Chemical alarm cues in juvenile African catfish, *Clarias gariepinus* Burchell: A potential stressor in aquaculture?

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ABSTRACT

Previous studies on the effects of stocking density on the behaviour of African catfish have shown that at low densities, especially directly after restocking of tanks, increased aggression might occur. This aggression may directly affect the welfare of the fish. In addition, the resulting skin damage may also lead to the release of chemical alarm cues from the skin of the fish, possibly acting as a secondary stressor in a farming situation. Moreover, in a recirculation aquaculture system, a build-up of chemical alarm cues might occur. The objective of this study was to examine the effects of a single chemical alarm cue administration on the behaviour and growth performance of group-housed African catfish. Furthermore, the effects of a single passage over a biofilter on the behavioural response of African catfish to chemical alarm cues were tested. Although African catfish responded to chemical alarm cues with a short-term 35% increase in the number of active fish, no long-term effects were observed on both behaviour and growth performance of the fish. Furthermore, the results indicated that a single passage over a biofilter did not strongly alter the response of African catfish to the alarm cue, indicated by a 25% increase in the number of active fish. In conclusion, the results of this study indicate that chemical alarm cues, at the concentration applied in this study, cannot be considered a stressor for African catfish, although the effects of higher cue concentrations need further study. In addition, further study into the effects of chemical alarm cues on other, non-predatory, farmed fish is recommended.

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1. Introduction

The Ostariophysan chemical alarm cue or Schreckstoff system has been demonstrated in a wide variety of fish species (Smith, 1992), including the African catfish (Van de Nieuwegiessen et al., 2008). The system is characterised by distinct epidermal club cells which contain the chemical alarm cue. These cues may be released as a result of mechanical skin damage, e.g. through a predator attack. When detected by nearby conspecifics, these chemical alarm cues generally elicit species specific anti-predator responses, e.g., increased shoaling, freezing, and refuging (Brown and Godin, 1999; Brown et al., 1995; Mathis and Smith, 1993). However, some (predatory) species may use these signals as foraging cues (Chivers et al., 1996; Mathis et al., 1995). In general, a combination of increased activity and an increase in vertical area use is considered a foraging response (see Godin, 1997; Smith, 1997).

Although there is an extensive knowledge on the ecological role of chemical alarm cues, in a farming situation the potential effects of these cues are largely unexplored. Chemical alarm cues are likely present in aquaculture systems, released through agonistic behaviour, handling, or high stocking densities. Especially in recirculating aquaculture systems (RAS) chemical alarm cues may pose a problem. In contrast to flow-through systems, RAS are closed systems which re-use

water with mechanical and biological treatment between each use. Because of the closed character of a RAS a range of compounds, including chemical alarm cues, may accumulate.

Previous studies on the interaction between stocking density and behaviour of African catfish revealed that at the lower densities applied in practice, increased aggression occurs, especially directly after stocking (Van de Nieuwegiessen et al., in press). This may potentially lead to the release of chemical alarm cues. To gain insight into the possible consequences of this alarm cue release in a farming situation, the objective of this study was to examine the effects of a single alarm cue administration on the behaviour and growth performance of group-housed African catfish. Since it is unclear if the water treatment within a RAS affects the biological activity of chemical alarm cues, the effects of a single passage over a biofilter on the behavioural response of African catfish to chemical alarm cues were assessed.

2. Materials and methods

2.1. Animals and experimental conditions

All of the procedures involving animals were conducted in accordance with the Dutch law on experimental animals and were approved by the Wageningen University Animal Experimental Committee (DEC). Full sib juveniles of *Clarias gariepinus* Burchell ($N = 160$, mixed sex, average weight ($\pm$S.E.) = 104.0 ± 1.11 g) were obtained from a

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commercial fish farmer (Fleuren & Nooijen viskwekerijen BV, Someren, The Netherlands). Prior to the experiment, fish were held at the central fish facility (De Haar Vissen, Wageningen University, The Netherlands). Fish were randomly assigned to 16 70 L-aquaria (10 fish/tank) allowing them to adapt to the recirculating aquaculture system during a 14-day adaptation period.

Each tank was equipped with a single air-stone attached to the tank outlet. An extra length of airline tubing was attached to the air-stone, allowing for the injection of experimental stimuli from behind a black plastic blind to assure that the behavioural response of the fish was not influenced by people present.

During both the adaptation period and the experimental period, the aquaria were filled with recirculating UV-treated tap water with a water temperature of 25 °C, pH 7.5–8.0, NH_4^+ 0 mg/l, NO_2^- < 0.03 mg/l, NO_3^- < 150 mg/l and the conductivity ranged between 2000 and 4000 µs/cm. The photoperiod was 12 L:12D. Flow rate for each aquarium was set at 8 l/min. Fish were fed a commercial pelleted feed (Skretting ME-3, Fontaine les Vervins, France, 45% crude protein, 11% crude fat, 2% crude fibre, 8% ash) twice a day by hand. Feeding started at 09:00 h and 17.00 h and continued until apparent satiation.

2.2. Stimulus preparation

African catfish skin extract was prepared from a single donor fish (female, 217.9 g). The donor fish was killed humanely with 0.8 g/l tricaine methanesulfonate (MS-222, Cresent Research Chemicals, Phoenix, USA) and 1.6 g/l $NaHCO_3$. Using a surgical knife, skin sections were immediately removed from either side of the donor and placed in 50 ml of chilled demineralised water. Blood and muscle tissue were made certain not to contaminate the solution. Skin sections were then homogenized using a homogeniser (LaboCAT X1030, LaboCAT B.V., Zevenbergen, The Netherlands), the solution filtered through glass wool (to remove any remaining tissue), and the final volume adjusted by adding demineralised water. A dilution of 0.1 cm^2 skin/ml of demineralised water was used (Lawrence and Smith, 1989). 15 ml of this stimulus was applied in a 70 L tank. Given the area of skin typically damaged during predation, this concentration is most likely an ecologically valid one (G.E. Brown, personal communication). No information is currently available on alarm cue concentrations in fish farms. By using a commonly applied alarm cue concentration, the behavioural responses of African catfish can be compared to responses found in other studies. Future studies should determine alarm cue concentrations under farmed conditions and test for the effects of such concentrations.

2.3. Experimental procedures and measurements

After 14 days of adaptation, four experimental treatments were studied with a 2 by 2 factorial design. Factor 1 was directly adding conspecific skin extract in the tank of the fish (yes or no). Factor 2 was the water filtration system (RAS or flow-through). Four replicates per treatment were applied. The experimental period lasted for 14 days.

African catfish were first tested to a control (demineralised water) and subsequently tested to either conspecific skin extract or a second control (demineralised water). One hour before the control trial the tanks were disconnected from the recirculation/flow-through system, to avoid diluting the skin extract. Control trials and skin extract/second control trials were 60 min apart. Control and skin extract/second control trials consisted of a 15 min pre-stimulus and 15 min post-stimulus observation period. Prior to the pre-stimulus period, 50 ml of tank water was withdrawn and discarded and an additional 50 ml of tank water was withdrawn. At the start of the post-stimulus period, either 15 ml of demineralised water or 15 ml of skin extract was injected and slowly flushed into the tanks using the retained 50 ml of tank water. Dye tests showed that this procedure resulted in a homogeneous distribution of the chemical stimuli throughout the tank within 30 s. Control trials were conducted before skin extract trials to exclude a possible masking of the

response to the control trial by the skin extract stimuli. During both the pre-stimulus and post-stimulus observation period the percentage of animals swimming was continuously studied. Furthermore, the number of escape attempts (defined as an animal moving to the water's surface exposing its head to at least the gill cover) was measured. Behavioural response to either the control or conspecific skin extract/second control trial was calculated by subtracting the pre-stimulus response from the post-stimulus response.

Following these behavioural observations, all tanks were reconnected to the recirculation/flow-through system. The chemical alarm cues added to the tanks either entered the RAS or were flushed out of the flow-through system. The tanks receiving the double control either came in contact with chemical alarm cues after the cues passed the biofilter (in the RAS treatment) or never came in contact with chemical alarm cues (in the flow-through system). By comparing the behavioural response of these tanks the biological activity of chemical alarm cues after passing a biofilter was assessed. The behaviour of the fish was therefore studied, in blocks of 5 min, for 3 h after the tanks had been reconnected to the systems. For long-term behavioural changes, additional behavioural video recordings were made at days 1, 3, 7, and 12 from 12.00 h–12.30 h. The percentage of animals swimming (a displacement of the body, while browsing, moving, eating and air-breathing), percentage of animals resting (moving passively through the water or lying still at the bottom of the tank) and numbers of escape behaviour were recorded. Every minute the total number of fish swimming and total number of fish resting were counted as well as the total number of visible fish. The activity patterns were expressed as a percentage of the total number of fish counted. Escape attempts (frequency) were recorded by all occurrence sampling and expressed as number of escape attempts per fish per hour. The quality of the video recordings did not allow for a proper quantification of agonistic behaviour. Therefore, as an indicator of aggression, the number of bite wounds on the body of the fish was determined at the end of the experiment. This indirect measurement of agonistic behaviour was shown to have a strong correlation with the number of aggressive acts of African catfish (Almazan-Rueda et al., 2004). At the end of the 2-week experimental period, all fish were individually weighed.

Feed intake of the fish expressed per metabolic weight unit was calculated as Feed intake (g/$kg^{0.8}$ per day) = FI / (Wmean / 1000)$^{0.8}$, where FI (g/d) is the average feed intake per fish per day and Wmean is the geometric mean body weight, which was calculated as Wmean(g) = $\sqrt{(Wi*Wf)}$, where Wi and Wf are the initial and final average individual fish weight (g). Specific growth rates (SGR) were calculated as SGR (%/d) = [(lnWf − ln Wi)/t] × 100, where t is the experimental duration (days) and Wi and Wf are the initial and final average individual fish weight (g). Feed conversion ratio (FCR) was calculated as FCR = FItot / (Wf − Wi), where FItot (g) is the total average feed intake per fish during the experimental period.

2.4. Data analysis

The results are expressed as means (±S.E.M). In this study, tanks were considered the experimental unit. All data was analysed using a t-test or 2-way ANOVA. The error terms of these ANOVA analyses were tested for homogeneity of variances and normality, using respectively the Levene's test and the Shapiro–Wilk test. Preliminary analysis of swimming activity and escape behaviour indicated no effects of the day of recording. Therefore, data of all 4 days was pooled. Results were considered statistically significant when P-values were below 0.05; P-values between 0.05 and 0.10 were called trends.

3. Results

3.1. Behavioural responses to chemical stimuli and water filtration system

During 15 min after exposure to the chemical stimuli, African catfish responded to chemical alarm cues with an increase in the