Primary Research Paper

Attraction of the amphipod *Gammarus pulex* to water-borne cues of food

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Abstract

We examined the ability of the amphipod *Gammarus pulex* to detect chemical cues released from potential food sources. Therefore, response of *G. pulex* to chemical cues from food was tested in paired-choice laboratory experiments. Comparisons were made between artificial and natural leaves, with and without the importance of aufwuchs, and with different components of the aufwuchs community. Our study demonstrated that *G. pulex* actively chose its food and that *G. pulex* is most strongly attracted to the aufwuchs on discs rather than to the leaf itself. Fungi and bacteria are more important in the food selection process than algae probably because fungal and bacterial cues are more specific cues for decaying leaves than algal cues, since algae also grow on mineral substrates and then do not contribute to leaf decomposition.

Introduction

Successful food gathering is critical to every organism to provide the essential energy for maintenance, growth and reproduction. Many organisms must balance the benefits of feeding with associated risks from predation. Predation imposes a selective pressure on prey to evolve mechanisms that reduce the risk of being eaten. *Gammarus* species (Crustacea: Amphipoda: Gammaridae), a dominant invertebrate in Northern European streams, ponds, and shallow lakes, are important benthic macroinvertebrate prey of many fish and other predators (MacNeil et al., 1999). Different *Gammarus* species can detect predators from chemical cues released from fish and injured conspecifics. Responses by *Gammarus* species include decreased activity and other changes in behavior (e.g. Williams & Moore, 1985; Wudkevich et al., 1997; Wisenden et al., 1999; Åbjörnsson et al., 2000; Baumgärtner et al., 2002), changes in precopulary mate guarding (e.g.

Mathis & Hoback, 1997), and reduced growth and survival (e.g. Lürling et al., subm). Gammarid activity is influenced by light, with organisms more active at night (Williams & Moore, 1985; Allen & Malmqvist, 1989; Holomuzki & Hoyle, 1990). This behavior may have evolved as an anti-predator response since under low light conditions prey are less vulnerable to visual predators (Lürling et al., subm). However, activity and swimming duration under low light but in the presence of predators is lower than in the absence of predators (Andersson et al., 1986), which may mean a cost by reducing the time spent foraging for food.

Gammarus sp. are considered opportunistic feeders, but have been demonstrated to be selective when given a choice (Friberg & Jacobsen, 1994; Graça et al., 2001). *Gammarus pulex* is highly mobile and uses a random food search (Graça et al., 2001) and is able to locate patches of preferred food in a heterogeneous environment (Graça, 1992). However, gammarids are prey for many invertebrate and fish species and, therefore,

have to survive and locate food in variable environments, often in situations with low light or low visibility. These conditions may strongly limit the use of visual cues for locating high quality food patches. Because prey organisms face a fundamental trade-off between food acquisition and risk of predation (e.g. Anholt & Werner, 1999), there may be a strong selective pressure on gammarids to develop chemosensory food detection capabilities that allow for both restricted movement and location of high quality food patches.

Most crustaceans are equipped with chemosensory sensilla (Derby, 1989; Zimmer-Faust, 1989) to gather chemical information about their habitat (e.g. Mead et al., 2003). Chemosensory reception may be an important mechanism for gammarids to detect water-borne chemical cues about food location. Chemically mediated food location has been documented extensively in crustaceans, but most studies have focused on marine species (Carr, 1988; Zimmer-Faust, 1989); information on attractant-mediated behavior in freshwater crustaceans is limited (e.g. Van Gool & Ringelberg, 1996). Studies of freshwater gammarid food preference are limited to effects of food choice and growth (Sutcliffe et al., 1981; Graça et al., 1993a, 1993b, 2001).

In general, gammarids are detritus-feeders that prefer to eat partially decomposed ('conditioned') leaves rather than freshly fallen leaves, most likely due to the presence of aufwuchs present on conditioned leaves (Bärlocher & Kendrik, 1975; Graça et al., 1993a, 1993b). We conducted two paired-choice experiments, to test the hypotheses that G. pulex (1) are capable of detecting food items by using chemical cues, and (2) prefer conditioned food items that include a microbial aufwuchs community.

Materials and methods

Test organism

Gammarus pulex were collected in the spring (2002) from Heelsumse Beek (5°45′ E, 51°59′ N, altitude 10 m asl), a non-polluted and natural stream in the vicinity of Wageningen, the Netherlands, using dipnets. They were transferred to the laboratory and kept in 30 l aquaria in a climate controlled room at 18 °C and a 16:8 light:dark cycle at a light level of 80 μmol quanta m^{-2} s^{-1}. Individuals of 4–7 mm in length, measured using a binocular, were selected for the study and were acclimatized to artificial pond water (Dutch Standard Water, DSW: Netherlands Normalisation Institute, 1980) for 1 week in a sequential diluting process. Each aquarium contained approximately 100 individuals that were fed surplus leaves from the sampled stream.

Food preparation

Fallen poplar leaves (Populus canadensis) were collected in the fall, leached for 7 days in tap water to remove the most active leachable substances, and air dried. Small discs (∅ 2.5 cm) were punched out of the leaves, taking care to avoid large veins. Leaf discs were sterilized by placing them for 24 h under ultraviolet radiation and were then conditioned as appropriate for the experiments.

In experiment I, we tested for G. pulex response to leaf versus glass fiber (GF/C Schleicher and Schuell, ∅ 2.5 cm) discs and to conditioned versus unconditioned leaves. We conditioned leaf and glass fiber discs by incubating them for 7 days at 20 °C under dark conditions in aquaria containing Dutch Standard Water (DSW) and an inoculum obtained from leaves from Dreijen Pond (Wageningen, the Netherlands), to allow for bacteria and fungi colonization. Discs were then transferred to aquaria containing DSW and an inoculum of the green algae Scenedesmus obliquus and conditioned for 14 days at 20 °C and a 16:8 light:dark cycle of 85 μmol quanta m^{-2} s^{-1}. The inoculum was derived from a chemostat culture held at 20 °C, in continuous light of 85 μmol quanta m^{-2} s^{-1}, and at a dilution rate of 1.0 day^{-1}.

The laboratory conditioned discs were tested against each other, and against naturally conditioned leaves. This natural food was obtained by collecting poplar leaves from the same stream where the gammarids were collected, from which discs (∅ 2.5 cm) were punched.

In experiment II, we tested G. pulex preference for poplar discs that had been conditioned in the laboratory with either algae or bacteria and fungi. Conditioning with bacteria and fungi was conducted using the same methods described above but under dark conditions at 20 °C. Conditioning

with algae was accomplished using similar methods as before with the exception of a 28 day conditioning period to ensure thorough conditioning of the leaves.

Experiment I

Pilot experiments were performed to design the optimal experimental procedure. Individual *G. pulex* that had been starved for 1 week prior to the experiment, were allowed to choose between two discs, using sight, touch and chemoreception. Six different choice pairs, listed in (Table 1), were tested.

For each test pair, one individual *G. pulex* was placed in the test aquarium ($20 \times 20 \times 11.5$ cm) filled with 2 l of DSW. To ensure identical starting positions, organisms were maintained for 3 min in a perspex tube ($\varnothing$ 2.5 cm) within the aquarium prior to the release. Two test discs were suspended in the water 8 cm apart, the tube was removed, and each specimen was observed for 15 min (see Fig. 1 for experimental setup). A disc was considered to be preferred when a specimen moved to and stayed on it for 1 min. Our pilot experiments indicated that this method of scoring ensured the highest percentage of responding individuals. All pairs were tested in three replicate aquaria in a climate-controlled room (20 °C, light intensity of 75-μmol quanta m^{-2} s^{-1}). Ten to fifteen individuals were tested per replicate for a total of 32–45 individuals per test pair.

Data were analyzed using a χ^2 test with non-responding individuals excluded from the analyses (Van den Boom et al., 2002). We tested the null hypothesis that *G. pulex* had no preference for either disc ($P(disc\ 1) = P(disc\ 2) = 0.5$) using a χ^2 test. We also tested for differences in percent of animals responding per treatment using a one-way analysis of variance (ANOVA), followed by a Tukey *post-hoc* comparison test ($p < 0.05$). All statistical analyses were conducted using SPSS.

Table 1. Combinations of different discs and conditioning from which *Gammarus pulex* could choose in experiment I

| Test	Choice 1	Choice 2	Test goal
1	conditioned leaf disc	conditioned leaf disc	control
2	conditioned leaf disc	unconditioned leaf disc	conditioning leaf
3	conditioned filter	unconditioned filter	conditioning filter
4	conditioned leaf disc	conditioned filter	leaf versus filter
5	unconditioned leaf disc	unconditioned filter	leaf versus filter
6	conditioned leaf disc	natural leaf disc	control
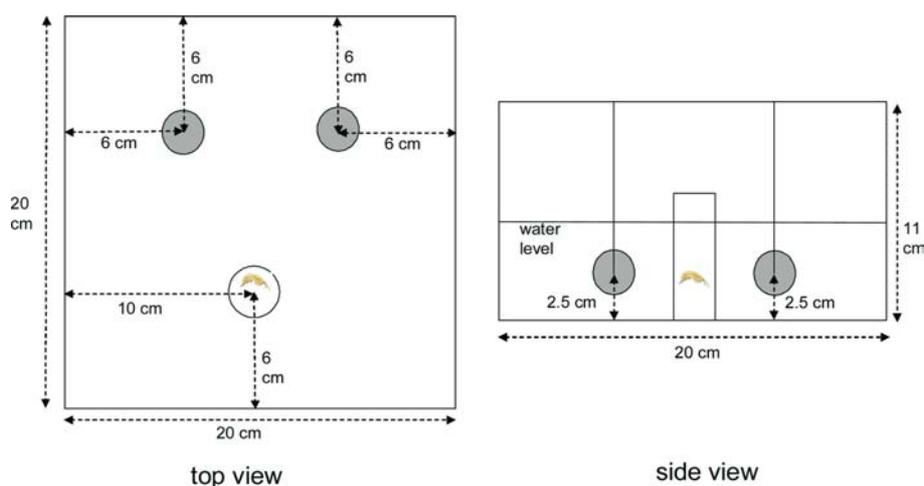
Figure 1. Experimental set-up for both experiments (a) shows top view and (b) shows side view. Test aquaria measured $20 \times 20 \times 11$ cm and were filled with 2 l of Dutch Standard Water. Open circle in (a) and bar in (b) represent the perspex tube in which *Gammarus pulex* were placed prior to release. Grey circles in both figures represent substrate discs (experiment I) or tea-eggs containing substrate discs (experiment II). Discs and tea-eggs were suspended from wires placed over the aquaria.

Table 2. Combinations of different conditioning from which *Gammarus pulex* could choose in experiment II

Test	Choice 1	Choice 2
1	bacteria/fungi	algae
2	bacteria/fungi	unconditioned
3	algae	unconditioned

Experiment II

We tested *G. pulex* preference for poplar leaves conditioned with either bacteria/fungi or algae versus unconditioned leaves using only chemoreception to make their selection (Table 2). In general, the same method as in experiment I was used but with some changes. We used commercially available steel tea-eggs (mesh size 500 μm) to prevent *G. pulex* from seeing leaf discs. Leaf discs were placed in separate tea-eggs and were suspended in the test aquarium. Individuals that had been starved for 1 week prior to the experiment were observed for 20 min. When a specimen stayed for longer than 1 min within 1 cm of a tea-egg, this tea-egg was considered to be preferred. For each trial, between 33 and 45 individuals were tested. Same statistical tests as in experiment I were applied.

Results

Gammarus individuals usually explored the aquarium after release from the perspex tube with occasional rest along the sides of the aquarium before continuing swimming. They usually found food discs within 3 min and stayed on the chosen disc for the remainder of the observation period.

Results from experiment I show that *G. pulex* does not make a choice between similar conditioned leaf discs (control pairs), or between conditioned leaf and filter disc (Fig. 2). When offered choices between conditioned and unconditioned substrates *G. pulex* significantly preferred the conditioned discs (χ^2 test; $p < 0.01$). When offered the choice between unconditioned leaf and filter discs *G. pulex* chose the leaf disc (Fig. 2; χ^2 test; $p < 0.05$). *G. pulex* did not display a preference between laboratory and naturally conditioned leaf discs.

Percent of individuals responding varied significantly with treatment with *G. pulex* less responsive to unconditioned than to conditioned discs (ANOVA $F_{5,12} = 5.59$; $p = 0.007$). Most non-responding individuals moved little remaining in the vicinity of the release location, with a few individuals displaying erratic swimming behavior.
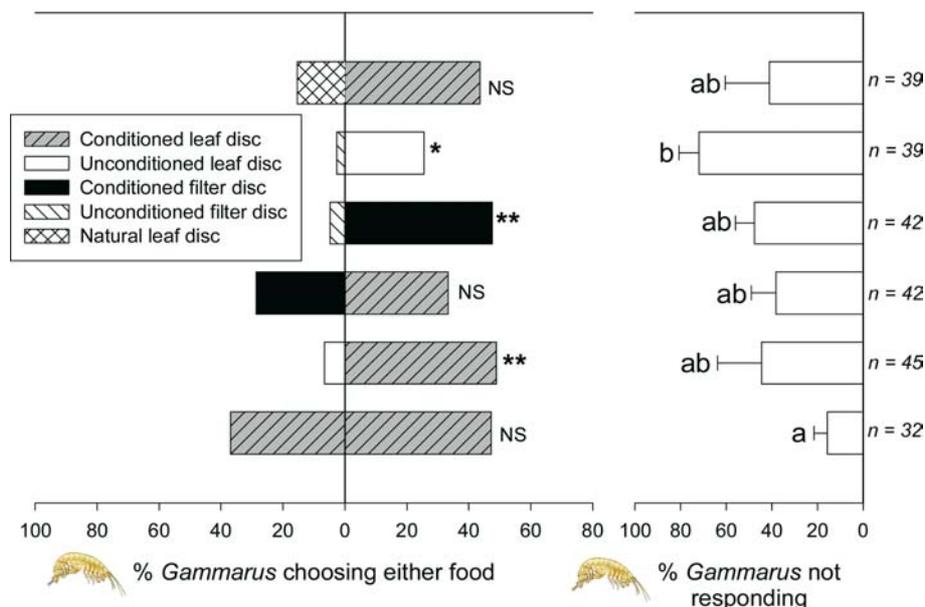

Figure 2. Gammarus pulex substrate and food conditioning choice (left panel) and percent response (right panel) in Experiment I (*$p < 0.05$, ** $p < 0.01$; tested with a χ^2 test). Error bars represent standard deviations; lower case letters indicate homogeneous groups (Tukey *post-hoc* comparison test, $p < 0.05$).

Most responding individuals remained on the preferred disc once they had made a choice.

In experiment II, *G. pulex* preferred leaves conditioned with fungi and bacteria over leaves conditioned by algae (Fig. 3; χ^2 test; $p < 0.10$). More animals responded when both tea-eggs contained conditioned leaves than when only 1 was conditioned (Fig. 3; one-way ANOVA $F_{2,6} = 6.60$; $p = 0.030$).

Discussion

Within a period of 15 min, *G. pulex* appear to respond more to chemical signals from aufwuchs than from leaves. Although, *G. pulex* chose unconditioned leaves over an artificial unconditioned substrate, conditioning produced a stronger response, even on an artificial substrate (glass fiber). Use of chemoreception has been documented in marine crustaceans and our study supports the hypothesis that freshwater *G. pulex* can detect food items through chemical cues alone.

Our results show that *G. pulex* is attracted to chemical cues released from microbial aufwuchs on conditioned substrates. Since they also respond to conditioned glass discs, the attraction is not only due to the leaves. More specifically, fungal/ bacteria aufwuchs is more attractive to *Gammarus* than coverage with algae. Fungal and bacterial cues, therefore, may be more specific cues for decaying leaves than algal cues, since algae also grow on mineral substrates and then do not contribute to leaf decomposition. Our findings are consistent with other observations that all showed a preference of *Gammarus* to fungi-conditioned leaves (Bärlocher & Kendrick, 1975; Sutcliffe et al., 1981; Graça et al., 1993a). This preference is explained from the presence of aquatic hyphomycetes that degrade the pectic polymers in leaf cell walls (Chamier & Dixon, 1982). These hyphomycetes breakdown leaf tissue, making it easier for *Gammarus* to consume and allowing for a faster overall consumption rate (Sutcliffe et al., 1981). *G. pulex* generally consumes softer leaf material while avoiding harder veins (Peeters pers. obs.). Colonizing fungi act merely as modifiers of leaf substrates rather than as food material (Kostalos & Seymour, 1976; Sutcliffe et al., 1981; Graça et al., 1993b).

Small molecules such as amino acids, nucleotides, and low molecular weight organic compounds from wounded or dead prey have been shown to attract marine crustaceans (e.g. Carr, 1988; Zimmer-Faust, 1989). Marine herbivores may also use chemosensation to locate food. For
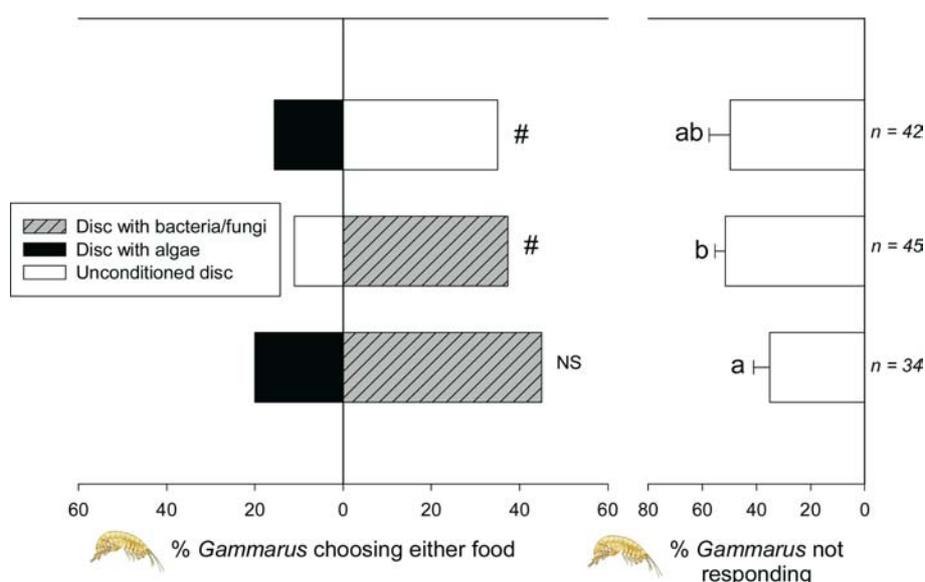

Figure 3. Gammarus pulex food conditioning choice (left panel) and percent response (right panel) in Experiment II (# $p < 0.10$). Error bars represent standard deviations; lower case letters indicate homogeneous groups (Tukey *post-hoc* comparison test, $p < 0.05$).

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example, sea urchins use water-borne cues to move towards palatable seaweed (Mann et al., 1984), and marine herbivorous copepods may discriminate between algal and non-algal food based on their chemical scent (Poulet & Marsot, 1978). In freshwater systems, the crayfish *Cambarus* sp. used chemosensory perception to locate its food (Sherba et al., 2000), and *Daphnia* perceived algae-associated chemicals to locate edible food (Van Gool & Ringelberg, 1996). Chemoreceptive food search behavior may be linked to anti-predator behavior such that locomotion and search time are reduced while lowering the risk of encounter with predators (Zimmer-Faust, 1989; Palmer, 1990). Moreover, it has been suggested that the ability to detect predators may coincide with the ability to detect food (Kats & Dill, 1998). Gammarids are sensitive to kairomones released from predators and to alarm pheromones from injured conspecifics (Williams & Moore, 1985; Andersson et al., 1986; Mathis & Hoback, 1997; Wudkevich et al., 1997; Wisenden et al., 1999, 2001; Åbjörnsson et al., 2000; Baumgärtner et al., 2002, 2003). Our study demonstrates that *G. pulex* can also detect chemical cues from food supporting the hypothesis that chemosensory abilities are correlated.

In conclusion, our study demonstrated that *G. pulex* actively chose its food. *G. pulex* is most strongly attracted to the aufwuchs on the discs, and to a lesser extent by the leaf itself. Fungi and bacteria are more important in the process than algae.

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