

# Sensitivity of northern redbelly dace, *Phoxinus eos*, to chemical alarm cues

Angélique Dupuch, Pierre Magnan, and Lawrence M. Dill

**Abstract:** The northern redbelly dace, *Phoxinus eos* (Cope, 1862), is subject to predation by brook trout, *Salvelinus fontinalis* (Mitchill, 1814), in Canadian Shield lakes, particularly when individuals migrate to the pelagic zone at sunset to feed on zooplankton and fish shoals break up into single individuals. The objectives of the present study were to (i) determine whether northern redbelly dace react to skin extracts from conspecifics and thus potentially use chemical alarms to detect predators in nature, (ii) characterize the fright reaction in northern redbelly dace under different concentrations of alarm substance, and (iii) estimate the active space of the alarm substance in this species. Northern redbelly dace responded to skin extracts of conspecifics with a series of antipredator behaviours. The dace moved closer to the substrate and away from the area where the alarm substance was injected, increased both the cohesion and polarization of their schools, and performed more dashing and freezing behaviours. The observed responses were closely correlated with the concentration of the skin extract solution, suggesting that individuals could use this sensitivity to different concentrations of alarm substance to estimate the risk of predation in nature. The dilution experiment allowed us to estimate that 1 cm<sup>2</sup> of northern redbelly dace skin contains enough alarm substance to generate a response of individuals in 110 558 L of water, which corresponds to a cube with sides of approximately 4.8 m. Indirect evidence suggests that the northern redbelly dace could use chemical alarm cues to assess the risk of predation by brook trout in nature.

**Résumé :** Le ventre rouge du nord, *Phoxinus eos* (Cope, 1862), est une proie naturelle de l'omble de fontaine, *Salvelinus fontinalis* (Mitchill, 1814), dans les lacs du bouclier canadien, en particulier lorsque les individus migrent dans la zone pélagique au coucher du soleil pour se nourrir de zooplancton et que les bancs se dispersent. Les objectifs de cette étude étaient (i) de déterminer si le ventre rouge du nord réagit à des extraits de peau de conspécifiques et ainsi, a le potentiel d'utiliser un système d'alarme chimique pour détecter les prédateurs en nature, (ii) de caractériser la réaction de peur chez le ventre rouge du nord à différentes concentrations de substance chimique d'alerte et (iii) d'estimer l'espace actif de la substance chimique d'alerte chez cette espèce. Les ventres rouges du nord ont répondu à des extraits de peau de conspécifiques par un ensemble de comportements anti-prédateurs. Les individus se rapprochaient du substrat et s'éloignaient de la zone d'injection de la substance chimique d'alerte, augmentaient la cohésion et la polarisation de leurs bancs et faisaient plus de mouvements brusques et d'immobilisations prolongées. Les réponses observées étaient fortement corrélées à la concentration de la substance chimique d'alerte, suggérant que les individus pourraient utiliser cette sensibilité à différentes concentrations de substance chimique d'alerte pour estimer le risque de prédation en nature. Les expériences de dilution nous ont permis d'estimer que 1 cm<sup>2</sup> de peau de ventre rouge du nord contient suffisamment de substance chimique d'alerte pour générer une réponse des individus dans 110 558 L d'eau, ce qui correspond à un cube de 4,8 m de côtés. Des évidences indirectes suggèrent que le ventre rouge du nord pourrait utiliser les substances chimiques d'alerte pour estimer le risque de prédation par l'omble de fontaine en nature.

## Introduction

Chemical alarm signalling is among the strategies used by fishes to counter predation (Smith 1992) and can be divided into two distinct categories: signals released by prey that are disturbed but not injured, called disturbance signals, and those emitted when the prey is captured by a predator, called

damage-released alarm cues (Chivers and Smith 1998). There are numerous examples of damage-released alarm cues in different groups, and especially in ostariophysians (reviews by Pfeiffer 1977; Smith 1992; Chivers and Smith 1998). "Schreckstoff", a term used specifically for ostariophysians (Von Frisch 1938, 1941) and, more generally, alarm substances (also called alarm pheromones), are chemi-

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cal compounds localized in club cells of the epidermis and released into the water after mechanical damage of the skin, e.g., after a predator attack (Smith 1992). When these alarm substances are detected by nearby conspecifics or other fish species (i.e., cross-species response), they elicit a set of anti-predator behaviours including increased school cohesion and predator inspection, dashing, freezing, looking for shelter, and decreased foraging (Mathis and Smith 1993; Brown and Smith 1997; Chivers and Smith 1998; Nordell 1998; Chivers et al. 2000; Bryer et al. 2001; Mirza et al. 2001; Mirza and Chivers 2003).

The northern redbelly dace, *Phoxinus eos* (Cope, 1862), is subject to predation by brook trout, *Salvelinus fontinalis* (Mitchill, 1814), in Canadian Shield lakes (Magnan 1988; Lachance and Magnan 1990; East and Magnan 1991; Lacasse and Magnan 1992). This species exhibits onshore-offshore diel migrations between the littoral and pelagic zones of these lakes (Naud and Magnan 1988; Comeau and Boisclair 1998). The fish swim in shoals in the littoral zone during the day and migrate to the pelagic zone at sunset, where shoals break up into single fish, and then return to the littoral at sunrise (Naud and Magnan 1988). The dace also show a preference for a densely covered habitat rather than a sparsely covered one in the littoral zone during the day (Naud and Magnan 1988). Northern redbelly dace probably migrate to the pelagic zone at sunset to increase feeding efficiency on zooplankton. Brook trout are primarily visual foragers (Magnan and FitzGerald 1984). The foraging benefit for individuals remaining in the offshore zone may be offset by an increased risk of predation by brook trout during daylight hours, thus resulting in diel onshore-offshore migrations. Chemical alarm signalling could be particularly important for dace when visual cues are limited, especially when swimming at night in the pelagic zone.

The first objective of the present study was to determine whether northern redbelly dace react to skin extracts from conspecifics, thus indicating the potential use of chemical alarm cues to avoid predators in nature. The second objective was to characterize the fright reaction of the northern redbelly dace under different concentrations of alarm substance. Most laboratory studies that demonstrated the existence of chemical alarm cues were based on responses to the presence-absence of a stimulus (i.e., distilled water control versus skin extracts) and thus did not address whether fish adjust their response in relation to the perceived degree of predation risk. Finally, few studies have attempted to determine the amount of alarm substance released when a predator captures a prey animal (Chivers and Smith 1998). The third objective of the study was thus to estimate the active space of the alarm substance in northern redbelly dace, i.e., the volume in which the stimulus concentration exceeds the detection threshold of the receiver (Lawrence and Smith 1989).

## Materials and methods

### Experimental fish and holding conditions

Northern redbelly dace were collected with a beach seine in Lake Mastigou (Mastigouche Reserve, Quebec, Canada; 46°40'N, 73°20'W) in September 1999 and May 2000. Lake Mastigou also contains brook trout and white sucker, *Cato-*

*stomus commersoni* (Lacepede, 1803). Northern redbelly dace are subject to trout predation in this lake (Lacasse and Magnan 1992).

Dace captured in September 1999 were used for pilot experiments and to collect skin extract (see below), while fish captured in May 2000 were used for experiments, which lasted from 13 June to 18 July. The fish were held in a temperature-controlled, dechlorinated freshwater tank (900 L) under a 12 h light : 12 h dark regime. Water quality was tested regularly throughout the study and fish appeared to be in good health. Water temperature was maintained at  $14 \pm 1$  °C and fish were fed ad libitum once a day with commercial trout pellets (Corey Aquaculture 2.0 GR) plus a food supplement (Tetra-Min Flakes).

### Chemical stimuli

We prepared skin extract from six northern redbelly dace ( $5.54 \pm 0.48$  cm total length, mean  $\pm$  SD). As Smith (1973) showed that male fathead minnow, *Pimephales promelas* Rafinesque, 1820 (an ostariophysian), lost all cells containing alarm substance during the breeding season, we used dace captured outside their reproductive season. After killing the fish by a blow to the head (in accordance with the Canadian Council on Animal Care guidelines), we removed a skin filet from both sides of each fish (total area of skin collected, 18.09 cm<sup>2</sup>, determined by planimetry with an image analyzer). Immediately after removal, we placed the skin samples in 100 mL of chilled glass-distilled water. We ground the skin samples with a domestic food grinder for 1 min and filtered the homogenate to remove solid particles. From this initial stock solution, we prepared 5-mL skin extract aliquots of different concentrations (0.01%, 0.1%, 1.0%, and 10.0% of the initial solution) with distilled water. These solutions (hereafter called "alarm substance") were frozen immediately after preparation. Preliminary experiments indicated that freezing the alarm substance solution did not alter the fishes' response (also see Waldman 1982).

### Experimental setup and protocol

The experiments took place in a 40-L glass aquarium (35 cm  $\times$  25 cm  $\times$  30 cm depth) filled with 0.5 cm of gravel and 30 L of water and aerated with an air-stone fixed on the left side. A plastic tube allowed us to inject the alarm substance (15 mL) near the air-stone with a 25-mL syringe from behind a black curtain placed around the aquarium. Fish positions and behaviours were filmed from the top (1.20 m) and the side (0.8 m) of the aquarium with two Hi-8 video cameras (RCA and Sony CCD-TR400) connected to a recording system that allowed us to film experiments for periods of 20 min (10 min before and 10 min after alarm substance injection).

Groups of 10 fish ( $5.46 \pm 0.2$  cm) were used in each experiment. To allow experimental fish to acclimate to the experimental aquarium, they were first transferred from the 900-L holding tanks to a 40-L glass aquarium (similar to the experimental one) for 24 h. These fish were then transferred to the experimental aquarium for 24 h before the beginning of an experiment. Dace were fed a few hours before the beginning of an experiment so that hunger would not affect their behaviour (Morgan 1988a, 1988b). For each alarm substance concentration, five different groups of fish were

tested (giving five replicates per concentration). A fish was used only once in our experiment; we used 10 fish per replicate  $\times$  five replicates per concentration  $\times$  five concentrations for a total of 250 experimental fish. The testing order of the different concentrations was determined randomly as follows: 1.0%, control, 0.1%, 0.01%, and 10.0%. The five replicates of each concentration were done consecutively (one experiment per day). After each experiment, the aquarium was washed and the water changed to minimize the exposure of experimental fish to the alarm substance from the previous trial. We used distilled water as a control stimulus.

### Fish response to the alarm substance

We used six descriptors to quantify fish behaviour before and after injection of the alarm substance: indices of vertical and horizontal position in the aquarium, school cohesion and polarization, and numbers of dashing and freezing behaviours. Indices of vertical and horizontal position and schooling cohesion and polarization were measured every 30 s, while the total numbers of dashing and freezing behaviours were counted for the 20 min of the experiment.

Vertical and horizontal indices were calculated following Waldman (1982). The vertical index ( $V_I$ ) was calculated as

$$V_I = (+1)(N_{\text{TOP}}) + (0)(N_{\text{CENTER}}) + (-1)(N_{\text{BOTTOM}})$$

where  $N_{\text{TOP}}$  represents the number of fish in the top third of the aquarium,  $N_{\text{CENTER}}$  the number in the central third, and  $N_{\text{BOTTOM}}$  the number in the bottom third. This index can range from +10 (all fish in the top third) to -10 (all fish in the bottom third). The horizontal index ( $H_I$ ) was calculated as

$$H_I = (+1)(N'_{\text{LEFT}}) + (0)(N'_{\text{CENTER}}) + (-1)(N'_{\text{RIGHT}})$$

where  $N'_{\text{LEFT}}$  represents the number of fish in the left third of the aquarium,  $N'_{\text{CENTER}}$  the number in the central third, and  $N'_{\text{RIGHT}}$  the number in the right third. The horizontal index can range from +10 (all fish in the left side) to -10 (all fish in the right side).

Schooling cohesion and polarization were calculated following Heczko and Seghers (1981). A grid divided into 1-cm squares was placed over the television monitor. The  $X, Y$  coordinates of each fish's snout were plotted, and the centroid of the school was determined by calculating the mean  $X, Y$  coordinates every 30 s of the videotape. The mean distance of individual fish from the centre of the school was used as an index of school cohesion ( $C_I$ ). A decrease in the mean distance to the centre of the school indicated an increase in school cohesion. The measure of schooling polarization was obtained by determining the heading of each fish (based on  $360^\circ$ ) and calculating the mean heading (Batschelet 1981) for the school. The schooling polarization index ( $P_I$ ) is the mean deviation of fish from the mean heading. When this index decreased, the schooling polarization increased. Dashing behaviour was defined as very rapid, apparently disoriented swimming, while freezing behaviour was defined as very little movement for more than 30 s (Lawrence and Smith 1989). These two behaviours are well-known antipredator behaviours in cyprinid fish (Chivers and Smith 1998).

### Active space of alarm substances

The "active space" is the volume in which the concentration of the stimulus exceeds the detection threshold of the receiver (Lawrence and Smith 1989). To estimate the active space, one must first estimate the lowest alarm substance concentration (detection threshold) that initiates a response in a given volume ( $V = 30$  L in our experiments). Then, knowing the surface area of the skin used to prepare the various dilutions ( $18.09$  cm<sup>2</sup>) and the volume of alarm substance used in the experiments ( $v = 15$  mL in our experiment), it is possible to estimate the surface area of skin per millilitre ( $S$ ) that generated a response in the receiver for our experimental setup. So, the active space (volume) of water generated by  $1$  cm<sup>2</sup> of skin can be estimated as

$$\text{active space} = \frac{V}{Sv}$$

### Statistical analyses

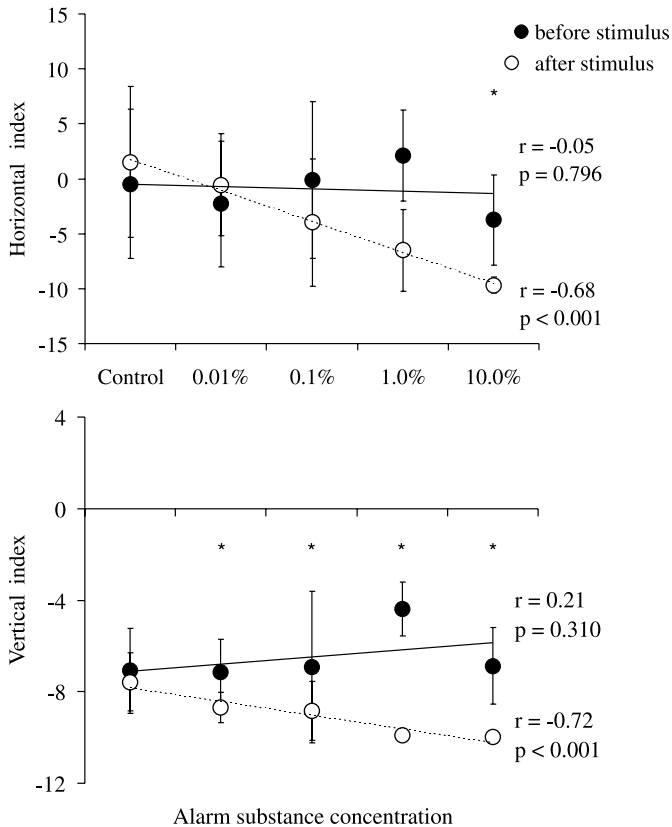
For each response variable and alarm substance concentration, we used the Wilcoxon paired-sample test to determine if and for which concentration the differences between pre- and poststimulus observations were significant. We also used Pearson product-moment correlations to determine if the intensities of the fish responses were related to the alarm substance concentration (dose response). The data on dashing and freezing were  $\log(X + 1)$  transformed to fulfill the conditions of normality and homogeneity of residuals (Montgomery and Peck 1982). The correlation analysis was more powerful than the single Wilcoxon paired-sample test because it used all observations of each given response variable and concentration value as a continuous variable. In both analyses, the sample unit was the experiment. Although we recognize that this is an important issue, we did not attempt to correct probability values for multiple tests given that the Bonferroni correction can be extremely conservative (Moran 2003; Peres-Neto et al. 2003).

## Results

### Effects of the alarm substance on fish behaviour

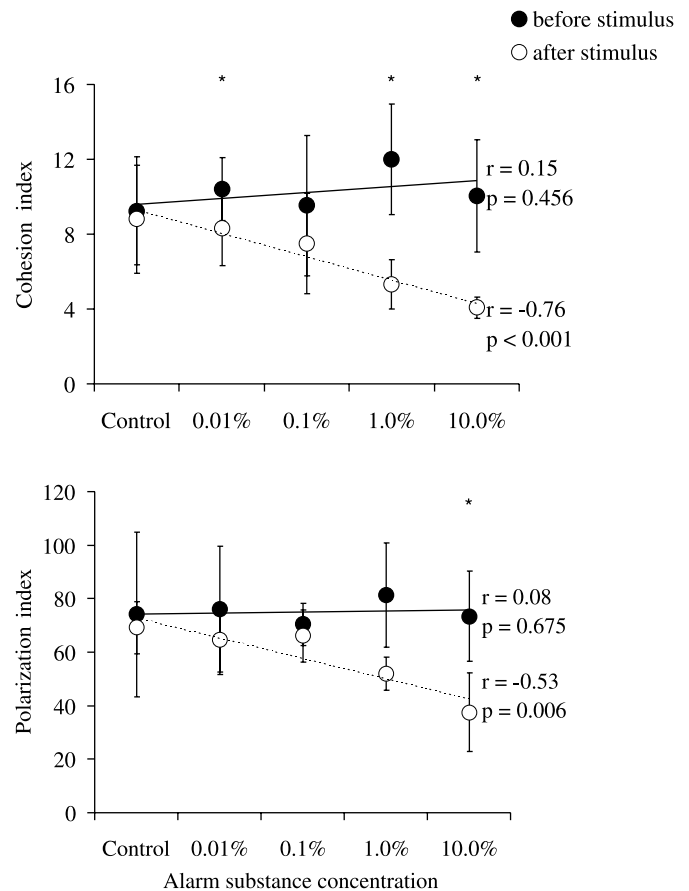
The fish swam freely throughout the aquarium during both the acclimation period and the control treatments (distilled water). We did not find any significant difference between the pre- and poststimulus periods for any of the response variables in the distilled water control treatment (Wilcoxon paired-sample test,  $p > 0.05$ ) (Figs. 1–3) with the exception of the number of dashings, which was significantly higher in the poststimulus periods than in the pre-stimulus periods (Wilcoxon paired-sample test,  $p < 0.05$ ) (Fig. 3). However, we did not find any significant correlation between the prestimulus values and the tested concentration treatments for any of the response variables (Figs. 1–3) (before stimulus; Pearson product-moment correlation,  $-0.29 < r < 0.21$ ,  $p > 0.05$ ). Based on the measured indices, the fish swam over all of the horizontal space (Fig. 1) and below the middle depth of the aquarium (Fig. 1) in groups with low cohesion and polarization (i.e., in shoals rather than in schools; sensu Pitcher 1986) (Fig. 2), and they performed few dashing and freezing behaviours (Fig. 3).

**Fig. 1.** Variations of horizontal and vertical indices for northern redbelly dace, *Phoxinus eos*, before (solid line) and after (broken line) the introduction of alarm substance at five different concentrations (control (distilled water), 0.01%, 0.1%, 1.0%, and 10.0%). Each data point is the mean  $\pm$  SD of five replicates;  $r$  is the Pearson correlation coefficient with  $p$  value. An asterisk indicates a significant difference between pre- and poststimulus observations as determined by a Wilcoxon paired-sample test;  $z = -2.02$ ,  $p < 0.05$ .



In contrast, the addition of the alarm substance significantly affected fish behaviour both qualitatively and quantitatively (Figs. 1–3). Based on the paired-sample tests, the horizontal and polarization indices were the least sensitive to the treatment effects (no significant differences between the pre- and poststimulus periods for skin extract concentrations of 0.01%, 0.1%, or 1.0%; Wilcoxon paired-sample test,  $p > 0.05$ ). However, all of the response variables were significantly correlated with the concentration of the chemical stimulus (Figs. 1–3). Following the addition of the alarm substance, the fish moved toward the bottom (Fig. 1) (vertical index decreased;  $r = -0.72$ ,  $t = 5.03$ ,  $p < 0.001$ ) and into the right third (Fig. 1) (horizontal index decreased;  $r = -0.68$ ,  $t = 4.47$ ,  $p < 0.001$ ) of the aquarium (away from the injection site) while reducing the number of movements (Fig. 3) (number of freezing behaviours increased;  $r = 0.67$ ,  $t = 4.37$ ,  $p < 0.001$ ) and swimming more erratically (Fig. 3) (number of dashing behaviours increased;  $r = 0.78$ ,  $t = 5.02$ ,  $p < 0.001$ ) compared with their behaviour prior to the injection of the alarm substance. Dashing and freezing behaviours did not occur randomly after the introduction of alarm substance; the fish responded by dashing during the first

**Fig. 2.** Variations of cohesion and polarization indices for *P. eos* before (solid line) and after (broken line) the introduction of alarm substance at five different concentrations (control (distilled water), 0.01%, 0.1%, 1.0%, and 10.0%). Each data point is the mean  $\pm$  SD of five replicates;  $r$  is the Pearson correlation coefficient with  $p$  value. An asterisk indicates a significant difference between pre- and poststimulus observations as determined by a Wilcoxon paired-sample test;  $z = -2.02$ ,  $p < 0.05$ .



minutes and then freezing. Furthermore, fish swam in a more cohesive (Fig. 2) (index of cohesion decreased;  $r = -0.76$ ;  $t = 4.25$ ;  $p < 0.001$ ) and polarized (Fig. 2) (index of polarization decreased;  $r = -0.53$ ,  $t = 2.99$ ,  $p = 0.006$ ) school following addition of the alarm substance.

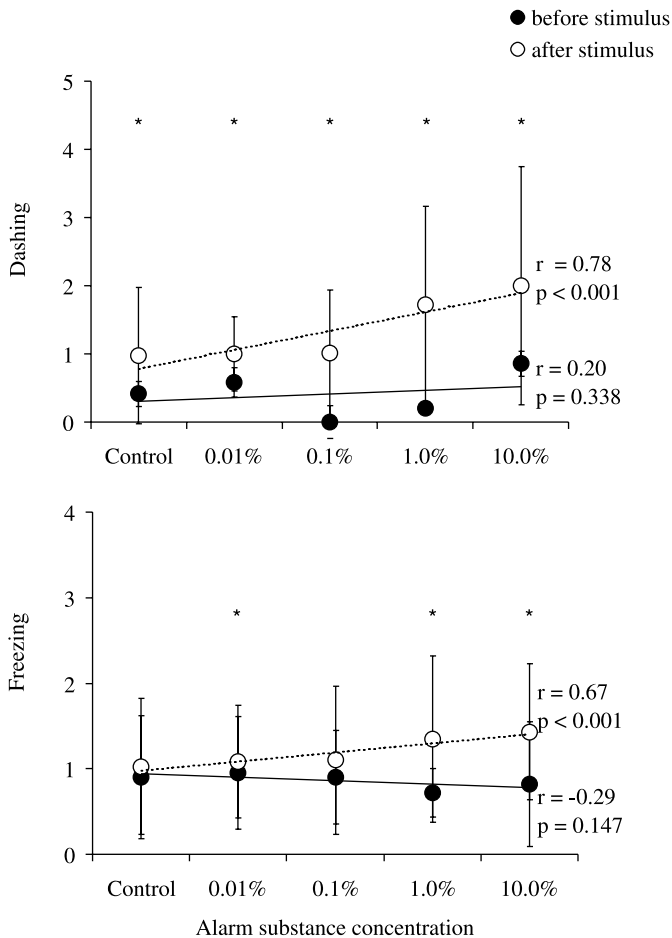
#### Temporal evolution of fish behaviour during experiments

For the two most sensitive indices, the intensity (as shown by mean values) and the consistency (as shown by standard deviations) of fish responses, as well as the time needed to return to the prestimulus state, were directly related to the concentration of the alarm substance (Figs. 4 and 5). The higher the concentration of the skin extract, the stronger, faster, and more consistent the response among individuals and the longer it took for them to return to the prestimulus index level.

#### Active space of the alarm substance

The skin extract diluted 1 : 10 000 still produced a reaction in northern redbelly dace for the most sensitive response

**Fig. 3.** Variations of numbers of dashing and freezing behaviors for *P. eos* before (solid line) and after (broken line) the introduction of alarm substance at five different concentrations (control (distilled water), 0.01%, 0.1%, 1.0%, and 10.0%). Values are  $\log(X + 1)$  transformed. Each data point is the mean  $\pm$  SD of five replicates;  $r$  is the Pearson correlation coefficient with  $p$  value. An asterisk indicates a significant difference between pre- and poststimulus observations as determined by a Wilcoxon paired-sample test;  $z = 2.02$ ,  $p < 0.05$ .

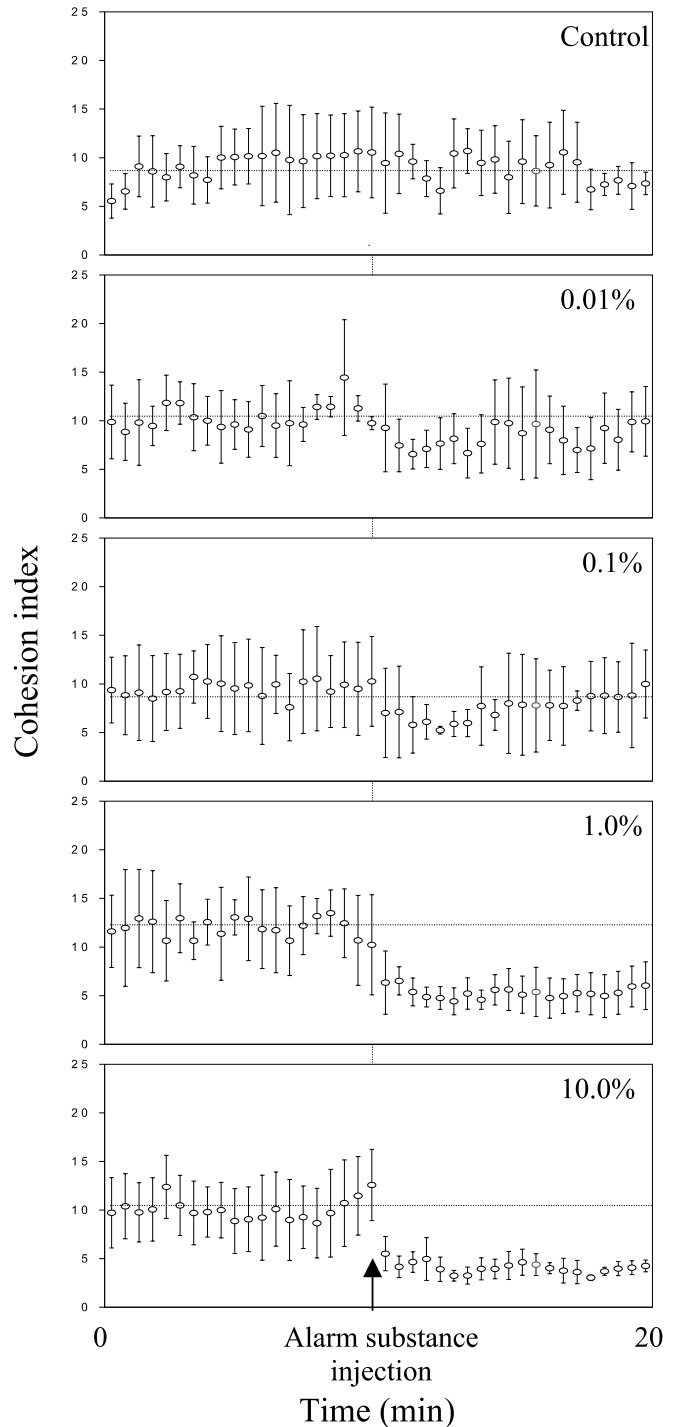


variables (cohesion and vertical indices and numbers of dashing and freezing behaviours). We thus considered this dilution to be the threshold for generating a significant response in our experimental setup. Since the original stock solution contained  $18.09 \text{ cm}^2$  of skin diluted in 100 mL of water, and the stock solution diluted to 1 : 10 000 still produced a significant reaction ( $S = 18.09 \times 10^{-6} \text{ cm}^2/\text{mL}$ ) when 15 mL was added to aquaria containing 30 L of water, we estimated that  $1 \text{ cm}^2$  of northern redbelly dace skin contains enough alarm substance to generate a response in individuals in 110 558 L of water, corresponding to a cube having sides of approximately 4.8 m.

**Discussion**

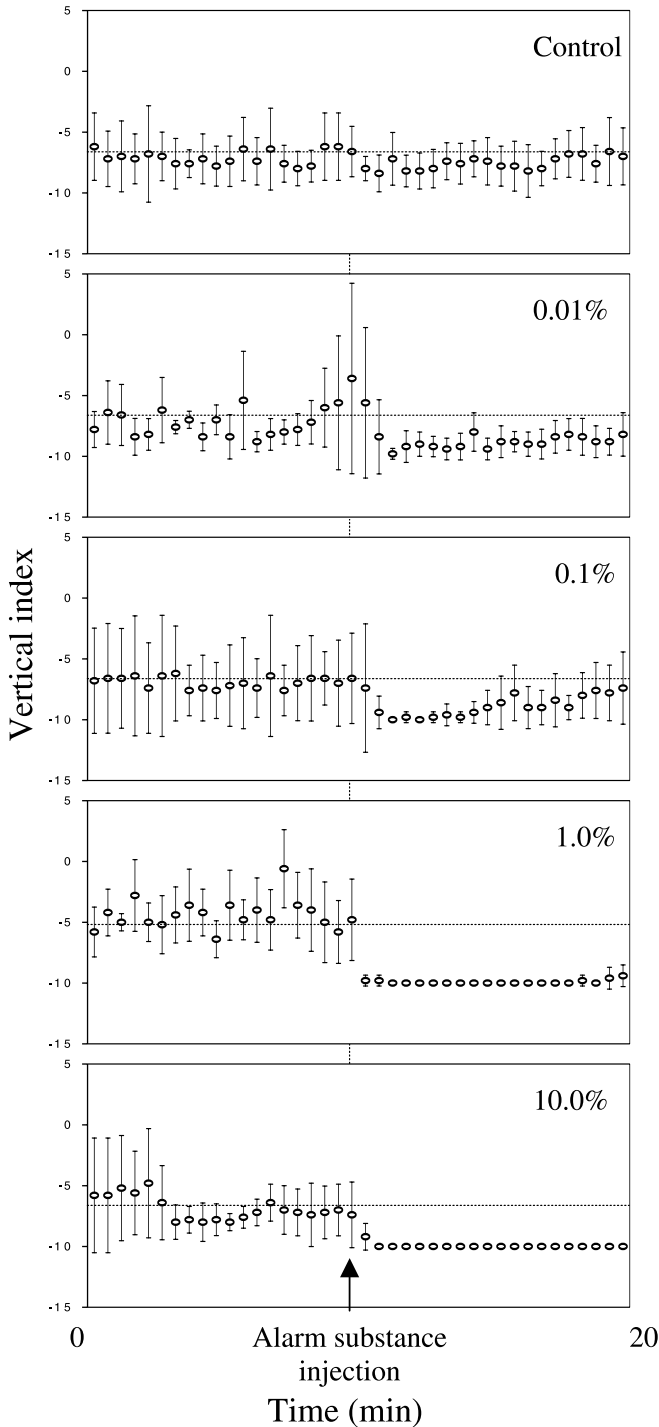
Our study showed that the northern redbelly dace responds with a fright reaction to skin extract of conspecifics. Overall, the dace moved closer to the substrate and away from the area where the alarm substance was injected, in-

**Fig. 4.** Temporal change of the cohesion index for *P. eos* during experiments at five different alarm substance concentrations (control (distilled water), 0.01%, 0.1%, 1.0%, and 10.0%). Each data point is the mean  $\pm$  SD of five replicates.



creased both the cohesion and polarization of their schools, and performed more dashing and freezing behaviours (dashing during the first minutes after the introduction of the alarm substance and then freezing). Von Frisch (1941) observed the same sequence of events when European minnows, *Phoxinus phoxinus* (Linnaeus, 1758), were exposed to water containing substances from injured minnows: the fish

**Fig. 5.** Temporal change of the vertical index for *P. eos* during experiments at five different alarm substance concentrations (control (distilled water), 0.01%, 0.1%, 1.0%, and 10.0%). Each data point is the mean  $\pm$  SD of five replicates.



first dashed toward the bottom and then aggregated in a corner of the aquarium. Thus, our results indicate that northern redbelly dace possess a chemical alarm system that could potentially be used to detect cues from injured conspecifics in nature. All of these responses are consistent with anti-predator behaviours observed in other species exposed to skin extracts of conspecifics (Chivers and Smith 1998) and

with behaviours typically seen when the risk of predation is high (Lima and Dill 1990). A schooling individual that performs dashing behaviours is likely to increase the confusion effect because the predator will have more difficulty focusing on a single individual (Pitcher and Parrish 1993). An individual performing dashing is also likely to inform other members of the school of the danger. It is known that schooling fish can gain information about the risk of predation through predator inspection behaviour and the behaviour of other fish (Pitcher 1992; Godin 1997). Northern redbelly dace are usually seen in schools of 50 to approximately 300 individuals in the littoral zone of our study lakes (A. Dupuch and P. Magnan, personal observations). It is thus possible that social facilitation might have played a role in our experiments, as in the field. However, we assumed that this factor did not confound the observed results because it was controlled (i.e., fish density fixed at 10 in all experiments). The antipredator benefits of schooling are also well known. A more cohesive school structure decreases the probability of individual fish being preyed on through a dilution effect and communication among fish (Heczko and Seghers 1981; Pitcher and Parrish 1993). The school may also react by adopting cooperative antipredator behaviour (Chivers et al. 1995). Finally, searching for shelter, as evidenced by dace moving close to the substrate and away from the alarm substance, and freezing are also clear antipredator behaviours (Brown and Godin 1999).

The dilution experiment showed that the observed responses of northern redbelly dace are closely correlated with the concentration of the skin extract solution. Among the large body of literature on chemical alarm signalling (reviewed by Chivers and Smith 1998), the studies that tested the effect of different skin extract concentrations found a positive correlation between the alarm substance dose and the intensity of the fright reaction (Gandolfi et al. 1968; Waldman 1982; Lawrence and Smith 1989; Lozada et al. 2000; Hagen et al. 2002; Jachner and Rydz 2002). Irving and Magurran (1997) studied the fright reaction of the European minnow at one skin extract concentration in an aquarium (distilled water versus 1 g/L) and two concentrations in a stream channel (distilled water versus 1 and 10 g/L). They observed that the magnitude of the response to skin extract was lower in the stream channel than in the aquarium, and even though the response persisted for slightly longer following exposure to the higher concentration, this difference was not significant. However, it is possible that dilution in the stream channel, which had about 30 times more water than the aquarium, reduced the fishes' response to skin extract and thus the difference between the two concentrations. Taken together, the results of our variable concentration experiments and those from the literature indicate that fish species with a chemical alarm system are very sensitive to different concentrations of skin extract and could potentially use this sensitivity to assess the risk of predation in nature. Some authors have suggested that the intensity of antipredator behaviours should be proportional to the perceived predation risk level if the risk varies in time and space (Dill and Fraser 1984; Gilliam and Fraser 1987; Tonn et al. 1992). In general, predation risk will be inversely related to distance from the predator (Lawrence and Smith 1989) and thus, in a chemical warning system, concentration could in-

dicating distance. We might expect that prey will respond to high concentrations of alarm substance as if predators were closer (high predation risk) and to low concentrations of alarm substance as if predators were more distant (low risk of predation). Recent studies that examined the question of graded versus nongraded responses to alarm cues found that fathead minnows and rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), show no graded response but are capable of detecting cues well below their population-specific behavioural response thresholds (Brown et al. 2001a, 2001b; Mirza and Chivers 2003). Below this threshold, individuals may exhibit a suite of threat-sensitive trade-offs and (or) "covert" (sensu Smith 1999) behavioural responses (Mirza and Chivers 2003). Prey that did not respond "overtly" (sensu Smith 1999) to lower concentrations might have used this energy for other processes such as foraging or finding mates (Brown et al. 2001a, 2001b; Mirza and Chivers 2003).

There was some variability in the responses to the alarm substance among fish groups, as indicated by the large confidence limits around our response variable means, especially at the lower concentrations (Figs. 4 and 5). The observed trend towards lower response intensities may be due to variation in individual behavioural response thresholds. Such individual variability in the intensity of responses has been reported in previous studies (Gandolfi et al. 1968; Pfeiffer 1974; Waldman 1982; Brown et al. 2001a; Mirza and Chivers 2003). For example, Gandolfi et al. (1968) found variations in individual alarm substance detection thresholds: while all fish detected the alarm substance at high concentrations, only a minority reacted at low concentrations. This variation in individual threshold may be due to a variety of factors such as past experience, sex, physiological state, age, and habitat characteristics (Kats and Dill 1998). In our study, observed variation in the responses to alarm substance at low concentrations may be due to a mixture of overt and covert behavioural responses (sensu Smith 1999), depending on the individual's detection threshold (e.g., Brown et al. 2001a; Mirza and Chivers 2003). Further experiments will be required to test this hypothesis.

The dilution experiment also allowed us to determine that 1 cm<sup>2</sup> of northern redbelly dace skin contains enough alarm substance to generate a response in 110 558 L of water. Gandolfi et al. (1968) found an active space of approximately 10 000 L/cm<sup>2</sup> skin in zebra danio, *Brachydanio rerio* (Hamilton, 1822), Lawrence and Smith (1989) found a value of 58 823 L/cm<sup>2</sup> skin in fathead minnow, and Mirza and Chivers (2003) found values between 135 246 and 270 491 L/cm<sup>2</sup> skin in rainbow trout. These estimates are certainly useful to compare the sensitivities of different species detecting alarm substances in the laboratory. However, they give only a crude estimation of the potential of alarm substances in the field because many other factors may come into play, such as water movements, physicochemical characteristics, and the severity of skin damage.

This raises the question of the real use of chemical alarm systems by fish in nature. Surprisingly, the first observation of chemical alarm cues in fish, by Von Frisch (1938), was made in the field. Some studies have provided evidence that fish avoid minnow traps or areas labelled with skin extracts (Mathis and Smith 1992, 1993; Wisenden et al. 1994, 1995; Chivers et al. 1995; Brown and Godin 1999; Gliwicz and

Dawidowicz 2001; Mirza and Chivers 2001; Pollock et al. 2003). Recent studies have shown that predator-naive trout were better able to evade predators if they were previously exposed to alarm signal, thus suggesting a survival benefit to receivers (Mirza and Chivers 2001, 2003). For example, brook trout can learn to recognize predators through releaser-induced recognition learning and this learning enhances survival of individuals during encounters with a predator (Mirza and Chivers 2000). By recognizing predators and being able to assess predation risk (using visual and chemical cues), prey fish can exhibit antipredator responses, which may increase survival. Wisenden and Thiel (2002) also showed that minnow alarm substance attracted predators. In this context, the use of alarm substance could be a benefit because predator attraction and interference (competition, aggression, and others) may increase survival chances of prey. Yet, these results remain inconclusive because the skin extract concentration used in these experiments might not correspond to the effective alarm substance concentration released when a prey is injured by a predator. There is some indirect evidence that the northern redbelly dace would find its ability to detect skin extracts of injured conspecifics, as shown in the present study, useful to counter predation by brook trout in the field. Studies done on 69 lakes of the Canadian Shield have revealed that the mean percent mass of prey fish (mostly northern redbelly dace) in the stomach contents of brook trout was higher in lakes containing creek chub, *Semotilus atromaculatus* (Mitchill, 1818), or white sucker than in lakes containing only brook trout (Magnan 1988; Lachance and Magnan 1990; East and Magnan 1991; Lacasse and Magnan 1992). Although stomach content data are not a measure of predation risk per se, these results suggest that the risk of trout predation upon dace is mediated by a third species (chub or sucker) in these lakes. Proulx (1991) provided some evidence that the extent of dace feeding migrations to the pelagic zone was lower in five lakes containing brook trout and white sucker than in five others containing only brook trout, suggesting that northern redbelly dace can perceive different risks of predation in these lakes. As these migrations occur between sunset and sunrise, when visual cues are limited, chemical alarm signalling could thus be particularly important for dace. Brown and Godin (1999) suggested that prey assessing both the visual and chemical cues associated with a predator increase the quality of information obtained and consequently reduce their risk of mortality. So, it could be profitable for northern redbelly dace to use a chemical alarm system even during the day when fish have visual cues but swim in habitats more complex than the pelagic zone (i.e., plant cover in the littoral zone; Smith 1992).

In conclusion, our study has shown that northern redbelly dace respond to the alarm substance of conspecifics with a set of antipredator behaviours. The higher the alarm substance concentration, the stronger and longer the fright reaction, suggesting that dace can perceive different degrees of predation risk and adjust their behavioural responses accordingly. Future research will be required to more directly quantify the response of prey to conspecifics injured by predators in the field. For example, one could quantify the behaviour of prey in field enclosures having different volumes, with and without the chemical stimulus of a hidden

predator preying upon a conspecific (i.e., no visual cue), using an underwater camera system like the one used by Marchand et al. (2002).

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