Agonistic and reproductive behaviors in males of red hybrid tilapia, *Oreochromis niloticus* (Linnaeus, 1758) x O. *mossambicus* (Peters, 1852) (Osteichthyes: Cichlidae)

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Abstract

The red hybrid tilapia, *Oreochromis niloticus* (Linnaeus, 1758) x *O. mossambicus* (Peters, 1852) is a fertile hybrid used in the semi-intensive level of fish culture in the Northeast of Brazil. It is a territorial cichlid and is highly aggressive towards conspecifics during the breeding season. The purpose of this study was to investigate and describe the aggressive behaviour displayed by the males of this hybrid in non-reproductive and reproductive contexts. Behavioural observations revealed that aggression displayed by the reproductive males of red hybrid tilapia included threatening, undulation, parallel, lateral and frontal attacks, chasing, escape and submission. Possession of a territory influenced male aggressiveness, which was more intense in their own territory than that observed in a neutral situation. The males built nests, irrespective of female presence. All the behavioural patterns were in accordance with those previously described for one parental species, the Nile tilapia, *O. niloticus*.

Keywords: freshwater fish, *Oreochromis niloticus* x *Oreochromis mossambicus*, reproductive aggression, red hybrid tilapia.

Comportamento agonístico e reprodutivo nos machos de híbrido vermelho de tilápia, *Oreochromis niloticus* (Linnaeus, 1758) x *O. mossambicus* (Peters, 1852) (Osteichthyes: Cichlidae)

Resumo

O híbrido vermelho de tilápia *Oreochromis niloticus* (Linnaeus, 1758) x *O. mossambicus* (Peters, 1852) é um híbrido fértil utilizado na piscicultura numa escala semi-intensiva no Nordeste brasileiro. É um ciclídeo territorial e altamente agressivo frente a coespecíficos na época reprodutiva. O objetivo deste estudo foi investigar e descrever os comportamentos agressivos exibidos pelos machos deste híbrido em contextos não-reprodutivos e reprodutivos. As observações comportamentais mostraram que as agressões exibidas pelos machos de híbrido vermelho de tilápia incluíram ameaça, ondulação, confronto paralelo, lateral e frontal, perseguição, fuga e submissão. A posse do território influenciou a agressividade dos machos, cuja intensidade foi maior no próprio território em relação àquela observada em situação neutra. Os ninhos foram escavados pelos machos independente da presença das fêmeas. Todos os padrões comportamentais descritos estavam de acordo com os padrões previamente descritos para uma das espécies parentais, a Tilápia do Nilo, *O. niloticus*.

Palavras-chave: peixe dulcícola, *Oreochromis niloticus* x *Oreochromis mossambicus*, agressão reprodutiva, híbrido vermelho de tilápia.

1. Introduction

Aggressiveness is greater in fish during the breeding phase when males of many species establish and defend a territory for reproductive purposes (Turner and Huntingford, 1987). In cichlids, the territory is also considered a limited reproductive resource, whose occupation is aggressively defended by the males. During conflicts, territorial males exhibit agonistic behavior towards their opponents, which includes threatening, spreading

their fins and gill covers, thus resulting in increased body size (Yabuta, 2002; Telerph, 2004) and aggressive fights (Alvarenga and Volpato, 1995)

Body size affects the results of agonistic encounters between the males of many fish species (Enquist et al., 1990; Gonçalves-de-Freitas, 1999). Body size is considered as an attribute of crucial importance to the males of fish that are involved in conflict resolutions, since larger males become dominant (Turner, 1994). The male characteristics, such as relative body size and aggression, coupled with the possession of a good quality territory, could cause differences in mating success (Chellappa et al, 1999a,b). Aggression in fish is affected by other factors, such as familiarity (Giaquinto and Volpato, 1997), previous territory occupancy and reproductive period (Gonçalves-de-Freitas, 1999). Despite these effects on aggression, social interactions affect several processes of aquaculture interest, such as growth (Volpato and Fernandes,1994), stress (Fernandes and Volpato, 1993; Alvarenga and Volpato, 1995) and reproduction (Chellappa et al., 1999a,b).

Tilapia belongs to the Cichlidae family, is territorial and aggressive towards their conspecifics. The behaviour of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), has been extensively investigated. Studies on this fish behaviour patterns considered several aspects, such as communication (Giaquinto and Volpato, 1997), social stress (Fernandes and Volpato, 1993; Alvarenga and Volpato, 1995; Correa et al., 2003; Volpato et al., 2003; Barreto and Volpato, 2004), social hierarchy (Barki and Volpato, 1998), aggression (Alvarenga and Volpato, 1995; Giaquinto and Volpato, 1997; Correa et al., 2003); reproduction (Gonçalves-de-Freitas and Nishida, 1998), learning (Barki and Volpato, 1998; Moreira and Volpato, 2004), and others. However, most of the studies on the Nile tilapia are related to fish production or fish management due to the importance of this species in tropical systems (Vijayan et al., 1997; Barcellos et al., 1999a,b; Gontijo et al., 2003).

Despite these studies, few studies on the behaviour of the hybrid red Nile tilapia, *O. niloticus* (Linnaeus, 1758) x *O. mossambicus* (Peters, 1852), using this hybrid, have been carried out. The red hybrid tilapia is increasingly being used in semi-intensive level of fish culture in Northeast Brazil (Câmara and Chellappa, 1996, 2000). Therefore, an understanding of their aggressive behaviour, which is strongly associated with the reproductive cycle, can contribute information of interest, resulting in a more efficient and profitable cultivation of red hybrid tilapia.

The purpose of this study was to investigate the behaviour of the red Nile tilapia, markedly describing social interactions in agonistic and reproductive disputes, which included competition for breeding territory, effects of territory occupation, and behaviour patterns associated with the construction of nests during the initial phase of reproduction.

2. Material and Methods

2.1. Acquisition, maintenance and sex identification of the fish

Ninety individuals of both sexes of the red hybrid tilapia, *Orechromis niloticus* x *O. mossambicus* were obtained from the Fish Culture Station of the Agriculture College of the Federal University of Rio Grande do Norte (UFRN), Macaíba, RN, Brazil. The body length of red hybrid males was 163.6 mm ± 67.1 mm, with maximum and minimum lengths of 225 and 130 mm, respectively. The body length for the females was 122.2 mm ± 28.2 mm, with maximum and minimum values of 140 and 100 mm, respectively.

The fish were acclimatized in rectangular asbestos cement tanks (1.50 x 0.65 x 0.97 m). The temperature was maintained in the range of 26 and 28 °C, pH was maintained between 7.0 and 7.5, and dissolved oxygen between 7.0 and 8.0 $mg.L^{-1}$. Food was offered on a twice-daily basis, consisting of pellet ration for cichlids (tetra cichlids), corresponding to 3% of body weight. To assess sexual maturity, the genital pore region of the fish was coloured with methylene blue, which evidenced hyperemia (Goncalves-de-Freitas and Nishida, 1998). The following secondary sexual characteristics were considered for sex identification of the fish: the males had two orifices situated near the anal fin, the anus and the urogenital opening located on a small papilla. The females had three orifices, the anus, a transversal genital opening and a urinary opening.

This study consisted of three tests, which is specified below. All theses tests were conducted in glass aquariums, each measuring 60 x 40 x 40 cm, with a total water volume of 100 L.

2.2. Specific procedures

2.2.1. Aggressive behaviour (Test 1)

To describe the agonistic behaviour between the males of red hybrid tilapia during the initial breeding phase, eight males of equal size (166 mm) were placed in pairs in each aquarium in a neutral situation, where there was no territory established. All glass aquariums used in this study measured 60 x 40 x 40 cm with a total water volume of 100 L. The fish were observed in two sessions a day of 30 minutes each in the morning and in the afternoon (with an interval of four hours between the observations), during a period of one month, with a total of 60 observations. Continuous focal sampling method was adopted for the observations, using a digital chronometer. The behaviours displayed by the males during the conflicts were recorded and described according to studies of this species (Gonçalves-de-Freitas and Nishida, 1994) and of other cichlids (Chellappa, 1988; Neat, 1996; Chellappa et al., 1999a).

2.2.2. Agonistic behavior while establishing territory (Test 2)

Eight equally sized males were used (180 mm) to evaluate agonistic behaviour between males. Each aquar-

ium consisted of a 5 cm thick layer of crushed stone and sand as substrate, in a neutral situation where no fish resided. Two males were paired at the same time in each aquarium. The fish remained paired for two days.

Dominance was inferred from the analysis of the agonistic profile, which included attacks of the dominant fish toward the submissive counterpart. All submissive fishes were subjected to dominant aggression and were supposed to be stressed. These fishes were removed from the aquaria. Seventy-two hours subsequent to establishing territory, another equally sized male was introduced in each aquarium to give continuity to the test. In this situation, the eight males were tested, four as residents and four as non-residents. Residents were considered to be the four dominant fish that remained in the aquarium. The four introduced fish were considered as non-residents. Sixty observation sessions (as described in the previous test) were performed through continuous focal sampling, using a digital chronometer, to quantify the social interaction and the time spent by the fish respective to the nest site.

2.2.3. Nest building behaviour (Test 3)

After establishing territory, the fish started excavating the nests in the sand of the aquarium. To investigate the nest-building behaviour of the red hybrid males, four males and four females were used, besides the sexually mature pair in each aquarium. The total body length of the red hybrid males and females was 160 mm ± 67.1 mm and 122 mm ± 28.2 mm, respectively. Fifteen observation sessions were performed over a period of 30 minutes The fish excavated the crushed stone and sand on the bottom of the aquaria with their mouth and deposited this material away from the nest. Data pertaining to the distance where each male deposited the removed sand, the minimum and maximum time spent to build the nest, the diameter of the nest, and the time near and away from the nest were registered. The males were considered near to the nest when they remained close to the edge of the territory, at a distance of up to 20 cm from the center of the nest. The excavating behavior was measured by the number of times the fish removed the sand with their mouth.

2.3. Statistical Analyses

The following tests were used in the statistical analysis: Descriptive analysis (mean and standard deviation) of the measured variables and Students *t*-test for paired samples. Results were considered statistically significant at $p < 0.05$. Analyses were performed using the Statistica software program, version 6.0.

3. Results

The agonistic behavior exhibited by the males of red hybrid tilapia in a neutral area included threatening, undulation, parallel, lateral and frontal attacks, chasing, escape and subordination, which are described in Table 1.

The red hybrid males manifested social interactions during the initial phase of breeding, and the resident males emitted theses interactions at a higher frequency than the non-residents ($t = 3.84$, $p < 0.05$) (Figure 1).

In relation to the aggressiveness displayed by the males in a neutral situation, it was verified that the mean frequency of aggressiveness in their own territory was greater than that in a neutral setting ($t = 4.22$ $p < 0.05$) (Figure 2).

The nest-building activities began with the excavation of the substrate, when the fish removed the sand with their mouth and deposited it approximately 20 cm from the excavation site. The total number of nests excavated registered in this study was 476 and time of excavation was 30 ± 21.3 minutes. The frequency of excavation in the absence of females was 198 ± 7.1 times, and in the presence of females it was 278 ± 28.2 times. The excavated nest had a concave shape, resembling an inverted cone. The diameter of the nest was 167 ± 26 mm, with a depth of 49 ± 12 mm. The males initiated nest-building activities regardless of the presence of females. The time spent by the males at the nest was 24.6 ± 1.67 min, and the time away was 20.6 ± 0.82 min. The time spent by the females near and away from the nest was 2.15 ± 1.14 min and 22.6 ± 1.21 min, respectively. The time spent by the pair near and away from the nest was 4.6 ± 0.53 min and 11.14 ± 1.03 min, respectively.

Table 1. Description of behaviour displayed during conflicts between males of red hybrid tilapia, *Oreochromis niloticus* x *O. mossambicus*.

Behaviour	Description
Threatening	The fish spreads its fins fully and opens its gill covers as it approaches the opponent.
Undulation	The fish moves its body in a wave-like motion in an antero-posterior direction.
Parallel confrontation	The fish swim side by side, undulating their bodies, without physical contact.
Lateral attack	The fish bites the lateral region of the body of the opponent.
Frontal attack	Juxtaposition of the jaws and undulation of the tail region.
Chasing	The fish swims towards the other following its trajectory.
Escape	The pursued fish leaves the area of confrontation.
Subordination	The fish keeps its head elevated with its fins retracted and remains motionless.

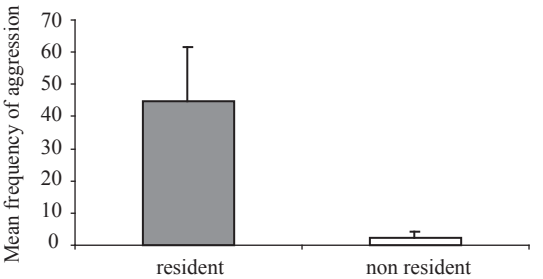

Figure 1. Mean frequency of attacks displayed by males of red hybrid tilapia as residents and non-residents.

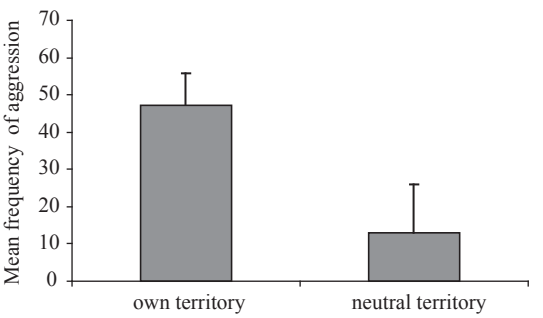

Figure 2. Mean frequency of attacks displayed by males of red hybrid tilapia in a neutral territory and in the own territory.

4. Discussion

Aggressive behavior is relatively common in territorial fish and many species of fish have a diversified repertory of responses during agonistic encounters (Turner, 1994). Many of these responses involve the spreading of fins and extending the gill covers, resulting in an apparent increase in body size, in addition to fin movement and biting, during which the opponents can demonstrate their fighting skills. In the Nile tilapia, *Oreochromis niloticus*, the aggressive males present agonistic behaviour patterns, such as threatening, undulation, parallel, lateral and frontal attacks, chasing and escape (Gonçalves-de-Freitas and Nishida, 1994; Gonçalves-de-Freitas, 1999). However, other agonistic profiles or denominations have been considered in the studies of the Nile tilapia (Alvarenga and Volpato, 1995; Giaquinto and Volpato, 1997; Volpato and Trajano, 2005). In the present study the males of the red hybrid displayed aggressive behaviour similar to that of *O. niloticus*. The agonistic behaviour of other cichlid fishes, such as angelfish, *Pterophyllum scalare* and the common tucurané, *Cichla ocellaris* includes movements of approximation, frontal attack, threatening, change of eye colour, biting, chasing, escape and submission (Chellappa et al., 1999a). Aggressive fish displays, such as the erection of the dorsal fin and lateral display are common in the Nile tilapia (Barki and Volpato, 1998; Gonçalves-de-Freitas, 2002). Besides these, the eye color is also used as an indicator of social rank in the Nile tilapia, *O. niloticus* (Volpato et al., 2003), and in other fish species (Beaching, 1995; O´Connor et al., 1999, 2000; Sutter and Huntingford, 2002). However, in the Nile tilapia, social and rank signals do not rely exclusively on visual cues. It has been demonstrated that chemical signals affect rank recognition in this species (Giaquinto and Volpato, 1997).

In the current study, it was observed that there was a gradual increase in the intensity of fighting as an encounter proceeded among male fishes. The fights often started with energy-saving movements, such as threatening displays, which were performed at a distance without physical contact. These movements were followed up by more energy-intensive behaviour that involved physical contact, such as lateral and frontal attacks and mouth locking. Alvarenga and Volpato (1995) showed that ventral nipping and lateral fights are the two main agonistic behaviors associated with high energy consumption in the Nile tilapia. In interactions where physical contact did not occur, such situation allowed the opponents to assess their relative strength, and to become subordinate at this point (Chellappa, 1988; Chellappa and Huntingford, 1989).

Territory is a fixed area from which other males are expelled by means of agonistic behaviour. Territoriality occurs when males adjust their fighting behaviour to their position in space. In the present study it was observed that breeding males restrict their activity to specific areas and chase any intruding males to the edge of the territory, using a combination of displays and attacks. In territorial species, the claiming of territory confers an arbitrary advantage on the resident, providing it with greater chances of winning, regardless of the size of the opponent (Maynard Smith, 1984). This has been reported as "previous territory effect" and is frequently encountered in fish (Gonçalves-de-Freitas and Nishida, 1994; Chellappa et al., 1999b). In the present study, though the males were of equal size, the residents were more aggressive and victorious in the conflicts. Furthermore, residency is considered to be one of the important factors that influences male aggressiveness and consequently the results of the conflicts (Chellappa et al., 1999b).

Occasionally males in a group can be arranged in terms of a linear ranking system or dominance hierarchy that reflects the pattern of agonistic relationships within them. Dominant males often get priority of access to limiting resources, such as territories, food and mates. This social status established by overt confrontations is maintained by less aggressive encounters, usually characterized by displays, which avoid serious damage between conspecifics (Turner and Huntingford, 1986; Volpato and Fernandes, 1994; Haller and Wittenberger, 1988). In the present study, during encounters between two male fishes, the subordinate (having lost in the previous encounters) often withdrew in response to an approach by the dominant male. In the Nile tilapia, *O. niloticus*, the dominant α males have priority in access to reproductive territories and in the courting of females in comparison

to the small subordinate males (Gonçalves-de-Freitas and Nishida, 1998). In this study, dominant males had accesses to the nest and to court females, which indicates that this ability is also encountered in the red hybrid.

The size of the nests built by male fish corresponds approximately to the total length of their body size. This result was similarly observed in a study performed with the species *Symphodus melops*, in which the nest size was positively correlated with male body size (Uglem and Rosenqvist, 2002). In the current study it was demonstrated that the red hybrid presents the same patterns of social relationship previously described for one parental species, the Nile tilapia, *O. niloticus*. Further studies on agonistic and reproductive behaviour of the red hybrid should be conducted in order to provide broader comparisons.

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