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Fish odour triggers conspecific attraction behaviour in an aquatic invertebrate

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Group living has evolved as an adaptation to predation in many animal species. In a multitude of vertebrates, the tendency to aggregate varies with the risk of predation, but experimental evidence for this is less well known in invertebrates. Here, we examine the tendency to aggregate in the freshwater amphipod *Gammarus pulex* in the absence and presence of predator fish odour. Without fish odour, the gammarids showed no significant tendency to aggregate. In contrast to this, in fish-conditioned water, they significantly preferred to stay close to conspecifics. Predation risk can, thus, influence gammarids social behaviour.

Keywords: *Gammarus pulex*; Crustacea; Amphipoda; shoaling; group; olfactory cues

1. INTRODUCTION

Aggregation of conspecific individuals is a widespread phenomenon in animals. On one hand, ecological factors, such as clumped food sources, habitat structure or microclimatic conditions can result in a patchy distribution of conspecifics. On the other hand, aggregation itself can be advantageous for the aggregated individuals. The best studied and probably most frequent benefit of grouping is the avoidance of predation (reviewed by Krause & Ruxton 2002). This might be because the probability of getting caught by a predator is decreased in a group (e.g. Codella & Raffa 1995) or because predators might be confused by a large number of prey items (e.g. Krakauer 1995). Besides the benefits of aggregations, there are also potential costs, like an increased competition for food or mating partners or an enhanced risk of being parasitized (Krause & Ruxton 2002). Thus, the tendency to aggregate should be strongly dependent on the ecological context. For example, many group living vertebrates, particularly fishes, form larger groups in the presence of predators (Pitcher & Parrish 1993). However, less is known about the tendency to aggregate associated with predation risk in invertebrates.

Here, the individual aggregation behaviour of the freshwater amphipod *Gammarus pulex* is examined under different levels of predation risk. Gammarids are opportunistic feeders preferring decomposed leaves (e.g. Graça *et al.* 1993), though they also

exhibit predation and cannibalism (MacNeil *et al.* 1997). Because gammarids are an important prey for several fishes (MacNeil *et al.* 1999; Mazzi & Bakker 2003; Perrot-Minnot *et al.* 2007), they have developed pronounced anti-predator behaviours. Their higher activity rates during the night (Williams & Moore 1985; Allan & Malmqvist 1989) are interpreted as an adaptation to avoid visual predators. Furthermore, gammarids actively avoid fish predators, which they recognize by their olfactory cues (Williams & Moore 1986; Dezfuli *et al.* 2003; Baldauf *et al.* 2007; Perrot-Minnot *et al.* 2007). While gammarids are often found in high densities in nature (e.g. Williams & Moore 1986), it is to our knowledge not known whether these aggregations are the result of an active preference for conspecifics. It is also unknown whether the aggregation behaviour of *G. pulex* is influenced by the risk of predation. To answer these questions, this study examined the tendency of individual *G. pulex* to aggregate with conspecifics both in the absence and presence of fish predator odours.

2. MATERIAL AND METHODS

(a) Experimental subjects

Several thousands of *G. pulex* were caught on 7 February 2008 from the brook Katzenlochbach near Bonn, Germany and transferred to the laboratory. Here, they were kept in two tanks (1 × w × h: 70 × 40 × 35 cm) filled with aerated tap water under a temperature and light regime that resembled February conditions (10 L: 14 D, temperature 12 ± 1°C). The tanks were equipped with dead leaves taken from the natural habitat to provide nutrition and shelter.

As predator stimulus, the odour of three-spined sticklebacks (*Gasterosteus aculeatus*), a fish present in the Katzenlochbach drainage system and known to prey upon *Gammarus* (Mazzi & Bakker 2003), was used. Twenty sticklebacks were caught from the institutional pond using minnow traps 7 days prior to the start of the experiments and transferred to a tank (80 × 40 × 35 cm) filled with aerated tap water under a standardized winter light regime (8 L: 16 D). Each evening after the last experiment, fish were fed with frozen *Chironomus* larvae, which were consumed within 30 min. Immediately before the start of each predator trial, stickleback-conditioned water was taken from the holding tank and added to the test tank (see below).

(b) Experimental design

Experiments took place from 8 to 11 February 2008 between 10.00 and 18.00 under daylight conditions. In four identical test aquaria (30 × 20 × 20 cm), a tea ball (3.5 cm in diameter) consisting of a fine metal grid was hung in the middle of the left- and right-side walls 1 cm above the bottom. Two lines drawn on the bottom divided the tank into three equal-sized compartments (10 × 20 cm). The outer compartments containing the tea balls served as choice zones, the middle compartment served as neutral zone. Tanks were filled with 1-day-old tap water to a height of 8 cm (called 'no predator treatment' from now on). After each trial, test tanks were rinsed using tap water. In half of the experiments ($n=58$), 200 ml stickleback-conditioned water was added to the tap water (called 'predator treatment' from now on). Ten haphazardly chosen gammarids, which were not in precopulatory pairs, were placed into one tea ball and the other tea ball remained empty. The metal grid allowed olfactory exchange with the surrounding tank water, while visual and vibrational contacts were limited. The side on which the gammarids were presented was alternated between trials to avoid side effects. Individuals infected with acanthocephalan parasites were discarded. All gammarids were only used once.

Ten minutes after the tea balls were introduced into the tank, a haphazardly chosen, unpaired test gammarid was put into a clear plastic cylinder (3.5 cm in diameter) placed in the middle of the tank. As soon as the test individual started to move, the cylinder was lifted. Thirty seconds after lifting the cylinder, it was scored whether the test individual was located in the choice zone with or without gammarids or in the neutral zone by observations from above the tank. This was repeated at 30 s intervals for a period of 5 min. Thus, we recorded 10 positions for each individual. The observer was unaware of the predator regime of the trials. After the experiments, the size of the test gammarids was estimated using a digital calliper. Sizes ranged between 3.43 and 7.93 mm and

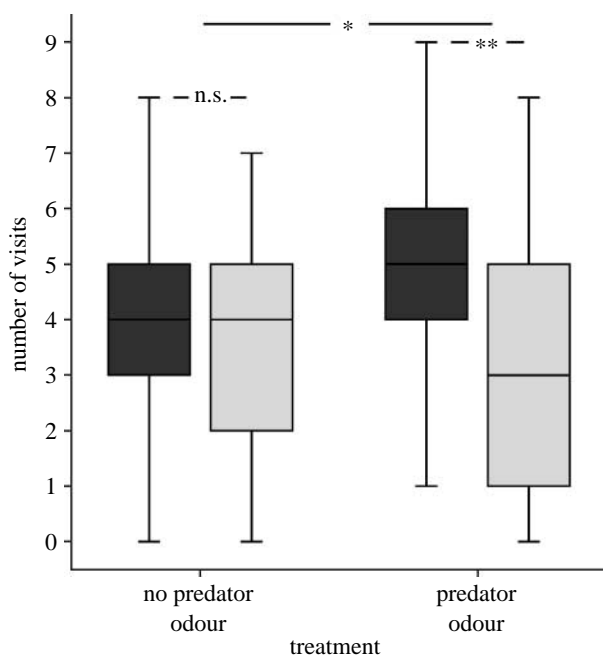


Figure 1. Choice of the gammarids in the two treatments (median \pm quartiles, percentiles). When no predator odours were present ('no predator treatment') gammarids showed no preference for conspecifics (black bar) or the empty tea ball (grey bar). In contrast to this, gammarids in the 'predator treatment' significantly preferred the choice zone in which conspecifics were presented (black bar) over the one without gammarids (grey bar). * $p < 0.05$; ** $p < 0.01$; n.s. $p > 0.1$. The number of visits is equal to occurrences in the respective choice zones scored at 30 s intervals during the 5 min trials.

did not differ significantly (Student's t -test: $n_1 = n_2 = 58$, $t = -0.056$, $p = 0.956$) between the no predator treatment (mean \pm s.d.: 5.3 ± 1.02 mm) and the predator treatment (5.31 ± 0.94 mm).

(c) Statistical analysis

To test whether the preferences of the gammarids differed between the two treatments, a preference index was calculated for each treatment by subtracting the number of observations in which the test individual was located in the choice zone without conspecifics from the number of observations in which it was located in the choice zone in which conspecifics were present. Analyses were performed using the SPSS v. 14.0 statistical package. Given test probabilities are two-tailed throughout.

3. RESULTS

In the no predator treatment, test individuals did not show a significant preference for the choice zones with or without conspecifics (Wilcoxon matched-pairs signed-ranks test: $n = 58$, $z = 0.946$, $p = 0.344$; figure 1). In contrast to this, in stickleback-conditioned water, gammarids significantly preferred the choice zone in which their conspecifics were presented (Wilcoxon matched-pairs signed-ranks test: $n = 58$, $z = 3.161$, $p = 0.002$; figure 1). The preference indices in the two treatments differed significantly from each other (Mann-Whitney U -test: $n_1 = n_2 = 58$, $z = -2.078$, $p = 0.038$; figure 1). The preference indices were not significantly correlated with the test gammarids body size (Spearman's correlation, both $n = 58$, $r = 0.103$ and 0.223 , respectively, both $p > 0.05$).

4. DISCUSSION

This study reveals changes in the individual tendency of gammarids to be attracted to conspecifics depending on the presence of fish predator's olfactory cues. Gammarids showed a significant preference for staying in close proximity to conspecifics when stickleback-conditioned water was added to the tank. In neutral water without predator odours, the distribution of gammarids revealed no significant preference for one of the two sides. Our results are in concordance with previous findings, which suggested that aggregated distributions of gammarids in predator-free habitats are mainly determined by the patchy distribution of appropriate food sources (e.g. Williams & Moore 1986; Graça *et al.* 1993). In contrast to this, aggregated distributions of gammarids in habitats containing fish predators should at least partly be influenced by the gammarids' tendency to aggregate under these circumstances. The finding that changes in predation risk triggers aggregation behaviour has been shown in vertebrates (e.g. Krause & Godin 1994), but the present study is, to our knowledge, one of the few studies presenting experimental evidence of a comparable behavioural change in invertebrates. Some recent studies on aggregation preferences in crustaceans ended up with complex results (e.g. Baumgärtner *et al.* 2002; Evans *et al.* 2007; Linden 2007). The present study suggests that the presence of predator cues may influence the tendency to aggregate in invertebrates and should be considered in examinations referring to this problem.

Our experiments confirmed the findings of previous studies that gammarids are able to perceive the presence of fish predators by using olfactory cues (e.g. Wudkevich *et al.* 1997; Baldauf *et al.* 2007; Kaldonski *et al.* 2007; Perrot-Minnot *et al.* 2007). The predators in most of these studies were fed with gammarids. Therefore, fish odours as well as degradation products of the eaten gammarids might have played a role. In the present study, sticklebacks were caught from a *Gammarus*-free habitat. Fish were fed each evening with chironomids, which were consumed within 30 min. The first experiment the next day started approximately 16 hours later, making it improbable that chironomid odour was still present in the water. Furthermore, chironomids do not prey on gammarids, but might sometimes serve as a food source (e.g. Baumgärtner *et al.* 2002). Thus, stickleback-related olfactory cues are the most probable factor inducing anti-predator behaviour. As we could not determine the composition of the odour, we cannot distinguish whether gammarids reacted to the smell of fish-digested chironomids or to the odour of the stickleback itself. However, functionally both odours would lead to increased anti-predator behaviour. Another open question is which cues the test gammarids used to locate their conspecifics in the present experiment. Although visual or vibrational cues cannot be excluded completely due to the experimental set-up, previous studies (e.g. Krang & Baden 2004) suggest that olfactory cues play the key role in communication in amphipods.

Some studies have shown an influence of body size, age or sex on the distribution of gammarids

(e.g. Bollache *et al.* 2000). In the present study, test gammarids were haphazardly chosen from a natural population without distinguishing between age classes or sexes. The body size of the test individuals varied by 4 mm and did not differ significantly between the treatments. Furthermore, precopulatory couples were excluded from the experiments. The set-up was thus conservative with respect to the effects of sex or size of the test individuals.

Parasites are well known to influence the behaviour of gammarids. Recent studies have shown that an individual infected with the cystacanth stage of the acanthocephalan *Pomphorhynchus laevis* does not avoid the smell of a fish predator but rather prefers it (Baldauf *et al.* 2007; Perrot-Minnot *et al.* 2007). It is conceivable that parasitization might also influence the gammarids' aggregation behaviour. To avoid this effect, this study was conducted in the late winter when the number of infected individuals is low (Dezfuli *et al.* 1999; Dudinak & Spakulova 2003). Furthermore, only uninfected individuals were used, leaving the question of parasites' influence on gammarids' aggregation behaviour open for future studies.

Summarizing, this study provides experimental evidence of a predator-induced switch in conspecific attraction behaviour of individual gammarids. This suggests that aggregation may serve as an anti-predator behaviour in this species. Degradation products of eaten conspecifics were not necessary to obtain this result.

This study conforms to the Animal Behaviour Society guidelines for the use of animals in research and to the legal requirements of Germany.

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