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Less water renewal reduces effects on social aggression of the cichlid *Pterophyllum scalare*

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ABSTRACT

Changes in aquatic environments can affect fish behavior. Water renewal, for example, can dilute chemical signals, which are a social communication tool used by some fish species, and this dilution increases aggressive interactions in the group. Fierce and prolonged fights can affect fish welfare by increasing the probability of injuries, stress, and detrimental impacts on fish health. We tested whether the amount of water changed was associated with social aggression in the angelfish *Pterophyllum scalare*, a popular ornamental Amazonian cichlid. We designed an experiment with social groups (3 fish) that were assigned to one of three treatments (with 15 replicates of each treatment): 1) 50% water renewal; 2) 25% water renewal; or 3) 0% renewal (i.e., water removed and returned to the aquarium as a control). These treatments were referred to as T50%, T25%, and T0%, respectively. Fish behavior was video-recorded immediately before water renewal (baseline) and also 1 min, 1 h, 2 h, and 24 h after water renewal. The frequencies of attacks (overt fights) and displays (threats) were compared using the general linear model (GLM), with the treatment as the categorical factor, observation sessions as the repeated measures, and social rank as a continuous factor. Attacks increased after the water was changed, and they were higher in T50% than in T25% ($P = 0.0001$). In T25%, aggression returned to baseline levels after 1 h ($P = 0.32$), but remained increased after 24 h in T50% ($P = 0.000001$). Changing only a small volume of water at a time was therefore found to prevent exaggerated aggressive interactions among *P. scalare* specimens and to reduce the probability of injuries, stress, and detrimental impacts on fish welfare.

1. Introduction

Animal welfare can be affected by changes in the rearing environment. Among fish, changes in the aquatic environment can directly affect several types of communication (Rosenthal and Lobel, 2006) and can have negative consequences on behavior, particularly because chemical communication is impaired by changes in water characteristics. Fish use chemicals to signal their reproductive state (Miranda et al., 2005; Stacey and Sorensen, 2006), to recognize conspecifics (Reebs, 1994; Thünken et al., 2009), to warn conspecifics of predators (Jordão and Volpato, 2000), to identify size (Giaquinto and Volpato, 2005), to find well-nourished males in sexual selection (Giaquinto, 2010), and to communicate social rank in a social hierarchy system (Giaquinto and Volpato, 1997; Gonçalves-de-Freitas et al., 2008). These signals are released with the urine (Hubbard et al., 2014) and with bile acids (Giaquinto et al., 2015). In aquaculture systems, water needs to be renewed to remove food leftovers, feces, and toxic nitrogen compounds, as well as to maintain adequate environmental quality for fish health (Goldstein, 2001). Water renewal in fishkeeping and aquaculture management can wash away chemical signals, resulting in social instability and increased aggressive interactions in the group (e.g. Gonçalves-de-Freitas et al., 2008).

Aggressive interaction is part of the natural behavior of several fish species (Damsgård and Huntingford, 2012). However, in some artificial circumstances, aggressive interaction becomes fierce and prolonged (e.g. Barreto et al., 2015; Boscolo et al., 2011). The subsequent fights cause bodily injury (Martins et al., 2012), fin damage and scale loss (Maan et al., 2001), increased stress levels (Boscolo et al., 2011; Huntingford et al., 2006), metabolic impairment (Sloman et al., 2000), and reduced probability of survival (Barreto et al., 2015). All of these

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changes present problems for fishkeeping (Damsgård and Huntingford, 2012), particularly among fish whose natural behavior includes competition for social rank. Therefore, finding ways to mitigate such effects is important both for fish welfare and for more successful aquaculture and fishkeeping.

Cichlids are commonly raised in aquaculture as finfish and for ornamental trade (Huntingford et al., 2012). Species from the family Cichlidae exhibit territorial behavior and form a spatial dominance hierarchy in which social rank is established by aggressive interactions (Turner and Huntingford, 1986). Social hierarchy is maintained by displays, which are behaviors that require low energy expenditure and which result in a low likelihood of injury. These displays also allow fish to recognize their social ranks and communicate them using a set of signals, including visual signals (e.g. Beeching, 1992; Boscolo et al., 2011; Korzan et al., 2008; Volpato et al., 2003), acoustic signals (Longrie et al., 2008), and chemical signals (Giaquinto and Volpato, 1997; Gonçalves-de-Freitas et al., 2008; Keller-Costa et al., 2015). When one or more signals are impaired, fish can not recognize social rank, and increased rates of aggression can therefore follow (Boscolo et al., 2011). Although cichlid species have a complex visual system (Fernald and Wright, 1985), chemical communication is crucial for many species of fish. Giaquinto and Volpato (1997), for example, showed that an interruption in the interchange of water between two contestants' aquaria caused Nile tilapia to continue fighting, and social rank was not established. Moreover, Gonçalves-de-Freitas et al. (2008) showed that continuous water renewal destabilized social hierarchy and increased attacks among Nile tilapia males by reducing the amount of information received by the subordinate fish. They also found that, after establishing their social rank, fish again escalated to overt fights after three hours—the point at which water renewal reached 50%. These findings suggest that, in conditions in which water is not continuously renewed, social hierarchy can be established again after some time, thus reducing aggressive interaction in the group. In ornamental trade fish, aquarium water needs to be changed and replaced by clean water (albeit not continuously) as part of environmental management. Still, there are many questions regarding water change practices. Could water dilution have a transient effect if a small quantity of water is renewed? Are aggressive interactions associated with the amount of water renewed? The hypothesis tested here is that the amount of water renewed affects the time required to re-establish social hierarchy. This study tested the effect of changes in aquarium water on aggressive behavior of the cichlid angelfish *Pterophyllum scalare*, a popular ornamental cichlid species (Huntingford et al., 2012). We predicted that the amount of water renewed would positively correlate with aggression among individuals. Thus, by knowing the effect of the amount of water changed during aquarium management, we can offer a way to reduce the detrimental effects of artificial environments on social fish.

2. Methods

2.1. Fish housing

Pterophyllum scalare Schultze 1823 juveniles were obtained from the ornamental fish facility at the Aquaculture Center of the Jaboticabal campus of São Paulo State University, or UNESP (Centro de Aquicultura da UNESP, Jaboticabal, São Paulo, Brazil). The fish were acclimated to the lab for 15 days in 500 L polypropylene tanks (ca. 1 fish/10 L) with water at 28 ± 1 °C and a 12L:12D light regime (from 7:00 a.m. to 7:00 p.m.). Juveniles were used because *P. scalare* is a monomorphic species, and it is difficult distinguish the sex by external traits. Aggressive interaction can be different for adult males and females, since androgen levels increase during adult life in males (Oliveira and Almada, 1998; Oliveira, 2004), although androgens are also increased in female cichlids (e.g. Renn et al., 2012; Taves et al., 2009; Tubert et al., 2012). The use of juveniles prevented the influence of sex on aggressive behavior, and their use was possible because juveniles exhibit aggressive interactions in this phase as well (Carvalho et al., 2012). Fish were fed TetraColor Tropical Fish Food Granules (Tetra) twice a day (8:00 a.m. and 6:00 p.m.) until apparent satiation. Water quality was maintained using biological filters (Canister; filtering 400 L/h) and constant aeration.

2.2. Experimental design

The effect of different amounts of water renewal on aggressive interaction was tested in social groups of three angelfish (ranked as alpha, beta, and gamma fish) whose sex was not identified. The fish ranged from 30 to 50 mm in standard length (SL) and 0.5–5.5 g in weight. The social groups were formed and were observed once a day for four days to identify social rank establishment. On the fourth day, the social groups were randomly assigned to one of three treatments: 1) 0% water renewal (control); 2) 25% water renewal; or 3) 50% water renewal, treatments hereby referred to as T0%, T25%, and T50%, respectively. The highest water change was based on Gonçalves-de-Freitas et al. (2008), who found that Nile tilapia increased aggressive interactions after 50% water renewal; half of this amount (25%) was therefore chosen to test for water renewal at a smaller volume. In all treatments, 50% of the water volume was removed from the aquarium through a plastic tube placed inside the aquaria. In T0%, the same water taken from the aquarium was returned to it as a control. In T25%, half of the water returned to the aquaria was clean water and half was the original water, resulting in 25% renewed water. In T50%, the water returned to the aquarium was 100% clean water, meaning that, after the treatment, 50% of the water in the tank was original and 50% was clean water.

The water handling process lasted the same amount of time in all treatments (~10 min). Clean water was collected from a fish-free aquarium similar to those used in the tests, with a biological filter and aeration and at the same temperature. The water was gently poured in using a glass beaker so that interference with fish behavior would be minimized. We tested 15 replicates (social groups) of each treatment. As a result, 135 fish were used in this study.

2.3. Aggressive interactions

In aggressive interactions between pairs, subordinate fish death can result from continuous and one-directed strikes from the dominant fish. Thus, social groups of 3 fish were used, as this is the minimum number of individuals required to form a representative social hierarchy without risk of death. Fish were grouped together for three days, which has been determined to be sufficient time for *P. scalare* to establish a social hierarchy (Gómez-Laplaza and Morgan, 2003). On the fourth day, the aggressive behavior was recorded immediately before the water was changed or handled (the baseline amount), and then 1 min, 1 h, 2 h, and 24 h after the water was changed to evaluate the effect of this change on aggressive interactions at several time intervals, and also to evaluate the recovery time to baseline aggression levels. The video recordings lasted 10 min and were always run between 2:00 p.m. and 6:00 p.m. to avoid possible influences from circadian rhythm (as recommended by Martin and Bateson, 2007). This period was chosen because it was long after the first feeding (8:00 a.m.), which prevented any influence from food competition (Gómez-Laplaza and Morgan, 2003; Grobler and Wood, 2013). The fish were recorded on camcorders (Sony High-Definition Handycam), which were placed on tripods in front of each aquarium.

The aggressive behaviors were based on the ethogram described by Carvalho et al. (2012) for *P. scalare*, and included chases, lateral threats, frontal displays, undulation, nipping, and mouth fights. Aggressive behavior was labeled either as attacks (nipping, mouth fights, and chases) or as displays (lateral threats, undulation, and frontal displays). Attacks are overt fights that involve direct physical contact and are usually followed by elevated energy expenditure, whereas displays are moments of aggressive which do not involve physical contact and