Antipredator and alarm reaction responses of silver catfish (Rhamdia quelen) juveniles exposed to waterborne ammonia

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Ammonia has relatively toxic effects on fish and other aquatic organisms. This study examined whether juvenile silver catfish exposed to alarm substances released by conspecifics and predators in water with different ammonia levels modify their behaviour in response to the perceived risk of predation. We used juvenile catfish that were naive to predators. The fish were raised from the larval stage in the laboratory and kept in 40-L aquaria at waterborne NH₃ concentrations of 0, 0.05, 0.1, or 0.2 mg L⁻¹ for 10 days. The alarm substances used were predator odour and skin extract from conspecifics. The juveniles were transferred to 2-L aquaria for the antipredator and alarm reaction behavioural tests, which were performed on days 1, 5 and 10 after initial exposure to ammonia. The test aquaria contained a shelter at one end of the tank. The trials consisted of a 10-min prestimulus and a 10-min poststimulus observation period. The results of the study suggest that naive juvenile catfish are able to identify predators and skin extract from conspecifics by odour. In addition, waterborne NH₃ levels modify the antipredator response of this species.

Key words: Alarm substance, Behaviour, Predator odour, Skin extract, Un-ionised ammonia.

Introduction

Ammonia enters aquatic systems as a component of industrial and agricultural waste and of effluents. It is also produced by fish as a metabolic waste and may represent a major problem in fish farms. Ammonia diffuses easily through fish respiratory membranes and damages the gill epithelium (Miron et al., 2008). Consequently, it impairs gas exchange between the animal and the water, and it destabilises the osmoregulatory system (Wood, 2001). High waterborne ammonia levels also disrupt cerebral amino acid and energy metabolism and cause brain swelling (Walsh et al., 2007). The symptoms of ammonia poisoning in fish include hyperventilation, abnormal swimming, searching the surface, increasing ventilation rate, loss of balance, convulsions and, ultimately, death (Suski et al., 2007).

Ammonia has a major effect on the ability of white muscle to generate large amounts of energy quickly through anaerobic metabolism (Schulte et al., 1992). White muscle is known to be very susceptible to ammonia toxicity, and an increase in waterborne ammonia levels decreases the swimming performance of fish (Shingles et al., 2001; Tudorache et al., 2010). In an ecological context, reduced fast-start performance can affect escape and predation. Walker et al. (2005) showed that more rapid fast starts increase the probability of evading predators. Thus, a reduction in speed could lead to a higher risk of being captured by a predator. Consequently, predator species that are not affected by ammonia concentrations in the water, such as avian or mammalian predators, could have a significantly higher probability of successful predation on brown trout in ammonia-polluted freshwater (Tudorache et al., 2008).

The failure of prey to recognise and respond to a predator increases the probability of being captured during an encounter with a predator (Kiesecker et al., 1996). In freshwater fishes,
the pattern of antipredator responses to detectable threat cues is influenced by the condition or state of the individual (Clark, 1994; Pollock et al., 2006) and by its life-history stage or body size (Willette, 2001; Marcus & Brown, 2003; Harvey & Brown, 2004; Elvidge et al., 2010). The chemical-sensory recognition of the predator by the prey may be essential if the predator is hidden or if it hunts from ambush. If the diet of the predator produces chemical cues that allow recognition of the predator by the prey, then these cues may provide important information to naive prey (Chivers et al., 1996). If a predator attacks a fish and causes substantial damage to the skin of the prey, specialised cells in the epidermis release a chemical called alarm substance that functions as an alarm signal. Other prey can detect the presence of the alarm substance through olfaction. The prey then displays a stereotyped pattern of predator avoidance behaviour. It thereby reduces the risk of predation (Scott et al., 2003). Studies with fishes of the superorder Ostariophysi, which includes the Cypriniformes, Characiformes, and Siluriformes, suggest that nitrogen oxides function as chemical alarm cues and that the nitrogen oxide functional group acts as the chief molecular trigger (Brown et al., 2000, 2003).

Silver catfish (Rhamdia quelen Quoy & Gaimard, 1824) larvae respond to the skin extract of conspecifics and to the odour of the piscivorous Hoplias malabaricus (Kochhann et al., 2009). High waterborne ammonia levels affect common carp (Cyprinus carpio) swimming activity and feeding behaviour (Israeli-Weinstein & Kimmel, 1998) and predatory behaviour and fast-start performance in brown trout (Salmo trutta) (Tudorache et al., 2008). However, no previous studies have addressed the antipredator and alarm reaction responses of prey in an environment containing ammonia. Our hypothesis is that ammonia could affect the recognition of alarm substances released by conspecifics as well as the achievement of antipredator behavior. The aim of this study is to examine whether water contaminated with different ammonia levels affects the behaviour of silver catfish juveniles exposed to alarm substances released by conspecifics and to predator odour.

Material and Methods

Silver catfish juveniles were obtained from a population formed by artificial reproduction at the fish culture laboratory of the Universidade Federal de Santa Maria in southern Brazil and maintained at the Fish Physiology Laboratory of the same institution for 40 d prior to the experiments. The fish were kept in 40-L aquaria and fed three times daily with commercial food (Purina, 45% crude protein). Water was siphoned from the aquaria once a day, and all waste was removed with suction.

Exposure to ammonia

Silver catfish juveniles were exposed to NH$_3$ concentrations of 0.0, 0.05, 0.1, and 0.2 mg L$^{-1}$ for 10 days, 40-L aquaria (N = 10, three replicates each). These levels were chosen because even-higher levels did not induce mortality in silver catfish up to the 15th day of exposure, but an increase in the level of waterborne NH$_3$ proportionally reduced the growth rate in this species (Miron et al., 2011). Approximately 20% of the water in the aquaria was replaced daily and the level of un-ionised ammonia (NH$_3$) in the added water and the pH of the added water had previously been adjusted to the values determined by the experimental design. Behavioural tests were performed on days 1, 5 and 10 after the beginning of NH$_3$ exposure.

Preparation of alarm substances

The silver catfish skin extract was prepared according to the method of Brown & Smith (1998). Individuals were selected and immediately killed with a blow to the head. The skin was removed from both sides of each fish (total 5 g), rinsed with distilled water and placed in 50 mL of cold distilled water. The mixture of water and skin was homogenised and filtered through glass wool. The filtrate was diluted to a final volume of 400 mL with distilled water. The skin extract was stored in 25 mL samples at -20°C prior to use. Distilled water was stored in 25-mL samples at the same temperature for use as a control. To obtain predator odour, one specimen of H. malabaricus (500 g) was collected from the wild, quickly transported to the laboratory, kept in an 250-L aquarium for three days at 24°C and fed five silver catfish juveniles (ca. 5.5 cm in length) daily. The predator was then transferred to a continuously aerated 20-L aquarium and kept in the aquarium for 15 hours. Water from the aquarium was stored in a refrigerator at 5°C and subsequently used as the predator odour stimulus. The H. malabaricus specimen was returned to the wild.

Sampling and water analysis

The water quality was analysed daily. Temperature (25.0 ± 0.5°C) and dissolved oxygen (7.35 ± 0.06 mg L$^{-1}$) were measured using an oxymeter (YSI model Y5512), pH(7.5 ± 0.5) was measured with a pH meter (400A Quimix). The levels of total ammonia (NH$_3$ + NH$_4^+$) were determined according to Eaton et al. (2005), and the NH$_3$ level was determined according to Piper et al. (1982).

Experiment

Juveniles (3.2 ± 0.2 cm and 1.1 ± 0.1 g) were transferred from the 40-L aquaria to 2-L polyethylene aquaria, where they remained isolated for 24 h before testing began. The experimental aquaria contained a marked midline and a shelter (4x4x4 cm) located at one end of the tank. Each behavioural observation (N = 12 fish for each test, in individual aquaria) lasted 20 min. The observations were conducted using procedures similar to those in Kochhann et al. (2009). The trials consisted of a 10-min prestimulus and a 10-min poststimulus observation period. A 1-mL stimulus sample (skin extract (SE), predator odour (PW) or distilled water (DW)) was added after the prestimulus period. The juveniles were fasted for 24 hours prior to the experiment. The time that the fish spent in the shelter and the number of midline crossings (an indication of locomotor activity) during the pre- and poststimulus periods were recorded as described by Scott et al. (2003).

The data are expressed as mean ± SEM. The relationship between the measured parameters and waterborne ammonia was calculated using SigmaPlot 11.0 software. If no significant
relationship was found, the homogeneity of variances among groups was tested using the Levene test. Because the variances were homoscedastic, the data were compared using two-way ANOVA (stimulus sample X waterborne ammonia levels) followed by the Tukey test. Statistica version 7.0 software was used for these tests. The minimum significance level was set at \( P < 0.05 \).

This study was approved by the Ethics Committee on Animal Experimentation of UFSM under registration number 25/2007.

**Results**

Overall mean time in the shelter and number of midline crossings by juvenile silver catfish during the 600 s (10 min) prestimulus period was 372 ± 46.8 s and 10 ± 3, respectively. Nine per cent of the juveniles did not use the shelter, but there was no significant relationship between time in the shelter and number of midline crossings because only 11% of these juveniles that did not use the shelter showed higher number of midline crossings than the overall mean. These behavioural observations were not significantly altered by exposure to waterborne \( \text{NH}_3 \).

Juvenile catfish exposed to PW spent significantly less time in the shelter than DW fish \( (p < 0.05) \) (Fig. 1). Overall, waterborne \( \text{NH}_3 \) exposure increased two-fold the number of juveniles exposed to PW that increased the time spent in the shelter \( (p < 0.05) \), and the time spent in the shelter on day 1 by the juveniles exposed to PW increased in proportion to the level of waterborne \( \text{NH}_3 \) (Fig. 1a; \( r^2 = 0.81 \)). No such relationship was observed 5 or 10 days after \( \text{NH}_3 \) exposure and the time spent in the shelter in these days was not affected significantly by PW compared to DW in juveniles exposed to waterborne \( \text{NH}_3 \) (Fig. 1b, c). Exposure to PW increased significantly the number of line crossings \( (p < 0.05) \) (Fig. 2). The increase in waterborne \( \text{NH}_3 \) levels did not change significantly the number of line crossings after PW compared to DW (Fig. 2), but decreased significantly the number of juveniles that showed more line crossings after PW \( (p < 0.05) \). Silver catfish juveniles exposed to SE significantly reduced the time spent in the shelter, compared with DW fish, on day 1 \( (p < 0.05) \) but not on days 5 and 10 (Fig. 3).

Waterborne \( \text{NH}_3 \) exposure did not change significantly the number of juveniles exposed to SE that increased the time spent in the shelter, but the time spent in the shelter by the juveniles exposed to SE on day 1 increased in proportion to the level of waterborne \( \text{NH}_3 \) (Fig. 3a; \( r^2 = 0.87 \)). This relationship was not observed 5 or 10 days after \( \text{NH}_3 \) exposure (Fig. 3b, c). Exposure to SE did not change the number of line crossings on any of the experimental days (Fig. 4). The number of line crossings on day 10 increased in proportion to the level of waterborne \( \text{NH}_3 \) (Fig. 4c; \( r^2 = 0.76 \)), but no such relationship was observed on days 1 or 5 (Fig. 4a, b) and waterborne \( \text{NH}_3 \) also did not change significantly the number of juveniles that showed more line crossings after SE.

**Discussion**

The typical antipredator behaviours of fish include reduction in activity and movement to the bottom (Wisenden et al., 2008), increasing use of shelters (Wisenden et al., 2010), shoal cohesion, avoiding alarm cues, changing the body shape and decreasing foraging (Chivers & Smith, 1998). Lawrence & Smith (1989) also recognise abrupt and fast movements, freezing and scanning as antipredator responses.

Silver catfish juveniles kept in water without \( \text{NH}_3 \) and exposed to PW exhibited a significantly higher number of line crossings.
crossings and spent less time in the shelter compared with unexposed fish. The juveniles exposed to SE from conspecifics also decreased the time spent in the shelter but did not change significantly the number of line crossings compared with unexposed fish. However, a previous study found that silver catfish larvae (27 mg) exposed to SE or PW increased the time spent in the shelter and decreased the number of line crossings (Kochhann et al., 2009), a usual antipredator response (Wisenden et al., 2008, 2010). These contrasting results might be a consequence of the different sizes of fish (larvae 0.027 g, juveniles 1.1 g) used in the two experiments. Apparently, the silver catfish larvae sought

**Fig. 2.** Change in the number of line crossings (mean ± SE) before and after exposure to predator odour (black bars) and distilled water (grey bars) on days 1 (a), 5 (b) and 10 (c) of exposure to different waterborne NH₃ levels.

**Fig. 3.** Change in time spent in the shelter (mean ± SE) before and after exposure to skin extract (black dots or bars) and distilled water (grey dots or bars) on days 1 (a), 5 (b) and 10 (c) of exposure to different waterborne NH₃ levels. * indicates a significant difference compared with distilled water (p <0.05).

A: $y = -144.94 + 1636.72x$, where $y = \Delta$ time spent in the shelter (s) and $x = \text{waterborne NH}_3$ levels (mg L⁻¹).
concealment because of their small body size, whereas the juveniles increased their amount of movement probably to avoid the PW stimulus. As locomotor activity in juveniles exposed to SE was similar, the fish probably stood somewhere else in the aquarium instead of the shelter. The Nile tilapia, Oreochromis niloticus, typically moves away (either rapidly or slowly) from the region in which an alarm substance was added, and this action precedes the freezing response (Barreto et al., 2010). Zebrafish, Danio rerio, increased the time and frequency of erratic movements proportionally to the concentration of the alarm substance (Speedie & Gerlai, 2008). Piaçu, Leporinus macrocephalus, also showed erratic movements, followed by school cohesion and immobility after exposure to SE (Barbosa Júnior et al., 2012). The responses of silver catfish juveniles to PW and SE observed in the present study could be related to the same kind of behaviour. Another tropical freshwater fish, Rivulus hartii, demonstrated significant size-dependent trends in response to heterospecific alarm cues, with smaller individuals exhibiting antipredator responses and larger individuals shifting their behaviour to increased levels of activity consistent with a foraging, or predatory, response (Elvidge et al., 2010).

The overall response of the silver catfish juveniles to the increase in waterborne NH₃ was the decrease of antipredator responses to PW or SE. No similar studies have been conducted on other species exposed to high levels of waterborne NH₃. However, brown trout exposed to 1 mg L⁻¹ NH₃ exhibited predatory strikes, having a significantly lower distance, speed and turning radius and a lower number of prey captured than unexposed fish (Tudorache et al., 2008).

The decrease of the antipredator behaviour of silver catfish juveniles was proportional to the waterborne NH₃ levels on some days and for some observed behaviours. The reason for this variation is not clear, but the effect of waterborne NH₃ on the behaviour of common carp, Cyprinus carpio, also varied with the time of exposure (Israeli-Weinstein & Kimmel, 1998). The loss of response to PW or SE of conspecifics by silver catfish juveniles exposed to waterborne NH₃ does not imply that this species would be more vulnerable to predation because ammonia influences predator-prey interactions by disrupting predatory behaviour, changing the predator’s intraspecific interactions and reducing the predation rate (Tudorache et al., 2008).

The results of this study suggest that silver catfish juveniles can use odour cues to identify predators and to identify skin extract from conspecifics. In addition, waterborne NH₃ levels modify the antipredator response of this species.

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Fig. 4. Change in the number of line crossings (mean ± SE) before and after exposure to skin extract (black dots or bars) and distilled water (grey dots or bars) on days 1 (a), 5 (b) and 10 (c) of exposure to different waterborne NH₃ levels. C: y = -9.54 + 47.01x, where y = Δ line crossings and x = waterborne NH₃ levels (mg L⁻¹).
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