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The effect of increased light intensity on the aggressive behavior of the Nile tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae)

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ABSTRACT. Animals show behavioral and physiological changes that emerge in response to environmental perturbations (i.e., emergency life-history stages). In this study, we investigate the effects of light intensity on aggressive encounters and social stability in groups of adult male Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758). The study compared the behavior observed under low (280.75 ± 50.60 lx) and high (1394.14 ± 520.32 lx) light intensities, with 12 replicates for each treatment. Adult fish were isolated in 36-L aquaria for 96 hours, and three males were grouped for 11 days in 140-L aquaria. Agonistic behavior was video-recorded (10 min/day) on the 3rd, 5th, 7th, and 9th day to quantify aggressive interactions and social stability. There was an effect of light intensity and day of observation on the total number of agonistic behaviors performed by the fish group. Besides, increased frequency of aggressive interactions (the sum of the four sessions) by the alpha, beta and gamma fish occurred at the higher light intensity. The dominance ranks of the fish remained unchanged across the observation sessions under both the low and high light intensities. We concluded that enhanced light intensity has a cumulative effect that increases the aggressiveness of the Nile tilapia but that this effect is not sufficiently strong to destabilize the social hierarchy.

KEY WORDS. Aggressiveness; aquaculture; environmental changes; social rank.

Factors producing long-term modifications to the aquatic environment can emerge from global climate perturbations, destruction of riparian vegetation, and the artificial environments found in aquaculture systems (Barrella *et al.* 2000). Changes in abiotic factors, such as water temperature, water level, photoperiod, and light intensity, can affect the physiology and behavior of teleosts (e.g., Britz & Pienaar 1992, Almazán-Rueda *et al.* 2005, El-Sayed & Kawanna 2007).

Light intensity is one of several abiotic variables that affect fish aggressive behavior (Sakakura & Tsukamoto 1997, Valdimarsson & Metcalfe 2001, Almazán-Rueda *et al.* 2004, Castro & Caballero 2004). Because this effect can interfere with the stability of the dominance hierarchy, understanding fish response to light intensity is important if specimens are reared artificially (Baldisserotto 2002). Some indirect effects of light intensity on the social hierarchy have previously been observed in fish. For instance, the hormone melatonin, the levels of which are reduced by high light intensity (Ekström & Meissl 1997), can control the physiological and behavioral profile of subordinate rainbow trout. According to Larson *et al.* (2004), low melatonin can reduce the signals produced by the subordinate fish and, in turn, destabilize the social hierarchy. Thus, we hypothesize that increased light intensity increases aggression and disrupts social stability in groups of the Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758). This hypothesis is based on the findings that the melatonin levels of Nile tilapia are reduced during photophase (Martinez-Chavez *et al.* 2008), and that reductions in this hormone increase aggressiveness in another cichlid species, *Aequidens pulcher* (Gill, 1858) (Munro 1986) and thereby produce social destabilization (e.g. Boscolo *et al.* 2011).

Although the social hierarchy is modulated by several factors related to aggressive motivation (Nelissen & Andries 1988, Quinn *et al.* 1996, Carvalho & Gonçalves-de-Freitas 2008), studies linking changes in environmental factors to the dominance hierarchy are scarce in fish. A number of previous studies have considered the availability of shelters (Kadry & Barreto 2010), the physical structure of the environment (Hofmann *et al.* 1999), the concentration of oxygen (Sneddon & Yerbury 2004), and the water level and flow (Sloman *et al.* 2001, Sneddon *et al.* 2006, Gonçalves-de-Freitas *et al.* 2008). However, the effect of light intensity on the stability of the social hierarchy has been investigated to a lesser extent, primarily in cichlids.

The Nile tilapia is one of the primary species reared in aquaculture worldwide (Popma & Lovshin 1995). As a cichlid, the Nile tilapia is territorial and exhibits a social hierarchy established by aggressive interactions (Gonçalves-de-Freitas *et al.* 2008, Carvalho & Gonçalves-de-Freitas 2008). Social dominance relationships can increase social stress as a result of increased aggressive interactions (Zayan 1991). Moreover, the luminosity interferes with reproduction, growth, metabolic rate, aggressiveness in the Nile tilapia (Biswas & Takeuchi 2002, Biswas *et al.* 2005, 2006, Carvalho *et al.* 2012), and this may affect the social hierarchy. Thus, we tested the effect of light intensity on the aggressiveness and social hierarchy in a cichlid fish, *O. niloticus*. Such studies can contribute to the scientific understanding of the mechanisms controlling aggressiveness in fish.

MATERIAL AND METHODS

The fish were initially kept in a fish pond (a 185-m^3 tank). Prior to the beginning of the study, the specimens were transferred to the laboratory and kept there for 15 days before experimentation in 500-L indoor tanks (ca.1 fish/5 L) with no chlorinated water, a temperature of approximately 27°C, controlled light intensity (749.8 ± 89.7 lx) and 12-hours light/dark cycles. Biological filters were used to guarantee water quality. The fish were fed commercial tropical fish food (28% of crude protein, Guabi/Pirá, Campinas, Brazil) twice a day.

Seventy-two adult male Nile tilapias were exposed to low (280.75 ± 50.60 lx) or high (1394.14 ± 520.32 lx) light intensity treatments. The fish were initially isolated for 96 hours in each light treatment to reduce the effect of previous social experience (during rearing) on aggressive behavior. Thereafter, three individuals were grouped in another aquarium for 11 days under each treatment, and their agonistic behavior was video-recorded (10 min/day at the 3rd, 5th, 7 th, and 9th day) to quantify agonistic interactions and the stability of the hierarchy. Twelve replicates were used for each treatment.

Animals were weighed, measured and sexed for grouping. The groups were formed using animals with similar standard length (one-way ANOVA, low light: df = 2, F = 0.02, p = 0.98; high light: df = 2, F = 0.03, p = 0.97) and body weight (one-way ANOVA, low light: df = 2, F = 0.12, p = 0.89; high light: df = 2, F = 0.39, p = 0.68) because size is a factor that affects agonistic behavior (Beeching 1992). The animals were identified by different cuts in the tail fin (Fernandes & Volpato 1993, Höglund *et al.* 2005). Sexing was performed by staining the genital papillae with methylene blue to reveal the opening of the oviduct in females (e.g., Carvalho & Gonçalves-de-Freitas 2008).

We used 40 cm x 30 cm x 40 cm (ca. 36 L) glass aquaria for isolation and 60 cm x 60 cm x 40 cm (ca. 140 L) glass aquaria for 3-male groups. Three walls of the aquarium were covered with opaque blue plastic to prevent visual contact between neighboring fish. The blue color was chosen because of its tendency to reduce cortisol levels in Nile tilapia (Volpato & Barreto 2001). The high light intensity used in the experiment was produced by four 9 W fluorescent light bulbs placed 55 mm above the surface of the water. The low light intensity was obtained from the standard illumination of the laboratory (four 40 W fluorescent light bulbs). Because the light intensity inside the aquarium can vary from the surface to the bottom and from the center to the sides, we divided the aquaria into 36 quadrants (three layers of 12 squares, each 9.0 x 9.0 x 9.0 cm) and recorded the light intensity at each point with a portable digital luximeter (model LD 240).The average light intensities inside the aquaria were then used as the representative values for the low and high intensity treatments.

Individual biological filters and constant aeration were provided for each aquarium. The pH was set at 7.2 and the ammonia level at 0.25 ppm. Water temperature was 26.38 ± 0.67°C, and the photoperiod was a 12 hours light/dark cycle, starting at 0700. Feed pellets (commercial food for tropical fish, 28% of crude protein, corresponding to 2% of biomass) were offered twice a day.

Fish manipulation (size measurement, weighing, cutting the tail fin and transferring fish from one aquarium to another) was always preceded by anesthesia with benzocaine (3 mg/L). At the end of the experiment, the fish were euthanized by a lethal dose of anesthetic (benzocaine: 27 mg/L) and dissected for gonad inspection and confirmation of sex and sexual maturity. Gonad maturation was assessed by macroscopic inspection. The morphological descriptions for the Nile tilapia in Babiker & Ibrahim (1979) were used.

We quantified agonistic interaction, based on the ethogram described for Nile tilapia in Falter (1983), as circling, chase, lateral fight, mouth fight, nipping, threat and undulation. We calculated the total amount of fighting behavior as the sum of all agonistic events. Aggressive acts were considered for each animal, and rank was defined by a dominance index (DI = given attacks/given + received attacks) as used by Oliveira & Almada (1996) for other cichlid species. DI varies from 0 to 1.0. Social position was classified according to the decreasing sequence of DI values as alpha (dominant), beta (intermediate) and gamma (subordinate). These ranks were determined within the group for each observation session.

The Shapiro-Wilk's test was used to assess data normality (Zar 1999) and F max was used to assess homoscedasticity (Ha & Ha 2011) after square root transformation of data. We compared the total amount of fighting behavior over time (four observation sessions) and light-intensity conditions with a mixed-model ANOVA followed by Fisher LSD post hoc test, with the light-intensity treatment as the independent factor and the observation session as a repeated measure. Complementarily, we compare the total frequency of aggression (the sum of aggressive acts performed during the four sessions) between treatments and ranks by two-way ANOVA, being the light intensity the independent variable and ranks, the dependent one. The persistence of individual rank over sessions in each light-intensity condition (social stability) was tested with Kendall's coefficient of concordance (e.g., Gómez-Laplaza & Morgan 1993). The statistical significance level was set at $\alpha = 0.05$.

This study was conducted according to the ethical principles adopted by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Ethical Committee of Animal Experimentation of the São Paulo State University (UNESP), Botucatu, SP, Brazil (protocol 52/06).

RESULTS

The Mixed-model ANOVA showed significant effect of the light intensity (df = 1, F = 4.69, p = 0.04) and observation sessions (df = 3, F = 4.10, p = 0.01) on the total amount of aggression for the whole group. The two first session were similar between them (LSD, p = 0.66), but significant different from the last two observations (LSD, p < 0.04). The last two were not statistically different (LSD, p = 0.73) (Fig. 1). There was no significant interaction between light intensity and observation sessions (df = 3, F = 1.96, p = 0.13). Complementarily, the analysis of total aggression by rank order, also showed a significant effect of the light intensity (2-way ANOVA, df = 1, F = 10.96, p = 0.0015), and rank (df = 2, F = 346.36, p = 0.000001). In this case, alpha, beta and gamma were all different among them (LSD, p < 0.000001) (Fig. 2). There was no significant interaction between light intensity and rank of fish (df = 2, F = 0.46, p = 0.63). A significant rank-order agreement over days was found for all groups tested, both at the low and at the high light intensities (Kendall's coefficient of concordance; low: W < 0.25, p > 0.28; high: W < 0.22, p > 0.34).

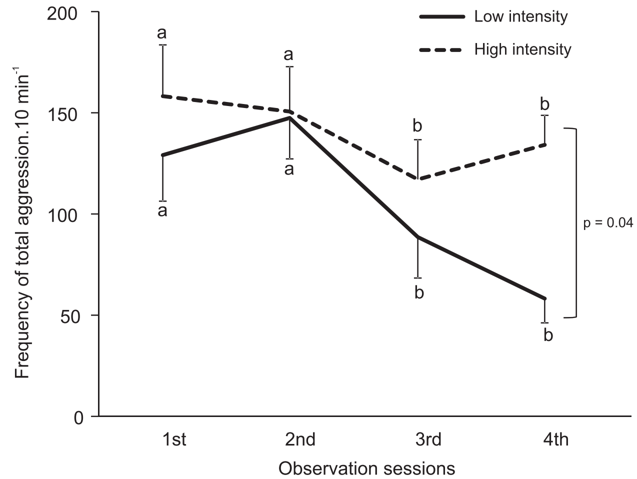

Figure 1. Mean (± SE) frequency of the total aggression of the whole group (3 fish) showed by session of observation. Letters compare observation sessions (repeated measures) and the lateral bar compares the light intensity. Different letters means significant difference (mixed model ANOVA, completed with Fisher LSD test).

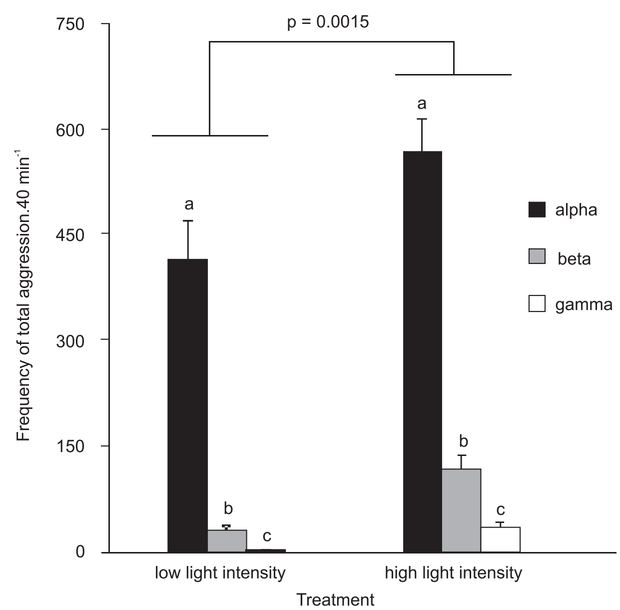

Figure 2. Mean (± SE) frequency of the total aggression of the whole period (sum of the 4 observation sessions) by hierarchical rank. Letters compare ranks and the upper bar compares the light intensity. Different letters means significant difference (2-way ANOVA, completed with Fisher LSD test).

DISCUSSION

The results of this study have demonstrated that aggressive behavior in groups of Nile tilapia was increased by high light intensity, but that this effect did not destabilize rank order. Moreover, the effect was widespread in the social group, regardless of social rank. According to Martinez-Chavez *et al.* (2008), melatonin in the Nile tilapia is controlled by the light regimen. This is a possible mechanism modulating aggressive behavior in this species, as Melatonin levels increase under low light- conditions (Ekström & Meissl 1997, Bayarri *et al.* 2002) and can reduce aggression in cichlids (Munro 1986). Moreover, high levels of melatonin induce subordination in rainbow trout (Larson *et al.* 2004). This behavioral modulation, however, contrasts with observations on the fish life stage. Carvalho *et al.* (2012) found that juvenile Nile tilapias have less motivation to fight under conditions of intense light. It is possible that juveniles are less aggressive when light is intense because they need to reduce predation risk, whereas adults need to defend their territory and attract females under those conditions (Carvalho *et al.* 2008). Studies on the behavior of the different life stages and the effect of melatonin on fish aggressive behavior, however, are rare in the literature.

High levels of aggressive interaction can harm fish health and welfare in aquaculture systems because more intense and prolonged contests produce a well-documented impact on energy expenditure and the risk of injuries to the contestants (e.g., Johnsson *et al.* 2006). Accordingly, the manipulation of light intensity can represent an approach to reducing aggressiveness in the artificial rearing of social fish, such as the Nile tilapia.

We expected that the high aggressiveness produced by high light intensity would destabilize the social hierarchy of the groups. In fact, several biotic and abiotic factors affect the aggressive interaction and dominance hierarchy, for instance, the same-sized fish group (Boscolo *et al.* 2011), the sex group composition (Carvalho & Gonçalves-de-Freitas 2011), the availability of shelters (Kadry & Barreto 2010), the concentration of oxygen (Sneddon & Yerbury 2004), and the water level (Gonçalves-de-Freitas *et al.* 2008). However, the effect of light intensity on the aggressive behavior was not sufficient to make the hierarchy unstable. Thus, the social challenges occurring among the alpha, beta, and gamma fish seem to be similar in the groups exposed to low and high light intensities.

Our result represents a component of the emergency life-history stage "take-it-or-leave-it", which corresponds to the behavioral and physiological strategies used by organisms to adjust to long-term perturbations of the physical environment. According to Wingfield (2003), vertebrates have evolved behavioral and physiological strategies to avoid the potential deleterious effects of predictable or unpredictable events. In this sense, the effect of light intensity on agonistic interactions indicates that fish change their behavior in response to abiotic perturbations. In Nile tilapia, this strategy is marked by an increase in aggressive interactions that maintains the social ranks in the group.

High light intensity has a cumulative effect on the aggressiveness of male Nile tilapia, but it is not sufficient to affect social stability. Therefore, light intensity is a factor that modulates aggressive behavior, and ambient light variation is a labile perturbation that can trigger emergency behavioral strategies in cichlid fish. Environmental factors should be controlled in aquaculture systems, and this finding can be considered in the design and management of rearing systems to minimize the undesirable effects of fighting on fish health and welfare.

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