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Daphnids respond to algae-associated odours

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Abstract. Using a Y-tube olfactometer, it was found that Daphnia galeata × hyalina moves to the arm with odour from either of two edible algal species (Scenedesmus acuminatus and Oscillatoria limnetica) rather than to the alternative arm with clean water. However, no differential response was observed when odours of the toxic cyanobacterium (Oscillatoria agardhii) were tested. We suggest that odours associated with edible algae attract Daphnia, whereas non-edible algae do not elicit attraction of Daphnia.

Introduction

While terrestrial ecologists recognized the importance of non-random search guided by info-chemicals, such as pheromones and kairomones (for terminology, see Dicke and Sabelis, 1988), aquatic ecologists were under the impression that zooplankton float passively, largely at the mercy of water currents. At the scale of the action radius of an individual plankton animal, the pelagic zone of lakes and oceans seems rather homogeneous. Hence, the need for oriented horizontal movements in response to chemical cues was considered to be low.

This has all changed during the past two decades. Pioneer cinematography studies by Strickler (1975) of copepods, catching algae or prey animals, have revealed various behavioural patterns that were influenced by chemical cues. Prey can distinguish vertebrate and invertebrate predators by smell and change behaviour accordingly. A good example is the initiation of diel vertical migration. Extensive migrations of copepods and cladocerans occur when fish predators are abundant (Bollens and Frost, 1989; Ringelberg et al., 1991), and the change in behaviour is elicited by predator kairomones (Ringelberg, 1991; Forward and Rittschof, 1993; De Meester, 1993; Loose, 1993). Other kinds of behaviour, such as swarming and somersaulting in Daphnia, are similarly influenced by kairomones (Pyanowska, 1994).

It is often suggested that chemical cues from potential food algae could be of importance because some are difficult to ingest or are nutritionally poor (e.g. Larson and Dodson, 1993). Cyanobacteria are well-known examples of unprofitable algae (Lampert, 1987). The products released by these prokaryotes decrease the feeding rate of Daphnia (Ostrofsky et al., 1983; Forsyth et al., 1992; Haney et al., 1995). Particle selectivity by contact chemoreception is present in copepods (Friedman and Strickler, 1975; Poulet and Marsot, 1978). However, the hypothesis of remote detection of algae by kairomones was not supported by experiments of DeMott and Watson (1991). Selectivity of particles in copepods is well established, but cladocerans are considered to passively select food particle size.

In this paper, we present the results of experiments to test whether Daphnia respond to water in which algae were kept for some time, and thus possibly containing algal-associated odours, when clean water was the alternative.
Method

A Y-tube olfactometer was used to test whether *Daphnia galeata x hyalina* can perceive odours of algae. This simple apparatus consists of a Y-shaped glass tube with two arms (at an angle of 75°) and one basal leg, each with a length of 13 cm and a diameter of 3.5 cm. The two arms served as inflows for water that was pumped into them at a rate of 7.8 ml min⁻¹. The water outflow was at the base of the leg. This Y-tube was placed horizontally in a water bath to reduce changes in temperature and, as far as possible, unwanted response-inducing patterns in light scatter and breaking. Experiments were carried out in a dark room and temperature was controlled at 17–19°C. Illumination was from above by fluorescent lights. The pattern of water flow in the Y-tube was studied by release of ink into one of the arms. One minute after release, this arm was completely filled and after the bifurcation point the flows of both arms did not mix, but remained separated up to the outflow.

Water used in the experiments originated from the hypolimnion of Lake Maarsseveen [for a description of this lake, see Ringelberg (1981) and Swain *et al.* (1987)]. This water was circulated over a sand filter to stimulate the breakdown of organic matter for at least 2 days before use.

Odour from three species of algae was tested: *Scenedesmus acuminatus*, the standard source of food in our *Daphnia* cultures; *Oscillatoria limnetica*, a cyanobacterium on which *Daphnia* easily grows (S.Repka, personal communication); and *Oscillatoria agardhii*, a toxic strain (M.Lürling, personal communication). *Scenedesmus acuminatus* was grown as a continuous culture, both *Oscillatoria* species were grown as batches. Algae were centrifuged and resuspended in the above-mentioned water from Lake Maarsseveen, for *S.acuminatus* at a concentration of 0.5 mg C l⁻¹, for both *Oscillatoria* at unknown (high) concentrations. After 1 day, the algae were removed by filtering over 0.45 µm and the filtrate was immediately used in the experiments against clean 0.45 µm filtered water from Lake Maarsseveen.

One day before tests, adult females of the hybrid *D.galeata x hyalina* were taken from our standard culture and put in clean Maarsseveen water with *S.acuminatus* at a concentration of ~0.5 mg C l⁻¹.

At the start of an experiment, the Y-tube was almost completely filled with clean water. An individual daphnid was put in a small beaker with clean water and introduced with this water into the leg of the Y-tube. Next, the leg was closed with a plug provided with the outflow. Finally, the whole Y-tube was covered by black paper, except for a small window above the outflow leg near the bifurcation. In this way, the daphnid was trapped optically in this part of the leg, thereby ensuring the same starting position of successive animals. After ~5 min of detainment, the inflow with clean water and test water containing odour of algae was started. One minute later, the *Daphnia* was released by gently lifting the black covering of the inflow arms. This resulted in the animal swimming into one of the inflow arms. Only daphnids that reached a distance of 9 cm into one of the arms were scored. Daphnids were used only once. After each run, the Y-tube was cleaned thoroughly with a brush and hot water. The inflow of algal odour was shifted to the other inflow arm after 10 runs, to prevent possible position effects. Although each daphnid was able to move freely over the whole cross-section of the outflow leg, it is possible that in
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some cases algae-associated odour was never experienced, because the water currents from both arms remained largely separated. This increased the chance of a type I error (Sokal and Rohlf, 1981).

The null hypothesis is derived directly from the design of the Y-tube: \( P(\text{odour}) = P(\text{clean}) = 0.5 \) (no preference), and the binomial test (Siegel and Castellan, 1988) was used to test this hypothesis.

**Results**

Experiments with clean water entering both arms of the Y-tube showed that the null hypothesis could not be rejected. Comparable numbers of daphnids reached the distance of 9 cm in either arm (47 and 53%, respectively; \( N = 34 \)). Thus, no environmentally induced position effect was present. To be certain, any such effect in the experiments was further reduced by switching the odour side after each 10 runs.

Results of the tests were in conflict with the null hypothesis that *Daphnia* showed no preference for water which previously contained edible algae. Preference for the arm containing odour of *S.acuminatus* was highly significant \( (P < 0.0001, N = 97; \text{Figure 1}) \) with 73% of the animals ending up at the arm containing the odour. The tests with odour of the edible cyanobacterium *O.limnetica* showed the same result: 70% of the animals were found in the arm with the algal flavour \( (N = 60, P = 0.0027; \text{Figure 1}) \).

When water that had contained the toxic cyanobacterium *O.agardhii* was used as odour source, no preference could be observed: 52% of the animals (not significant; \( N = 69 \)) ended up in the arm where odour was offered (Figure 1).

**Discussion**

Our results show that *D.galeata x hyalina* discriminates between clean water and water that previously contained algae. Because water with algae was filtered, leakage of the contents of damaged cells into the water cannot be excluded. However, even if leakage products smaller than 0.45 \( \mu \text{m} \) were responsible for the observed preferences, the results may still be ascribed to odour perception. Nevertheless, one may question whether responses to algae-associated chemicals, as shown under ideal, artificial conditions, promote foraging under more realistic conditions.

The differential effect of *O.limnetica* and *O.agardhii* species or strains with and without nutritional value, or without and with toxic properties, respectively, suggests that specific information can be derived. It may be expected that water from the toxic *O.agardhii* would have a repellent effect, but it elicited no significant response. Perhaps, in the case of toxic algae, the information derived from algae-associated chemicals is both 'attractive' and 'repellent'. Experiments comparing species simultaneously in a Y-tube olfactometer will increase insight into the potential of chemoperception in daphnids.

In general, the swimming behaviour of *Daphnia* seems to be quite erratic. However, they are very sensitive to temporal and spatial differences in light intensity, and small irregularities in the angular light field might be held responsible for poorly understood swimming behaviour. In our experiments, significant swim-
Fig. 1. Fraction diagram of responses of *D. galeata × hyalina* to algae-associated odours in a Y-tube olfactometer, choice between clean water and water that previously contained the algae indicated above the diagram. *N* values represent the numbers of *Daphnia* tested. Asterisks indicate the level of significance: **P < 0.003; ***P < 0.0001; n.s. = not significant.

...ing into one of the inflow arms of the Y-tube could be easily realized by an appropriate angular light distribution. However, careful control of the light field proved that no bias to either arm was present in the control runs. It also proved important to have a similar starting position in the outflow leg for all runs and this was realized by making use of *Daphnia*'s strong photo-orientation. The subsequent release and directional swimming into either arm was likewise predominantly realized optically. The arm chosen was influenced by the presence of odour in the inflowing water.

The results do not reveal what adaptive value must be attributed to the demonstrated chemoperception of algae-associated kairomones. It is obvious to suggest that swimming toward patches of high-quality algae is adaptive. This requires
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remote chemoreception in the first place, but oriented swimming should follow in order to bring the animal successfully to the source. In our setting, swimming was predominantly directed by an experimentally induced photobehaviour. Daphnids also tend to swim upstream (rheotaxis) and weak water currents might thus direct the animal to a source of attractive kairomones. The action radius of these daphnids must be considered in relation to the distance between patches, however. On a smaller scale, chemoperception might be used for continuous redistribution within a patch when dense aggregates of animals deplete an area of algae. Swarming is common in this species and, with regard to feeding, might offer the advantage of a more easy assimilation of algae that pass the gut repeatedly (Kersting, 1991). On the other hand, competition might become predominant in an aggregate when the concentration of algae is considerably diminished. Redistribution is then profitable. Relevant experiments must be designed to deal with these aspects of optimal foraging. The results of our experiments show that Daphnia has the potential to discriminate between ecologically relevant odours. At what spatial scale such information is used is an open question for future research.

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References


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