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Social relationship as inducer of immunological and stress responses in matrinxã (*Brycon amazonicus*)

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ABSTRACT

Matrinxã (*Brycon amazonicus*) is a freshwater neotropical fish species with a social interaction and aggressive behavior, especially in crowded environments. This species' social structure is established by agonistic interactions, which increase significantly at the fifth hour, when compared to the first and second hours of confinement, when each dominant fish competes with other individual for a conditioned territorialism. This social relationship also induces a complex physiological response in the organism, which generates an acute stimulation from the stressor agent. A stress situation modulates the physiology of the subordinate fish, which undergo significant increases in cortisol, glucose, hematocrit, and hemoglobin, when compared to the control fish. The immune system also indicates a modulation caused by cortisol, which results in an increase in neutrophils and a significant decrease in thrombocytes in subordinate fish, in comparison with control fish; however, the dominant fish show a significant increase in monocytes and a decrease in lymphocyte levels, when compared control fish. The agonistic interactions in *B. amazonicus* during crowding are not favorable to the physiology and immune system of the fish, mainly subordinate fish, and should be avoided in rearing systems.

1. Introduction

Matrinxã (*Brycon amazonicus*) is a freshwater neotropical fish species native to the Amazon basin, which lives in schools and has a migratory behavior (Andrade and Malta, 2006). Currently, it is one of the most used species in commercial rearing systems in Brazil and other South American countries (Gomes and Urbinati, 2005). Matrinxã production is mainly destined to fee-fishing or sold directly to the marketplace as food fish. Under crowding circumstances, matrinxã shows a noticeable social interaction and aggressive behavior.

In fishes, aggressive behavior has established for dominant relationships (Corrêa et al., 2003). Moreover, a given species builds a social structure which is characterized by the agonistic interactions, and fish in one same group may compete with one another (McCarthy, 2001). As a consequence, the dominant fish is empowered to monopolize the use of essential resources such as food and shelter, whereas the subordinate fish can be excluded from these resources, attacked and even killed (Gilmour et al., 2005) mainly in rearing conditions. Therefore, more pronounced physiological damages are normally found in subordinate fish (Buchner et al., 2004), including elevation in cortisol levels, increased the susceptibility to disease and drop in growth rates (Sloman et al., 2008). All these factors add to the losses in a rearing system.

The dominance relationship induces a complex physiological response in the organism such as hematological, metabolical and immune alterations, which constitute an acute stimulation from the stressor agent (Barton et al., 2002). The glucocorticoids, mainly cortisol, are secreted while a stress situation can modulate the immune system through specific receptors on the immune cells (Padgett and Glaser, 2003). Thus, it is possible to identify an elevation in plasma cortisol in subordinate fish submitted to an acute exposure to dominant individuals (Buchner et al., 2004). During stress, basal metabolism is increased, inducing the production of glucose from non-carbohydrate sources and increasing the plasma glucose concentration via gluconeogenesis (Barton et al., 2002). Several studies assume this increase to be a result of the requirement for energy during the agonistic interactions (Corrêa et al., 2003).

This study aims to investigate the influence of social relations as the inductors of stress and immunological responses in *B. amazonicus* by alterations in cortisol levels, glucose, hemoglobin, hematocrit, and leukocyte counts. Also, this work shall contribute to a better understanding of stress physiology in social fish in general and provide data that help to improve aquaculture conditions and decrease economic losses.

2. Materials and methods

B. amazonicus (Characidae, Characiformes) juveniles, sexually immature and of indefinite sex (mean mass and length were 11.62 ± 2.23 g and 10.18 ± 0.54 cm, respectively), were obtained from a commercial

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supplier and transferred to a 1000-L indoor tank with constant aeration, at the Ecotoxicology Laboratory in the Centro Universitário de Vila Velha (Vila Velha, Espírito Santo, Brazil). Fish were acclimatized for 15 days and fed daily with a commercial ration containing 36% protein, three times a day. During acclimatization, 50% of the total volume of water in the tank was replaced three times a week.

The experimental procedure was conducted according to Hirschenhauser et al. (2004). After acclimatization, 12 fish were individually distributed in 30-L aerated aquaria and maintained for 48 h before the experiment actually started. At the same time, 6 fish were maintained in a well aerated 400-L aquarium (at this population density no agonistic behavior is observed in the aquaria). After this period, the 6 fish from the 400-L aquarium were individually distributed in 6 30-L aquaria. In total, 6 aquaria with 2 fish each one (test group) and the others 6 aquaria with one fish (control group) were used.

The expected behavior was the dominance of one the two fishes in the test group, manifested as aggressive interactions. To measure the agonistic interactions between the two fish of each aquarium, the aquaria were filmed according to Adams et al. (2000). The laboratory windows and the aquaria were isolated using black plastic films to avoid variation in light. Each test aquarium was filmed after the first hour, for 5 min in a period of 1 h, totaling 5 films for an aquarium during the experiment. For the analyses of available information, the film corresponding to each test aquarium was subdivided into 1-minute sections. In this manner, the number of agonistic interactions for 5 sections of 1 min in each 1-hour period was calculated. Two types of agonistic behavior were mainly determined by dominant fish: frontal attack to caudal fin and to opercular region of the subordinate fish.

After 6 h of experiment, all the fish from each aquarium were anesthetized with benzocaine (100 mg/L), and the volume of blood necessary to allow useful measurements was drawn from the caudal vasculature with heparinized syringes. Blood glucose, hematocrit, and hemoglobin were immediately measured. Leukocyte count was estimated with an aliquot of blood spotted on glass blades. The remaining blood was centrifuged (1100 *g*, 10 min), and the plasma was stored at −20 °C for cortisol analysis.

Blood glucose and hemoglobin were determined using a biochemical commercial kit (Bioclin, Belo Horizonte, MG, Brazil). Hematocrit values were measured after centrifugation for 10 min in microhematocrit tubes. The differential leukocyte count was established by observation on blood-smear-containing blades stained according to the Panotico method. The cortisol level was measured in accordance with the chemiluminescence method using the Siemens/Bayer ACS-180 SE biochemistry kit, with a range from 2 to 750 mg/L.

For further analyses, the fish from test aquaria were classified into dominants and subordinates. The aggressive fish were considered dominant and the escaping fish subordinate. The differences between the parameters were compared against the control group by one-way analysis of variance (ANOVA) and the Dunnett's test ($p<0.05$). Data from differential leukocyte count was log-transformed before statistical analysis. The results of the agonistic interactions were compared between the hours filmed by one-way ANOVA and the Tukey's test ($p<0.05$). All results are expressed as means ± SD.

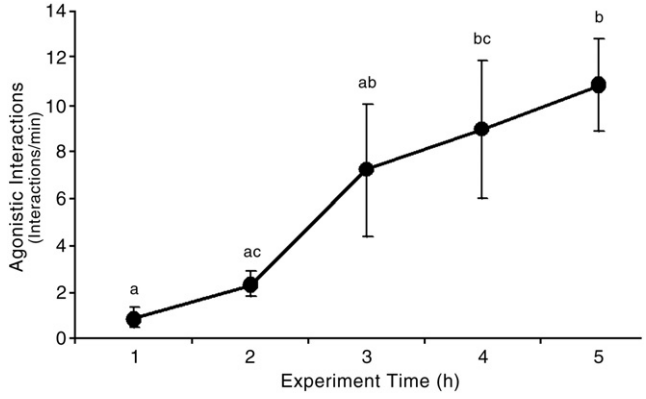

Fig. 1. Agonistic interactions were observed in *B. amazonicus* during six hours of social interaction, divided into one-hour periods. The results are means ± SD of 6 aquaria. Different letters indicate significant differences by one-way ANOVA and Tukey's test ($p<0.05$).

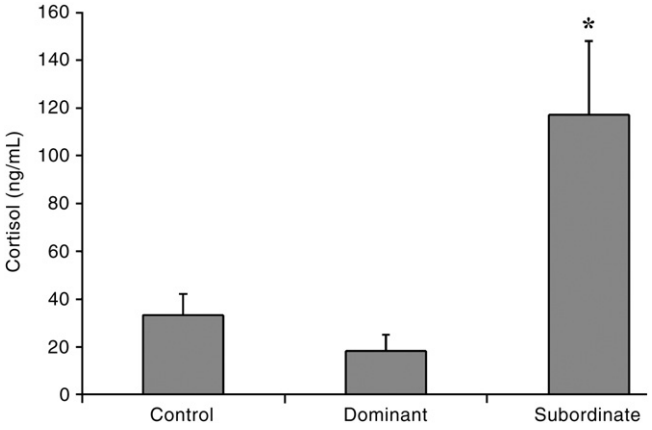

Fig. 2. Plasma cortisol value (ng/mL) in *B. amazonicus* after six hours of social interaction. The results are means ± SD of 6 fish per group.* Indicates significant differences from control group, determined by one-way ANOVA and Dunnett's test ($p<0.05$).

3. Results

The social relationship in *B. amazonicus* was characterized by a aggressive behavior, with the organization of fish into dominant and subordinate individuals. In four of the aquaria tested, the social relationship was characterized by the dominance of the resident fish, with the added fish being the subordinate ones. However, the opposite was observed in one aquarium, where the added fish were dominant. No dominance was ascertained in the sixth aquarium, therefore the data from those fish have been excluded from the physiological analysis.

Only dominant fish showed agonistic interactions, and the subordinate fish did not exhibit any aggressive behavior. In this experiment, a chasing behavior was observed in dominant fish and an escaping behavior in subordinate fish, which normally occurred within the volume of the grain stalk. The attacks, bites or attempts to bite occurred mainly on the sides of the body of the subordinate fish and directed toward the oppercular region and caudal fin. The agonistic interactions gradually increased during the experiment, and a significant increase was observed in the fifth hour, in comparison to the first and second hours (Fig. 1).

After the social-interaction period, subordinate fish had a significant increase in cortisol, plasma glucose, hematocrit, and hemoglobin in comparison to the control group. There were no significant alterations in those parameters in dominant fish, when compared to the control group.

The cortisol values were respectively 33.68 ± 8.84, 18.5 ± 6.7, and 117.5 ± 30.6 ng/mL in the control, dominant, and subordinate fish (Fig. 2). Plasmatic glucose concentrations in the control, dominant, and subordinate fish were respectively, 2.37 ± 0.62, 3.69 ± 0.46, and 16.80 ± 4.09 mMol/L (Fig. 3), whereas hematocrit values were 20.17 ± 1.28, 24.8 ± 1.83, and 25.6 ± 1.25%, respectively (Fig. 4). Hemoglobin values were 69.6 ± 7, 88.4 ± 7.6, and 123.6 ± 4.6 g/L, respectively, in the groups of fish, in the same order as above (Fig. 5).

The immunology in *B. amazonicus* was evaluated using cellular parameters, such as monocytes, thrombocytes, lymphocytes, and neutrophils. Monocytes showed a significant increase in dominant and in subordinate fish in comparison to the control. However, lymphocytes