CHEMICAL TRANSFER OF WARNING INFORMATION IN NON-INJURED FISH

by

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Summary

In this study we describe pacus, *Piaractus mesopotamicus*, detecting the presence of a predator by conspecific alerting pheromone. Moreover, we investigate whether this chemical information indicates the presence of a specific predator, or whether it indicates general disturbance. We exposed groups of pacus to the view of a predator fish (trahira, *Hoplias malabaricus*), a non-predator fish (piracanjuba, *Brycon orbignyanus*) or an aquarium without any fish (control), and then we transferred their water to isolated conspecifics. We set up six trials of each condition in which we analysed the dispersion and the distance from the visual stimulus in water-donor fish and the distance from the chemical stimulus in water-receiver pacus. This study showed that pacus visually identified the presence of another fish and recognised it as predator or non-predator. This is interpreted as an innate response. Such heterospecific detection affects the behaviour of pacus, which release chemicals that induce conspecifics to adopt a similar behavioural response. At least two chemicals might be involved, one of them possibly an alerting pheromone.

Keywords: chemical communication, disturbance pheromone, alerting pheromone, predator, fish behaviour, pacu, *Piaractus mesopotamicus*.

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Introduction

In predator-prey interactions, early detection of a predator may be crucial (Lima & Dill, 1990). In shoaling fish species, transfer of such information among conspecifics is achieved by different sensory modalities according to their habits and environment. Some species, *e.g.* minnows *Phoxinus phoxinus* (Magurran & Highan, 1988) and fathead minnows *Pimephales promelas* (Mathis *et al.*, 1996), give a fright reaction that warns conspecifics after detecting a predator. The fright reaction is clearly composed by visual displays, and these cues are widely used in fish communication (Guthrie & Muntz, 1993). However, considering the poor quality of aquatic images and the low transparency in turbid rivers, chemical cues are also expected to be used for transfer of information.

In fish, chemically mediated information among conspecifics is well reported. A range of studies has shown that many fish species release chemical alarm cues when injured (*e.g.* predator attack). Such alarm substance is stored in club cells located in the skin of most of Ostariophysan fishes, and it is released when these cells are burst by mechanical damage (for review see Pfeiffer, 1977; Smith, 1992; Chivers & Smith, 1998).

Although alarm substance has been largely investigated in fish, chemical communication in dangerous situations between non-injured conspecifics has scarcely been studied. The chemical released by stressed (but not injured) animals that is recognised by their conspecifics has been referred to as a 'disturbance pheromone', and is found in many aquatic organisms (Hazlett, 1989; Wisenden *et al.*, 1995; Mathis & Lancaster, 1998; Kiesecker *et al.*, 1999).

As far as we know, Wisenden *et al.* (1995) are the only investigators to experimentally describe such a disturbance pheromone in fish. They found that individual Iowa darters, visually stimulated by an artificial model of a predator, release chemicals that induce alert posture in conspecifics. But the term 'disturbance pheromone' is not specific to this type of disturbance. Thus, we adopt in this study the term 'alerting pheromone', as suggested by N.R. Liley (pers. comm.) to indicate the vigilance response to the cue.

The present study is the second to show such transfer of alerting information by chemical cues in fish. This was achieved in the Brazilian Characidae pacu, *Piaractus mesopotamicus*, using a natural predator as visual stimuli to induce release of chemical signals. Moreover, we investigated whether in such chemical cues pacus discriminate the heterospecific as a predator or not. Pacu is a schooling fish living in turbid rivers where vision is limited, and thus elaborate chemical communication may be expected.

Methods

Our investigative strategy consisted of exposing groups of pacus (4 fish per aquarium) to the view of a predator (trahira, *Hoplias malabaricus*) and transferring their water to isolated conspecifics. In a second condition we used a piracanjuba, *Brycon orbignyanus* (non-predator), instead of the trahira to test whether the effect of exposure to any fish produced alerting pheromone. Either trahira or piracanjuba were always turned to face the donor pacus. In a third condition, we used only the view of an aquarium with water but without any other fish. We set up six trials of each condition in which we analysed the behaviour of both donor and receiver pacus.

Fishes and holding conditions

We used 180-day old juvenile pacus, *Piaractus mesopotamicus* (Holmberg, 1887), grown in a hatchery and which had never experienced another fish species. These fish were transferred to the laboratory and housed in a 500-l tank (2 fish per l over this period). The trahira was captured one week before the experiment and kept alone in a 500-l tank. The piracanjuba was held in a 2000 l tank with three other tropical species for about two months (1 fish per 100 l). The lengths of the trahira and piracanjuba were about 15 cm each, and they were about three times larger than the pacus.

All the tanks were in a closed room with 12L:12D cycle and water temperature about 25°C. Commercial fish food was provided once per day in excess, but feeding was detected only in pacus and piracanjuba. The fish were food-deprived at least 24 h before the experiment. Food leftovers and solid fish excreta were removed at least once per week.

Experimental apparatus

We used glass aquaria $(28.0 \times 10.0 \times 18.0 \text{ cm}; 4.8 \text{ l})$ as either *donor* or *receiver* containers. Each *receiver* aquarium was connected to only one *donor* and the conditioned water was always transferred toward the receiver. Water was transferred through a pipe by gravity (80 ml per min) and reached the receiver aquarium at an outlet in a corner situated 1.75 cm from the bottom and 1.75 cm from the wall. The donor container was not refilled during the experiment to avoid the dilution of the released chemical substances. In pretrial studies, we tested the system by adding methylene blue in the donor containers: after 20 min of water transference, the transmitance in the receiver aquaria was equivalent to 88% of the donor one.

An aquarium with heterospecific fish (trahira or piracanjuba) or water only was set next to each donor aquarium and an opaque partition avoided visual contact between them before the tests. The receiver aquaria had no visual contact with either donor or heterospecific aquaria.

We used a dark curtain surrounding the whole aquarium system to prevent disturbance by external stimuli. Manipulation of the water flow was made outside this area to avoid handling interference.

Behaviour recording and analysis

Pacus were introduced into experimental aquaria approximately 16 h before the beginning of the experiment. Four pacus were placed in each donor and only one in each receiver aquaria. Although we used a shoal species in an isolated condition (a stressful element), this situation has been recognised to induce the fish to give a 'pure' response to chemical stimuli (Lawrence & Smith, 1989).

Two video cameras were set up for simultaneous recording of fish behaviour in both donor and receiver aquaria. We drew a 28.0×17.5 -cm grid divided into 3.5-cm squares on the frontal side of these aquaria to analyse fish position.

The pacus were videotaped for 25 min. At the 5th min the partition between the donor and the heterospecific fish (or water) aquarium was lifted and transfer of water started.

For videotape analyses, every 30 s we registered the position of the fish's eye in the grid. These data were then plotted on an X-Y axis. Mathematical analysis considered a set of 10 points per fish each 5 min. Thus, for the grouped (4 fishes) and the isolated pacus, 200 and 50 points were collected in 25 min, respectively.

The mean of the fish position on X axis and the mean on Y axis were the barycentric coordinates (calculated to each 5-min period along 25 min). From these data, three parameters were evaluated:

- (1) *Dispersion* is the mean of the distances between each position and the respective barycenter in both receiver and donor aquaria (Thines & Wandenbussche, 1966). The lower these values, the lower the dispersion (greater the cohesion) of the group.
- (2) *Distance from the visual stimulus* is the mean distance of the donor fish from aquarium with the visual stimulus (trahira, piracanjuba or water). Trahira and piracanjuba were too long to swim freely in the aquaria. Thus, they stayed motionless and the distance of the donor pacus to the aquarium with the heterospecific fish indicated approximately the distance between these fish.
- (3) *Distance from the water source* is the mean distance of the receiver fish from the water source (end of the pipe).

In the first 5-min period of observation (before manipulations), the investigated parameters were similar between the conditions, as follow. Mean values (\pm SE, N = 6) are expressed in cm. *Dispersion of the donor fish*: predator = 5.31 ± 1.14 ; non-predator = 5.96 ± 0.75 ; and control = 6.12 ± 0.94 (F = 0.20; p = 0.82). *Dispersion of the receiver fish*: predator = 3.12 ± 1.15 ; non-predator = 2.66 ± 1.54 ; and control = 4.20 ± 1.38 (F = 0.33; p = 0.72). *Distance from the visual stimulus*: predator = 10.51 ± 3.24 ; non-predator = 15.00 ± 1.74 ; and control = 10.22 ± 2.16 (F = 1.18; p = 0.33). *Distance from the water source*: predator = 7.24 ± 0.92 ; non-predator = 14.19 ± 4.05 ; and control = 12.71 ± 3.68 (F = 1.30; p = 0.30). Therefore, we analysed the effect of either visual stimulus or water transfer by subtracting the results of this first period from the respective values in the subsequent ones (post-pre differences). Thus, negative values indicate that pacus decreased the dispersion or the distance from the stimulus. The medians of these values were compared by Friedman's two-way analysis of variance followed by Dunnett's multiple comparison test (Lehner, 1996).

Results

No significant effect was detected between the conditions before test stimuli were imposed. However, significant changes occurred after presentation of the stimuli in all the parameters, except for the dispersion of the donor fish (Fig. 1).

The visual stimulus of either heterospecific species or only water did not significantly change the dispersion of the donor fish (Friedman, $\chi^2 = 4.75$, p = 0.93), but the donors were closer to the non-predator and farther away from the predator (Friedmann, $\chi^2 = 17.33$, p = 0.0017, Dunnett's multiple comparison test) (Fig. 1).



Fig. 1. Donor fish. Behavioural patterns elicited by visual stimuli in juvenile pacus as measured by change in dispersion (A) and change in distance from cue source (B). Behavioural changes were calculated by subtracting pre-stimulus (first 5 min) from poststimulus scores. Data are expressed as mean values (\pm SE) obtained from 6 trials. Different letters indicate statistically significant differences at p < 0.05 (Friedmann, corrected for Dunnett multiple comparisons of the medians).



Fig. 2. Receiver fish. Behavioural patterns elicited by chemical stimuli in juvenile pacus as measured by change in dispersion (A) and change in distance from cue source (B). Behavioural changes were calculated by subtracting pre-stimulus (first 5 min) from post-stimulus scores. Data are expressed as mean values (\pm SE) obtained from 6 trials. Different letters indicate statistically significant differences at p < 0.05 (Friedmann, corrected for Dunnett multiple comparisons of the medians).

After water transfer, the receiver fish changed their dispersion (Friedmann, $\chi^2 = 9.00$, p = 0.011, Dunnett's multiple comparison test) and distance from the water source (Friedmann, $\chi^2 = 11.64$, p = 0.003, Dunnett's multiple comparison test) (Fig. 2). In the latter case, predator and non-predator stimuli elicited different patterns of response from the receivers. Furthermore, the profile of the receiver's distance from the chemical source stimulus is very similar to that of the donor fish's response to the visual stimulus.

Discussion

This study showed that predator-naïve juvenile pacu, *Piaractus mesopotamicus*, can visually identify and distinguish other fish species as predatory or non-predatory. This is interpreted as an innate response. Furthermore, such heterospecific detection affects the behaviour of pacus, which release chemicals that induce conspecifics to adopt a similar behavioural response. At least two chemicals might be involved, one of them possibly an alerting pheromone.

Visual recognition of predators

The behavioural response of the donor fish to the view of a heterospecific was significantly different from that adopted after the view of an aquarium without fish. Moreover, these responses were species dependent: pacu approached the piracanjuba but retreated from trahira. Trahira is a predator fish (Nelson, 1994; Lowe-McConnell, 1995) while piracanjuba is a herbivorous one (Lowe-McConnell, 1995). Therefore naïve *P. mesopotamicus* distinguished by visual cues a predator from a non-predator fish.

Many studies have already reported the importance of vision as a mechanism for predator recognition in fishes (*e.g.* Mathis *et al.*, 1993). Such fish abilities to recognise predators may be genetic and modulated by early experience (Magurran, 1990; Mathis *et al.*, 1993; Chivers & Smith, 1998). In our study, because we used naïve pacus, the different responses of the donor pacus to the predator or to the non-predator strongly suggests that the discrimination have a genetic basis. Another consideration is that these heterospecifics are sympatric with pacu (Lowe-McConnell, 1995) and thus recognition of the trahira is an important component in the anti-predatory mechanisms evolved in this species. In fact, according to Malyushina *et al.* (1991) predator cues evokes innate responses in conspecifics and these responses are more pronounced when predator-prey have coevolved and coexist in a same environment (see this point in Kats & Dill, 1998).

Chemical transfer of warning information among non-injuried conspecifics

Magurran & Higham (1988) showed that minnows (*Phoxinus phoxinus*), which could see threatened conspecifics (but not the predator), modified their own behaviour. But while the visual transfer of information among

individuals of a shoal may be important, vision may also be limited in the aquatic environment (Mathis *et al.*, 1993), and thus other sensory abilities may be involved. The second important conclusion of the present study is that chemicals released by the fish may mediate such transfer of information.

When pacus were faced with heterospecifics (it did not matter if it was a predator or not), they released chemical cues that increased dispersion in conspecifics. In such case, however, the change in dispersion may be a mere consequence of the movements of the pacus approaching to (non-predator condition) or retreating from (predator condition) the chemical cue. Thus, the main biological response to chemicals released by conspecifics is not related to the dispersion of the fish, but to the direction it is moving to.

Chemical communication is widely used by fish in different contexts, including the alarm situation (for review see Liley, 1982; Smith, 1992; Chivers & Smith, 1998). However, most of these studies report chemicals released from damaged skin (club cells) (see reviews by Pfeiffer, 1977; Chivers & Smith, 1998). Literature is very scarce concerning chemical communication by non-injured fish. Some studies have suggested that stressed fish may release different chemical stimuli (Todd *et al.*, 1967; Malyushina *et al.*, 1991; Lebedeva *et al.*, 1994), but only Wisenden *et al.* (1995) have linked both predation threats to releasing of chemicals and chemical detection to changes in vigilance of conspecific fish. In this context, the present study is the second to clearly show behavioural modulation by chemicals of conspecifics induced by predator presence.

Piracanjuba attracted pacus directly by visual cues and these grouped pacus attracted conspecifics by chemical means. The sympatric occurrence of these species may suggest common habitat preferences and thus staying closer to each other may be of biological significance.

Two chemically-mediated responses were detected in this study (alert and attraction) and the participation of at least two chemicals is strongly expected. Nitrogenous waste product, possibly ammonium, has been proposed as component of the disturbance pheromone in crayfish (Hazlett, 1989, 1990), frogs (Kiesecker *et al.*, 1999) and Iowa darters (Wisenden *et al.*, 1995) and might be involved in the alerting responses described in this study. However, the two different responses reported here may indicate that at least another chemical may also be involved.

References

- Chivers, D.P. & Smith, J.R.F. (1998). Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. Écoscience 5, p. 338-352.
- Guthrie. D.M. & Muntz, W.T.A. (1993). Role of vision in fish behaviour. In: Behaviour of teleost fishes (T.J. Pitcher, ed.). Chapman & Hall, London, p. 363-439.
- Hazlett, B.A. (1989). Additional sources of disturbance pheromone affecting the crayfish in Orconectes virilis. — J. Chem. Ecol. 15, p. 381-385.
- (1990). Source and nature of disturbance-chemical system in crayfish. J. Chem. Ecol. 16, p. 2263-2275.
- Kats, L.B. & Dill, L.M. (1998). The scent of death: chemosensory assessment of predation risk by prey animals. Écoscience 5, p. 361-394.
- Kiesecker, J.M., Chivers, D.P., Marco, A., Quilchanos, C., Anderson, M.T. & Blaustein, A.R. (1999). Identification of a disturbance signal in larval red-legged frogs, *Rana aurora*. Anim. Behav. 57, p. 1295-1300.
- Lawrence, B.J. & Smith, J.R.F. (1989). Behavioral response of solitary fathead minnows, *Pimephales promelas*, to alarm substance. J. Chem. Ecol. 15, p. 209-219.
- Lebedeva, N.Y., Vosilene, M.Z.Y. & Golovkina, T.V. (1994). Aspects of stress in rainbow trout, *Salmo gairdneri.* J. Ichthyol. 23, p. 66-74.
- Lehner, P.N. (1996). Handbook of ethological methods. Cambridge University Press, Cambridge.
- Liley, N.R. (1982). Chemical communication in fish. Can. J. Fish Aquat. Sci. 39, p. 22-35.
- Lima, S.L. & Dill, L.M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68, p. 610-640.
- Lowe-McConnel, R.H. (1995). Ecological studies in tropical fish communities. Cambridge University Press, Cambridge.
- Magurran, A.E. (1990). The inheritance and development of minnow anti-predator behaviour. — Anim. Behav. 39, p. 834-842.
- & Higham, A. (1988). Information transfer across fish shoals under predator threat. Ethology 78, p. 153-158.
- Malyushina, G.A., Kasumyan, A.O. & Marusov, E.A. (1991). Ecological aspects of chemical signals in fish. — Scripta Technica, Inc. 31, p. 1-7.
- Mathis, A., Chivers, D.P. & Smith, R.J.F. (1993). Population differences in response of fathead minnows (*Pimephales promelas*) to visual and chemical stimuli from predators. — Ethology 93, p. 31-40.
- —, — & — (1996). Cultural transmission of predator recognition in fishes: intraspecific and interspecific learning. — Anim. Behav. 51, p. 185-201.
- & Lancaster, D. (1998). Response of terrestrial salamanders to chemical stimuli from distressed conspecifics. — Amphibia-Reptilia 19, p. 330-335.
- Nelson, J.S. (1994). Fishes of the world. John Wiley & Sons, Inc., New York.
- Pfeiffer, W. (1977). The distribution of fright reaction and alarm substance cells in fishes. Copeia 4, p. 653-665.
- Smith, J.R.F. (1992). Alarm signals in fishes. Rev. Fish Biol. Fish. 2, p. 33-63.
- Thines, G. & Vandenbussche, E. (1966). The effects of alarm substance on the schooling behaviour of *Rasbora heteromorpha* Duncker in day and night conditions. — Anim. Behav. 14, p. 296-302.

- Todd, J.H., Atema, J. & Bardach, J.E. (1967). Chemical communication in social behavior of a fish, the yellow bullhead (*Ictalarus natalis*). Science 158, p. 672-673.
- Wisenden, B.D., Chivers, D.P. & Smith, R.J.F. (1995). Early warning in the predation sequence: a disturbance pheromone in Iowa darters (*Etheostoma exile*). — J. Chem. Ecol. 21, p. 1469-1480.