studies on cichlid fish revealed a higher dyadic interaction among male-male than male-female contests (Yamamoto et al., 1999).

Aquaculture practices have imposed for the fast-reproducing fish (e.g. Nile tilapia) a monosex culture to avoid excessive number of fish in the tanks (Beardmore et al., 2001). This situation, however, should produce a high competitive environment and thus increase agonistic encounters and energy cost. Moreover, competition for reproduction territory is restricted to the Nile tilapia males, which defend territory and build nests on it (Gonçalves-de-Freitas and Nishida, 1998).

We predicted that group sexual composition, male-male or male-female, should determine different agonistic interactions and energy consumption. To evaluate this prediction, we studied the aggressive behavior and O_2 consumption in Nile tilapia according to sex composition of the pairs.

2. Material and Methods

Adult Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) were provided from a commercial supplier and held in a hatchery (185 m^3 tank). Fish were transferred from these tanks to the laboratory (500 L tanks; ca. 1 fish/5 L) and maintained for 30 days before experimentation. During this time no chlorinated water supplied the tanks at temperature close to 27° C, and a 12L:12D photoperiod was set. Biological filters guaranteed good water quality and food (ration for tropical fish) was provided to satiation every morning and afternoon.

The general design tested size-matched adult fish by using the resident-intruder paradigm (based on Figler and Einhorn, 1983), in which a resident male was paired with either a male or a female conspecific. Social interactions and O_2 consumption were quantified for each pair in these conditions.

Forty fish (intruders and residents) were socially isolated in glass aquaria (30 x 30 x 40 cm) for 60 hours (residency time). After, a male or female intruder was gently transferred to the respective resident aquarium, thus composing 10 male-male (MM) and 10 male-female pairs (MF). Each pair was maintained for 40 minutes, and agonistic interaction was video-recorded during the first 10 minutes and the last 10 minutes periods. O_2 consumption was calculated from the difference between O_2 quantity in the water before pairing and after the 40 minutes period of pairing.

Fish were observed in glass aquaria of 30 x 30 x 40 cm (27.5 L), with 3 walls covered with an opaque blue plastic to prevent visual contact among neighboring fish. The blue color was chosen because it has been shown to prevent stress in the Nile tilapia (Volpato and Barreto, 2001). Water mean temperature was 28 ± 1.0 °C and photoperiod was 12L:12D (7:00 AM-7:00 PM). Fish were fed ration for tropical fish corresponding to 0.5% of biomass given twice a day (at 8:00 AM and 5:00 PM).

Sexing was made by inspection of genital papillae stained with methylene blue showing the oviduct opening in females (Afonso and Leboute, 1993). Individual recognition of each fish into the group was possible because the resident fish was marked with a small cut in its caudal fin (as used in Fernandes and Volpato, 1993). Fish manipulation before isolation was preceded by anesthesia (Benzocaine- 12.8 $mg.L^{-1}$) and fish were killed by overdose of this anesthetic at the end of the experiment for sex confirmation.

The agonistic interaction was quantified in terms of frequency of each agonistic type from an ethogram based on descriptions of Falter (1983) and Alvarenga and Volpato (1995) to the Nile tilapia, as following:

Chase: one fish follows the opponent that swims to an opposite direction.

Circling: two fish with erected dorsal fin swim following each other, describing a circle, like a slow chasing.

Flight: the attacked or chased fish keeps away from the contest place.

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

Lateral threat: one fish with its fins spread and mouth opened approaches the opponent laterally, 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.

Perpendicular threat: the fish stays in the center of the aquarium while the other swims describing a circle around it. The fish in the center follows the opponent with its head, but remains in the center.

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

"Total fight" as also analyzed as the sum of all agonistic types, except "flight".

The O_2 consumption of each pair was set by determination of water O_2 concentration evaluated by Winckler's Method. Two water samples of 500 mL each were used: the first obtained 1 minute before the pair formation and the second 40 minutes after pairing. Water was collected by carefull siphoning to avoid bubble formation. The canulae for siphoning was already fixed in the aquarium to avoid disturbance to the fish. After the first water sample, an intruder fish was put into the resident's aquarium. A vegetal oil layer was added on the water to avoid gas exchange with the air. A hard plastic screen with the same area of the aquarium was fixed 2 cm below the oil layer to avoid contact of the fish with the oil.

Before statistical analyses, outlier values were identified as shown in Volpato (2007), removed from the sample, and replaced by the mean value. Data normality was analyzed by Shapiro Wilk's test and parametric and non parametric statistical tests applied accordingly. Latency and frequency of agonistic interactions between MM and MF in a same time period were compared by the Mann-Whitney test; the frequencies compared over time (0-10 minutes x 30-40 minutes) were compared by Wilcoxon's test for each group (MM and MF); O_2 consumption was compared by the Student's t independent test between MM and MF. Statistical significance was set at $\alpha = 0.05$. Statistical tests were based on Zar (1999).

3. Results

Animal standard length and weight were not statistically different between MM and MF groups and within each group. Length (mean ± SD, MM resident = 9.34 ± 0.76 cm; MF resident = 9.31 ± 0.85 cm; MM intruder = 9.17 ± 0.72 cm; MF intruder = 9.20 ± 0.84 cm; two-way ANOVA, F(1.36) = 0.04, P = 0.84). Weight (mean ± SD, MM resident = 29.60 ± 7.30 g; MF resident = 28.65 ± 9.57 g; MM intruder = 27.52 ± 6 g; MF intruder = 28.94 ± 9.50 g; two-way ANOVA, F(1.36) = 0.18, P = 0.67).

Latencies to start contests were similar between MM and MF groups (mean ± SD, MM = 27.40 ± 25.15 s; MF = 14.22 ± 21.19 s; Mann-Whitney test, U = 33.50, P = 0.21). Most of the interactions significantly decreased from the initial to the final period of pairing within MM and MF (Wilcoxon, $P < 0.01$; Table 1).

No significant differences in the frequency of resident or intruder's agonistic behavior were observed in the first 10 minutes period (Mann-Whitney test, $P > 0.10$; Table 1) for MM and MF groups. At the end of the 10 minutes session, however, the frequency of resident undulation was higher in MF than in MM conditions, and the frequency of intruder's flight was lower in the MF than in the MM group (Table 1). Moreover, MM's resident and intruder showed 8 out of 9 agonistic types in the second 10 minutes period; in MF condition, the resident showed 3 and the intruder, 2 out of 9 agonistic acts (Table 1).

O_2 consumption was not different between MM and MF pairs, with mean (± SD) of 1.93 ± 0.54 for MM and 1.77 ± 0.46 $mgO_2.gDW^{-1}.40/min$ for MF (Student's t independent test, t = 0.71, P = 0.49). Macroscopic gonad analysis showed males with well developed whitened testes and females with developed, yellow color and well vascularized ovaries, which showed enhanced oocytes, indicating sexual maturity (Babiker and Ibrahim, 1979; Paiva et al., 1988).

4. Discussion

In the present study, we showed that male Nile tilapia does not fight in different way in immediate interactions with a male or female intruder, although some differences could be noticed later in the interaction. Moreover, the metabolic cost of interaction is not related to the sex composition of the pair.

As there was no difference between MM and MF conditions, neither in the latency to start interactions nor in the frequency of agonistic items in the first 10 minutes period (Table 1), we concluded that residents consider every conspecific (male or female) as a potential competitor, thus fighting similarly, indifferent to the intruder's sex. Also, the resident's behavior changed at the end of the 10 minutes session, when undulation was higher in the MF group. Undulation is a type of behavior that can be present in both agonistic and reproductive interactions, and it is related to courtship in the Nile tilapia (Gonçalves-de-Freitas and Nishida, 1998; Castro, 2004) and other fish species (e.g. Haley, 1987; Jaroensutasinee and Jaroensutasinee, 2003). Besides, female intruders exhibited less flightiness than male intruders indicating more tolerance with male approaching, what should occur in courtship interactions.

The period that we considered to analyze dyadic interactions was short to permit appearance of complete reproductive behavior, such as nest building or complete courtship. However, all animals tested had developed gonads, enabling the male to show some events related to courtship when females are present. Thus, our results support the idea that males start to change their interaction pattern related to the opponent sex only after some time of interaction, because the opponent responds in a different way, or alternatively, such change should be associated with sex recognition.

In the first case, it is possible that intruder females give-up fighting sooner than intruder males, modulating the pattern of attacks from resident males in order to reduce them or change the type of aggressive acts.

Table 1. Mean frequency (± SD) of agonistic types given by the Nile tilapia as a function of sex composition of the group and time of pairing.

Agonistic type	Origin	Group condition	Observation period	
			0-10 minutes	30-40 minutes
Chase	Resident	MM	0	0.30 ± 0.48
		MF	0	0
	Intruder	MM	0	0
		MF	0	0
Circling	Resident	MM	0.22 ± 0.42	0.22 ± 0.63
		MF	1.00 ± 1.56	0*
	Intruder	MM	0.22 ± 0.42	0.22 ± 0.63
		MF	1.00 ± 1.56	0*
Flight	Resident	MM	0.20 ± 0.42	0*
		MF	0	0
	Intruder	MM	0.44 ± 0.50	3.90 ± 4.33^{b}
		MF	1.89 ± 4.33	0.44 ± 0.96*^{a}
Lateral fight	Resident	MM	7.10 ± 4.53	0.78 ± 2.20*
		MF	6.60 ± 4.84	0*
	Intruder	MM	7.10 ± 4.53	0.78 ± 2.20*
		MF	6.20 ± 4.76	0*
Lateral threat	Resident	MM	10.60 ± 4.20	8.18 ± 7.25
		MF	12.89 ± 6.35	3.90 ± 4.28*
	Intruder	MM	8.11 ± 4.82	1.33 ± 3.77*
		MF	11.30 ± 7.30	0*
Mouth fight	Resident	MM	8.90 ± 4.93	3.40 ± 7.17*
		MF	9.10 ± 7.31	0*
	Intruder	MM	8.90 ± 4.93	3.40 ± 7.17*
		MF	9.10 ± 7.31	0*
Nipping	Resident	MM	14.10 ± 5.26	9.30 ± 7.09*
		MF	11.22 ± 5.45	7.78 ± 7.36
	Intruder	MM	10.70 ± 4.90	0.56 ± 1.57*
		MF	6.80 ± 4.64	0*
Perpendicular threat	Resident	MM	6.70 ± 5.12	0.44 ± 1.26*
		MF	4.20 ± 5.05	0*
	Intruder	MM	5.80 ± 4.05	0.56 ± 1.57*
		MF	6.90 ± 6.84	0*
Undulation	Resident	MM	22.80 ± 10.77	3.56 ± 5.89*^{a}
		MF	20.20 ± 4.08	8.56 ± 4.00*^{b}
	Intruder	MM	20.00 ± 9.92	3.00 ± 4.03*
		MF	16.10 ± 9.39	0.67 ± 1.25*
Total	Resident	MM	72.30 ± 25.29	24.44 ± 16.89*
		MF	73.50 ± 21.65	26.00 ± 16.84*
	Intruder	MM	64.40 ± 26.12	13.78 ± 17.24*
		MF	63.60 ± 30.42	6.10 ± 12.64*

Different letters indicate statistical significance between MM (male-male) and MF groups (male-female) (Mann-Whitney test, $P < 0.04$). Asterisks indicate significant differences between the first and second observation period within each group (Wilcoxon, $P < 0.01$).

Accordingly, MF resident shows only 3 aggressive acts and the female intruder exhibits only undulation and flight. Moreover, in MM conditions, a most complete exhibition of agonistic interaction occurred, since 8 out of 9 types were exhibited by both resident and intruder fish, meaning a reciprocal control of combatants in agonistic interactions.

Changes in the aggressive patterns stated here are likely to be associated with sex recognition. Balshine-Earn and Lotem (1998), for example, found that sex visual recognition occurs in the cichlid *Neolamprologus brichardi* (Poll, 1974) that were kept together during a month. The authors observed higher aggressive attacks from males to neighboring males than to neighboring females, and a tendency to courtship females, showing that aggressive interaction is a good indicator of sex recognition in fish. Despite studies on sexual recognition for Nile tilapia are still scarce, Castro (2004) found that male-female visual contact enhances undulation and gonad development in the male Nile tilapia, thus showing some kind of visual information about sex of conspecific.

Assessment of individual characters during social interactions is well reported, including some studies on cichlid fishes (e.g. Turner and Huntingford, 1986). Moreover, Tinbergen (1972) has already shown, in his classic study on the three-spined stickleback (*Gasterosteus aculleatus*), that the initial response of male in a social interaction is to fight with any individual, until sex perception, leading to continued fighting or changing to courtship. Thus, the Nile tilapia recognizes the opponent's sex in a similar way to the stickleback, but more time is demanding for such recognition.

The energetic cost was similar in MM and MF groups. The technique used in this study is a good tool to assess energetic cost in fishes (e.g. Alvarenga and Volpato, 1995). Moreover, O_2 consumption in the pair registered in this study is quite similar to that found in juvenile pairs of Nile tilapia by Alvarenga and Volpato (1995), who registered 1.90 ± 0.14 $mgO_2.gDW^{-1}/h$.

Alvarenga and Volpato (1995) found a positive correlation (canonical analysis) between fights (lateral and ventral) and O_2 consumption. We did not test for correlations, but we did not find differences of such aggressive behavior types (here we considered nipping to any part of the body as a nip) between MM and MF conditions, which can explain the absence of significance for O_2 consumption.

It is possible that most O_2 consumption occurred at the beginning of pairing, because there was a significant reduction in most of the interactions from the first to the second period of observation, which is expected for agonistic interactions of fishes (Haller and Wittenberger, 1988). Another explanation for the absence of difference in O_2 consumption may be that agonistic and reproductive interactions have the same energetic cost for the Nile tilapia. As pointed out by Wootton (1998), both types of behavior have high costs for fishes.

In short, our results do not support the prediction that male-male contests use more energy than male-female contests, even after sex recognition, at least for short-term interactions. However, such effect of sex on agonistic interactions might be studied in chronic situations.

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