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Volatile Foraging Kairomones in the Littoral Zone: Attraction of an Herbivorous Freshwater Gastropod to Algal Odors

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Abstract Volatile organic compounds (VOCs) produced by algae and cyanobacteria are primarily responsible for odors in fresh waters. Among other functions, VOCs may serve as important infochemicals in biofilms of benthic primary producers. VOCs liberated by benthic, mat-forming cyanobacteria can be used as habitat-finding cues by insects, nematodes, and possibly other organisms. We developed a new gastropod behavioral assay that allows detection of food preference without offering food, thus allowing the distinction between taste, which requires direct contact with the food source, and the detection of odorous infochemicals, which work over distance. We demonstrated that VOCs released from disintegrated cells of a benthic, mat-forming, green alga (*Ulothrix fimbriata*) are food-finding cues ("foraging kairomones") that attract the herbivorous freshwater snail *Radix ovata*. A mixture of three C_5 lipoxygenase compounds and 2(*E*),4(*E*)-heptadienal that mimic the major VOCs released by *U. fimbriata* attracted the snails, whereas neither the mixture of C_5 compounds nor 2(*E*),4(*E*)-heptadienal were effective when given alone. This study suggests that VOCs can play a steering role as infochemicals in freshwater benthic habitats, as has been established for many organismic interactions in terrestrial ecosystems.

Keywords Attractant · Food choice · Infochemicals · Lipoxygenase products · Nor-carotenoids · Oxylipins · *Radix ovata* · Unsaturated aldehydes · *Ulothrix fimbriata* · Volatile organic compounds (VOC) · Pulmonate · Snail

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Introduction

Volatile organic compounds (VOCs) are commonly found to function as infochemicals in terrestrial ecosystems (e.g., Metcalf, 1987; Kessler and Baldwin, 2001). However, in aquatic ecosystems, they have only received attention in the limited context of water quality issues: VOCs produced by algae and cyanobacteria frequently cause unpleasant odors in source water and are a nuisance in water treatment, especially for drinking water. Important for most consumer complaints are the earthy–musty odor compounds geosmin and 2-methylisoborneol (Watson and Ridal, 2004). Planktonic (Watson, 2003) and benthic cyanobacteria (Izaguirre and Taylor, 1995) are the major sources of both compounds. Numerous other VOCs belonging to lipoxygenase, carotene oxygenase, and fermentation products have been detected in lake and river water (Jüttner, 1995). The large number of compounds described for fresh water contrasts with the sparse studies on their ecological functions.

Some possible biological functions of VOCs in aquatic ecosystems are becoming clear (Watson, 2003). Volatile aldehydes might play a role in an activated defense mechanism of marine diatoms (Miralto, et al., 1999). Wound-activated diatom cells release α-, β-, γ-, δ-unsaturated aldehydes (Pohnert, 2000; Pohnert et al., 2002) that drastically lower the hatching rate of copepod eggs and might serve as a defense at the population level (Ianora et al., 2004). An alternative role of polyunsaturated aldehydes is serving as repellents for crustacean grazers (Jüttner, 2005).

Aquatic systems are ideally suited for communication via chemical cues because infochemicals can be easily distributed in concentrations sufficient for a response (Wisenden 2000). In highly structured benthic ecosystems, VOCs remain more localized than in planktonic ecosystems and may form robust microzones (Blackburn et al., 1998). Infochemicals related to predation (von Elert and Loose, 1996), reproduction (Müller et al., 1971), and foraging (Thomas et al., 1980) are perceived by many groups of aquatic organisms, which use this information to optimize their fitness. Indeed, VOCs function as habitat-finding cues for both aquatic insects (Evans, 1982) and nematodes (Höckelmann et al., 2004).

In littoral marine and freshwater ecosystems, herbivorous gastropods are of major importance because they structure the algal community by strong top–down predation pressure; they also form an important link to higher trophic levels because they are preyed upon by both vertebrate (fish, birds) and invertebrate predators, e.g., crayfish (Turner et al., 2000). Gastropods generally lack good vision, and cannot easily locate food patches visually (Gal et al., 2004). Their locomotion is energetically demanding, primarily owing to the production of pedal mucus (Denny, 1980). Therefore, it should be adaptive for snails to rely on chemical cues to minimize these costs by directed chemotaxis toward potential food sources.

Chemical stimuli affect the behavior of gastropods, and aquatic snails use their sensitivity to chemical cues as a principal modality to detect distant objects in the environment (Croll, 1983). The osphradium, located in the mantle cavity, is considered to be the major chemosensory organ of freshwater gastropods, although it might also be involved in other sensory processes such as the detection of ambient CO_2 pressure (Wedemeyer and Schild, 1995). However, to date, it has not been investigated whether long-range chemical cues are involved in observed food preferences, which have been quantified only either as residence time on a particular food patch or as the amount of food consumed (e.g., Madsen, 1992; Brendelberger, 1995; Wakefield and Murray, 1998). Snail chemotaxis toward dissolved sugars, amino acids, and carboxylic acids has been determined only in the context of control mechanisms for snails that are intermediate hosts

for parasites (Thomas et al., 1980; Thomas, 1986). Therefore, it remains unclear how these dissolved chemical cues are related to the process of finding food.

Volatile organic compounds released from benthic algae are suitable for dispersion over distance, and are good predictors of the presence of algal food. We hypothesize that freshwater gastropods utilize algal VOCs as foraging kairomones (sensu Ruther et al., 2002) for finding food. To investigate this hypothesis, we first identified the VOCs released upon cell disintegration from the filamentous benthic green alga *Ulothrix fimbriata* (Bold). *Ulothrix* is a cosmopolitan genus commonly occurring in periphyton communities of marine and fresh waters (John, 2002). In a newly developed standardized assay, we then investigated the behavioral response of the common pulmonate snail *Radix ovata* (Draparnaud) to these VOCs to see if they could be utilized as food-finding infochemicals by the snails. We deliberately used snails naïve to *Ulothrix* odors to distinguish between genetically fixed food-locating abilities and preferences acquired by olfactory learning.

Methods and Materials

Cultures

An axenic culture of the filamentous benthic green alga *U. fimbriata* (Bold) SAG 36.86 (Chlorophyceae) was obtained from the Göttingen Algal Culture Collection, Germany. The alga was cultivated semicontinuously as a suspension at a dilution rate of 0.25 d^{-1} in WC medium (Guillard and Lorenzen, 1972) at a constant temperature of 20°C and a light intensity of 1×10^{16} quanta s^{-1} cm^{-2}. The culture was aerated by heavy bubbling with sterile, compressed air to prevent sinking and attachment of the benthic algae to the bottom of the culture flasks. Algae were harvested, concentrated by centrifugation at 4000 × *g*, and resuspended in filtered (0.45 μm) Lake Constance water. In the resulting food suspensions, carbon concentrations were adjusted to 0.5 mg particulate organic carbon per milliliter by using photometric light extinction at 800 nm and carbon extinction regressions previously determined (P. Fink, unpublished data). Juvenile *R. ovata* (Draparnaud) snails with shell lengths of 5–10 mm were collected during the summer in the littoral zone of Lake Constance, and acclimatized in the laboratory (at 20°C under constant dim light) before use in the food choice experiments. During the acclimation period, snails were fed Tetra PlecoMin® fish food pellets (Tetra, Melle, Germany). Before the experiments, animals were moderately starved for 24 hr by placing them in filtered (0.45 μm) Lake Constance water without food to increase their food-searching activity.

VOC Analyses

To standardize VOC extraction, algal biomass equivalent to 10 mg particulate organic carbon was disintegrated by a freeze–thaw cycle in 40 ml ultrapure water. After addition of 25% NaCl, VOCs were extracted by closed-loop stripping for 45 min and sorbed onto Tenax TA (Supelco) as described by Jüttner (1988b). VOCs were then thermally desorbed from Tenax TA and directly transferred onto a capillary column (DB 1301, 30-m length, 0.32 mm i.d., J&W Scientific, Folsom, CA, USA) of a combined gas chromatograph–mass spectrometer (Thermo/Finnigan GCQ). Helium was used as the transfer and carrier gas. VOCs produced by *U. fimbriata* were separated with the temperature program 4 min at 0°C, 5°C min^{-1} to 250°C, and 10 min at 250°C, and identified by comparing the retention times and mass spectra (EI at 70 eV) with those of reference compounds (Aldrich). The

major VOCs identified were quantified as described by Jüttner (1988b) by using the peak areas of characteristic mass fragments and a calibration curve for each compound. 3-Hexanone was chosen as the internal standard because it did not occur in *U. fimbriata* and exhibited intermediate volatility with respect to the VOCs of *U. fimbriata*.

Extraction of VOCs for Food Choice Assays

Volatile organic compounds adsorbed onto Tenax TA (see previous section) were eluted with 5 ml diethyl ether. The ether was gently evaporated to dryness with nitrogen gas, and the residue was immediately dissolved in 100 µl of ethanol. Samples were stored in gas-tight vials at −20°C for <48 hr to avoid loss. Before the food choice assay, 7 ml filtered (0.45 µm) Lake Constance water was added to each sample, and this solution was added to the containers placed in the aquarium (see below).

As a control, 40 ml of ultrapure water were stripped with 25% NaCl onto Tenax TA, eluted with diethyl ether, dried, and dissolved in ethanol. In another control, WC medium aerated for several days instead of ultrapure water was analyzed to exclude possible effects of contaminants introduced during cultivation.

Synthetic VOC Mixtures

Reference compounds (Aldrich) identified as the major VOCs of *U. fimbriata* were mixed to approximately match the concentrations in the algal VOC extract (Table 1). However, the (*Z*)-isomer of 2-pentenal and the (*E,Z*)-isomer of 2,4-heptadienal were not available. Thus, only the (*E*)- and (*E,E*)-isomers were used in the complete VOC mixture (Table 1). To resolve whether the attractant activity of *U. fimbriata* VOCs was dependent on specific substances or rather on multiple compounds, 2(*E*),4(*E*)-heptadienal and a mixture of the three C_5 compounds 1-penten-3-one, 1-penten-3-ol, and 2(*E*)-pentenal (C_5 mixture; Table 1) were also tested in the assay.

Food Choice Assays

The food choice assay was specifically designed to separate chemotaxis, mediated through foraging kairomones, from food preference resulting from taste-receptor-mediated effects on patch residence time. Therefore, it was important to offer the food source in a way that allowed for the release and detection of foraging kairomones, but prevented the experimental animals from accessing the food itself. The food choice assays were performed in an aquarium (320 × 170 mm, 180 mm deep, total volume 10 l) in a climate-controlled room at 20°C. The aquarium was filled with 1 l of filtered (0.45 µm)

Table 1 Composition of the synthetic VOC mixtures offered to *R. ovata* in the food choice assays

VOC	Supplier and product no.	Complete mixture[a] (µg per container)	C_5 mixture (µg per container)	C_7 compound (µg per container)
1-Penten-3-one	Aldrich E5,130-9	85	85	–
1-Penten-3-ol	Aldrich P860-2	84	84	–
2(*E*)-Pentenal	Aldrich 26,925-5	86	86	–
2(*E*),4(*E*)-Heptadienal	Aldrich 18,054-8	0.9	–	0.9

[a] The complete VOC mixture was designed to mimic the VOCs of *U. fimbriata.*

Lake Constance water. Special containers were designed to allow the introduction of algae and samples of extracted VOCs in water without disturbing the water body (Fig. 1a,b). The containers were modifications of the olfactometers described by Thomas et al. (1980) and consisted of two cylindrical Plexiglas® rings with radial bores (5 mm diam.) near the bottom side of the ring. The inner ring (40 mm diam) was closed at the bottom by gluing a circular Plexiglas® plate to the opening. The inner ring fitted exactly into the outer ring (Fig. 1a,b). Hence, the container could be opened by rotating the outer ring, thereby matching up the bores of the outer and inner rings (Fig. 1a). Likewise, the container could be closed by rotating the outer ring so that the bores did not match up; closing the container stopped the exchange of substances between the inside and the outside of the container (Fig. 1b). One container for the test extract was placed into the aquarium 10.5 cm from the center, and another container for the control extract was placed at the opposite end of the aquarium 10.5 cm from the center. Each container received 7 ml of sample in filtered lake water at the same temperature as the surroundings; the inner and outer water levels were the same. The water level was higher than the radial bores of the containers and lower than the opening at the top of the containers. The top of each container was covered with a circular glass plate. The assay was initiated by rotating the outer rings to open the containers, and introducing five juvenile *R. ovata* at the center of the aquarium. The relative distance (±1 cm) of each individual snail to both containers was recorded every minute for a total time period of 40 min. The initial scoring value for each snail at the beginning of the experiment (equal distance to both containers) was set at 0; scoring values ranged from −21 (closest to the control) to +21 (closest to the test sample). After opening the containers containing samples, the dissolved substances could leave the containers through the bores. This was verified by analyzing the aquarium water 30 min after opening a container containing 1-penten-3-one: 16.6% of the compound (1.7 μg l^{-1}) were released into the surrounding water body of the aquarium within half an hour.

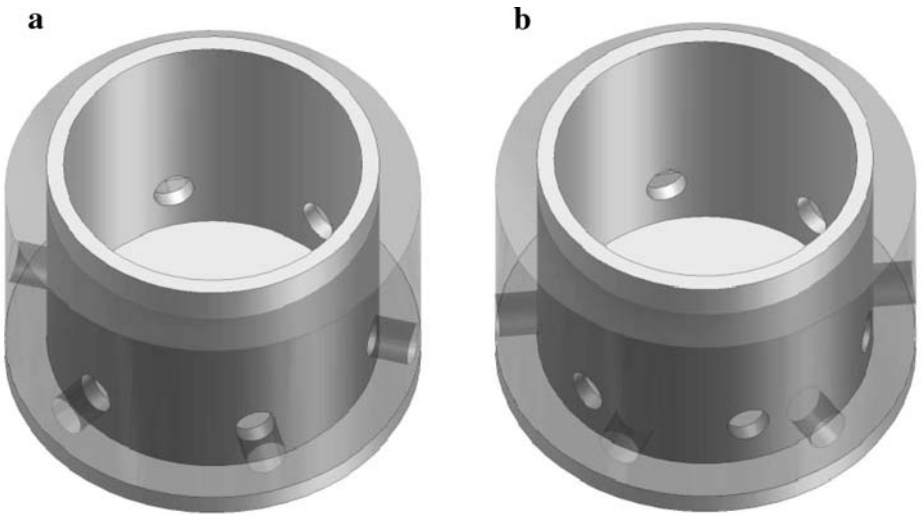


Fig. 1 Schematic drawing of the containers used in the food choice assays. (a) Open position. (b) Closed position

Statistical Analyses

Because the five individual snails in each experiment were not independent from each other, the mean of their distribution was calculated for every reading at intervals of 1 min. These mean values were plotted against the reading time and treated as one replicate experiment. All experiments were repeated at least five times, resulting in experimental series with $N = 5–10$ for each treatment. Between replicate experiments, the sides of the test and control containers were exchanged to exclude directional effects introduced by the experimental setup. In a series of control experiments ($N = 14$), both containers were filled with filtered lake water. The distribution of the snails in this control series was tested against the distribution of the snails in the test series, in which one container contained the test sample and the other contained a control sample. Data were tested to meet assumptions for analysis of variance (ANOVA; normal distribution, homogeneity of variance, Levene's test) and, subsequently, results were compared by using repeated-measurement ANOVA and the GLM module of Statistica® v.6 software package (StatSoft, Inc., 2004) and a significance level of $\alpha = 0.05$.

Results

Analysis of VOCs Released by *U. fimbriata*

Although intact *U. fimbriata* cells did not release any detectable VOCs into the surrounding water, freeze–thawed *U. fimbriata* cells released a variety of VOCs (Table 2). The most dominant group detected was lipoxygenase products released upon cleavage of polyunsaturated fatty acids, e.g., 1-penten-3-one, 1-penten-3-ol, (*Z*)-2-pentenal, (*E*)-2-pentenal, (*E,Z*)-2,4-heptadienal, and (*E,E*)-2,4-heptadienal. Minor compounds were the nor-carotenoids 6-methyl-5-hepten-2-one, α-ionone, β-ionone, and β-cyclocitral (Table 2). These minor compounds resulted in peak areas of <10% of the peak area of the major compounds and were not quantified in detail. We determined the concentrations of the major VOCs released by *U. fimbriata* upon cell disintegration (Table 3). The C_5 compounds 1-penten-3-one, 1-penten-3-

Table 2 VOCS liberated from *U. fimbriata* upon freeze–thawing

Compound group	VOC	M (g mol^{-1})	Rt (min)
Fatty acid pathway	1-Penten-3-one	84	10.03
	1-Penten-3-ol	86	10.71
	2(*Z*)-Pentenal	84	12.44
	2(*E*)-Pentenal	84	12.83
	2(*E*),4(*Z*) -Heptadienal	110	21.62
	2(*E*),4(*E*) -Heptadienal	110	22.06
	Nonanal	142	24.35
	Pentadecane	212	34.12
Isoprenoid pathway	6-Methyl-5-hepten-2-one	126	21.15
	β-Cyclocitral	152	27.35
	Geranylacetone	194	33.59
	α-Ionone	192	34.41
	β-Ionone	192	35.08

[a] Compounds are given with their molecular mass (M), grouped by their biosynthetic pathways, and sorted by retention time (Rt).

ol, and (*Z*)2-pentenal were the dominant compounds, e.g., at concentrations of up to 0.9 μg per mg algal carbon (1-penten-3-ol). (*E*)-2-pentenal, (*E,Z*)-2,4-heptadienal, and (*E,E*)-2,4-heptadienal were released at concentrations 10.4–18.7% of that of 1-penten-3-ol (Table 3).

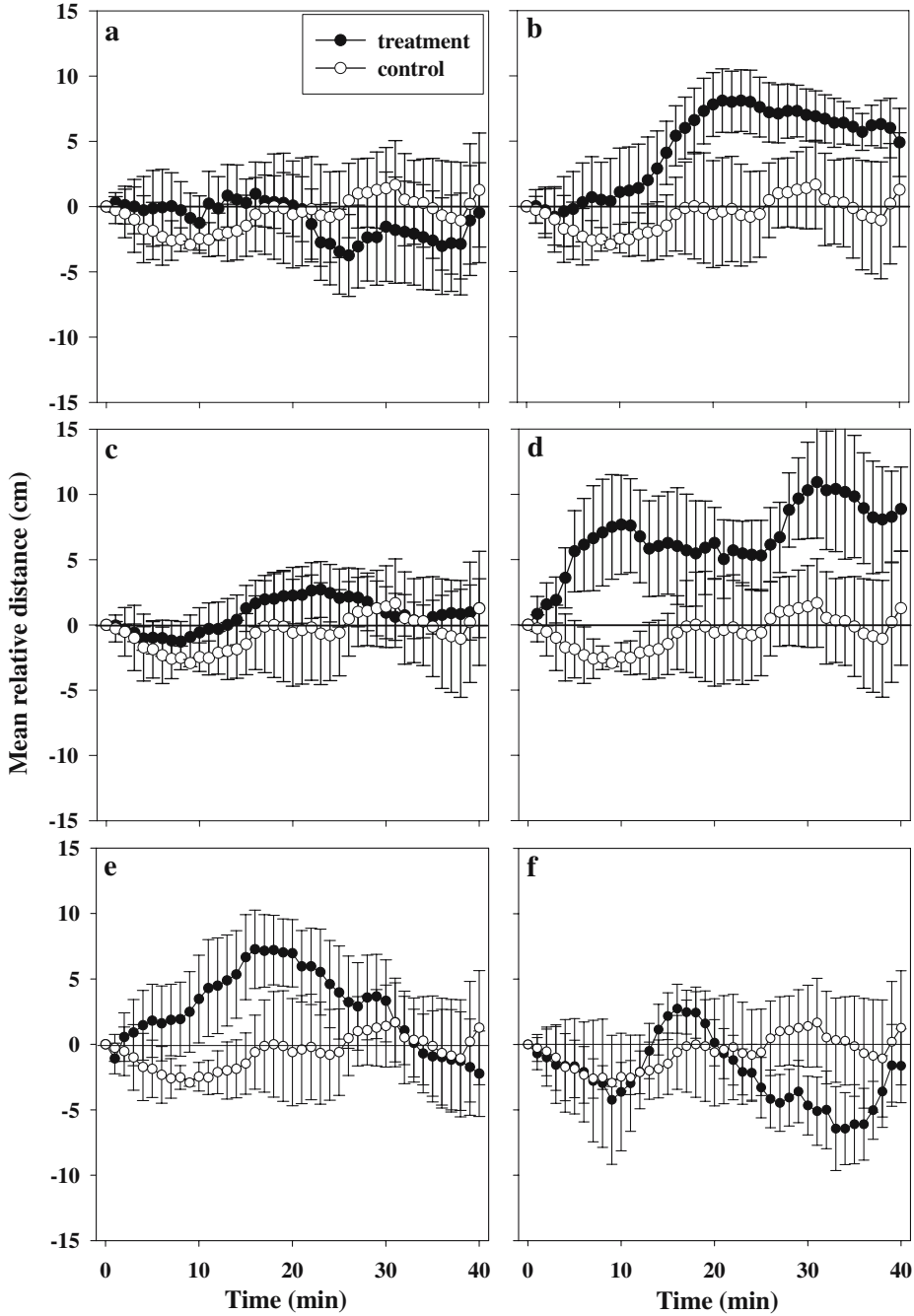
Food Choice Experiments with *R. ovata*

The results obtained in food choice experiments with intact and damaged *U. fimbriata* cells offered to *R. ovata* corroborated the hypothesized VOC release mechanism and the results of the VOC analysis: When a suspension of intact *U. fimbriata* cells was added to the test container and filtered lake water was added to the other container, juvenile *R. ovata* did not show any positive or negative chemotactic response to the algae (Fig. 2a; Table 4). Because intact algal cells did not release VOCs, the results indicate that the snails did not respond chemotactically to any other potential chemical gradient formed by the algae. Similarly, no response occurred when the supernatant of an exponentially growing culture of *U. fimbriata* was placed in the test container (Table 4), which indicated that exudates of actively growing cells do not evoke a chemotactic response. However, a positive chemotactic response was observed when VOCs released from the same amount of biomass of disintegrated *U. fimbriata* cells (trapped and eluted from Tenax TA), were placed in the test container; that is, the snails preferred the VOCs over the control (Fig. 2b; Table 4). This attraction was not caused by possible contaminants introduced during cultivation of the algae or by the VOC extraction process, because an equivalent VOC extract of aerated, sterile culture medium without algae was not preferred over the control (Fig. 2c; Table 4). Hence, the attraction of the snails clearly depended on the VOCs released from *U. fimbriata*. As expected from the results of VOC analysis, *R. ovata* preferred a synthetic mixture of pure C_5 and C_7 compounds designed to mimic the bouquet of released algal VOCs (Table 1) over the solvent control (Fig. 2d; Table 4). To determine whether *R. ovata* was attracted to the C_5 compounds or the C_7 compound or all, either a mixture of the three C_5 compounds or the C_7 compound 2(*E*),4(*E*)-heptadienal was offered (Table 1). Neither the mixture of C_5 compounds nor 2(*E*),4(*E*)-heptadienal alone significantly attracted *R. ovata* (Fig. 2e,f; Table 4). The results of the assays indicated that a mixture of C_5 and C_7 VOCs was needed to attract *R. ovata*, rather than a mixture of the C_5 compounds or 2(*E*),4(*E*)-heptadienal alone.

Table 3 Quantities of the six major compounds in the VOCS bouquet of *U. fimbriata*

VOC	Rt (min)	Release (ng mg POC^{-1})	Food choice assay (μg per container)
1-Penten-3-one	10.03	536	5.4
1-Penten-3-ol	10.71	924	9.2
2(*Z*)-Pentenal	12.44	488	4.9
2(*E*)-Pentenal	12.83	173	1.7
2(*E*),4(*Z*)-Heptadienal	21.62	170	1.7
2(*E*),4(*E*)-Heptadienal	22.06	97	1.0

[a] Compounds are sorted by retention time (Rt). The amount released is given as mean of $N = 3$ replicate measurements as nanograms of compound per milligram of algal particulate organic carbon (POC). In the food choice assays, the indicated amount of VOCs extracted from 10 mg algal carbon was added to the container. As the (*Z*)-isomer of 2-pentenal and the (*E,Z*)-isomer of 2,4-heptadienal were not available, the calibration for these compounds was done using the signal strengths of the respective (*E*)- and (*E,E*)-isomers.


a
b
c
d
e
f
treatment
control
Mean relative distance (cm)
Time (min)
Time (min)
15
10
5
0
-5
-10
-15
0
10
20
30
40

Discussion

Although diatoms (e.g., Pohnert and Boland, 1996; Wendel and Jüttner, 1996; Jüttner and Dürst, 1997) and especially cyanobacteria (Jüttner et al., 1983; Jüttner 1987; Watson and Ridal, 2004) have been the subjects of numerous studies of VOC production, the ecologically important group of green algae has been widely neglected in such studies. Green algae are phylogenetically related to higher plants, and therefore might be expected to produce similar VOCs. The so-called green leaf volatiles (GLVs) of terrestrial plants consist mainly of C_6 compounds (Croft et al., 1993) and play an important role in herbivory-activated defense mechanisms (e.g., Kessler and Baldwin, 2001; Halitschke et al., 2004). The VOCs released from the benthic green alga *U. fimbriata* upon cell disintegration differed from the GLVs released by terrestrial plants (Table 2). As in higher plants, lipoxygenase products were the major reaction products. Such products arise from the degradation of polyunsaturated fatty acids (Pohnert, 2002), but C_6 compounds, such as (*E*)-2-hexenal and (*Z*)-3-hexanal, which are primarily found in GLV mixtures from leaves, could not be unambiguously identified and, therefore, are not included in Table 2. The peaks assigned to the C_6 compounds in our study were small compared with the peaks of the C_5 and C_7 compounds; therefore, these C_6 compounds are not major VOCs in *U. fimbriata*. The position specificity of lipoxygenase/hydroperoxide lyases in *U. fimbriata* differed markedly from that of higher plants. In addition to VOCs resulting from fatty acid degradation, a variety of volatile nor-carotenoids were detected that are degradation products of carotenoids (Jüttner, 1988a; Simkin et al., 2004). Among others, β-ionone was found, which is an important component of flower odors (e.g., *Viola* sp.) and was shown to be a repellent for the freshwater nematode *Bursilla monohystera* (Höckelmann et al., 2004).

Using our newly developed assay system, we showed that *R. ovata* significantly preferred VOCs liberated from damaged *U. fimbriata* cells over control extracts. Cell damage was necessary for the liberation of the infochemicals perceived by the snails because neither undamaged algae nor a culture supernatant of exponentially growing *U. fimbriata* led to a chemotactic response. These results are in keeping with current ideas on the liberation of VOCs from algal phospholipids via rapid enzymatic degradation (Jüttner, 2001). The enzyme cascade is thought to start with a wound-activated lipase that cleaves algal lipids and releases polyunsaturated free fatty acids. These free fatty acids, which can be potent toxins for benthic herbivores (Jüttner, 2001), are in part rapidly oxygenated by a lipoxygenase that introduces dioxygen into the fatty acid molecule (Gardner, 1991). The hydroperoxides obtained are cleaved by specific lyases into a volatile compound and a nonvolatile short-chain oxo-fatty acid (Pohnert, 2002). Thus, cell damage seems to be a prerequisite for the formation of volatile oxylipins in filamentous green algae.

We unequivocally demonstrated that VOCs and not other compounds released upon cell lysis were responsible for the observed attractant activity, as the eluate of the Tenax adsorbent loaded with *U. fimbriata* volatiles was clearly preferred over a control eluate.

◀**Fig. 2** Mean relative distance (±S.E.) of the snails from the two containers in the food choice assays. One container with the test extract was placed into the aquarium 10.5 cm from the center (scoring value +21) and another container with a control extract was placed at the opposite end of the aquarium 10.5 cm from the center (scoring value −21). Test extracts (•): (A) undamaged *U. fimbriata* cells (N = 7). (B) VOC extract from disintegrated *U. fimbriata* cells (N = 5). (C) VOCs extracted from aerated algal medium (N = 10). (D) Synthetic complete VOC mixture containing three C_5 compounds and 2(*E*),4(*E*)-heptadienal (N = 6). (E) Synthetic VOC mixture containing the three C_5 compounds (N = 10). (F) Synthetic 2(*E*),4(*E*)-heptadienal (N = 6). Results from a series of control experiments (both containers with filtered lake water) are plotted for comparison (○).

Table 4 Results of repeated-measurement analyses of variances on the mean positions of five juvenile *R. ovata* in the food choice experiments

	SS	*df*	*F*	*P*	
Intact cells[a]					
Treatment	359.69	1	0.381	0.545	n.s.
Error	16,972.49	19			
Time	395.20	39	0.418	0.999	n.s.
Time × treatment	1106.98	39	1.172	0.221	n.s.
Error	16,998.87	702			
VOC extract[b]					
Treatment	3959.27	1	4.575	0.047	*
Error	14,712.65	17			
Time	2019.12	39	3.205	<0.001	***
Time × treatment	1234.34	39	1.959	<0.001	***
Error	10,710.56	663			
Aer. medium[c]					
Treatment	507.53	1	0.622	0.438	n.s.
Error	18,752.72	23			
Time	843.75	39	0.992	0.486	n.s.
Time × treatment	491.19	39	0.577	0.983	n.s.
Error	19,565.31	897			
Compl. mix.[d]					
Treatment	9239.39	1	8.501	0.009	**
Error	19,564.60	18			
Time	1449.77	39	1.501	0.027	*
Time × treatment	956.60	39	0.991	0.488	n.s.
Error	17,381.63	702			
C_5 mix.[e]					
Treatment	2715.27	1	2.185	0.154	n.s.
Error	27,344.71	22			
Time	1876.39	39	1.434	0.043	*
Time × treatment	2608.89	39	1.994	<0.001	***
Error	28,768.75	858			
C_7 compound[f]					
Treatment	498.39	1	0.623	0.440	n.s.
Error	14,397.08	18			
Time	753.09	39	0.804	0.799	n.s.
Time × treatment	1908.12	39	2.037	<0.001	***
Error	16,863.48	702			
Supernatant[g]					
Treatment	338.56	1	0.411	0.530	n.s.
Error	14,007.82	17			
Time	842.80	39	1.125	0.280	n.s.
Time × treatment	2341.34	39	3.125	<0.001	***
Error	12,738.88	663			

[a] Choice between control and intact cells of *U. fimbriata* ($N = 7$).

[b] Choice between control and VOC extract of *U. fimbriata* ($N = 5$).

[c] Choice between control and VOC extract of aerated WC medium ($N = 11$).

[d] Choice between control and the complete VOC mixture ($N = 6$).

[e] Choice between control and the synthetic C_5 mixture ($N = 10$).

[f] Choice between control and the synthetic C_7 compound ($N = 6$).

[g] Choice between control and the supernatant of an *U. fimbriata* culture ($N = 5$).

[h] All analyses compared the treatment (choice between a given test treatment and a control) with a series of control experiments ($N = 14$) in which snails had to choose between two containers with filtered lake water. Asterisks indicate significant differences at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***); n.s.: not significant.

Only volatile organic compounds adsorb to Tenax TA, so these compounds must have been responsible for the attractant activity of the eluate. This was further supported by the finding that a synthetic mixture of pure reference compounds designed to mimic the VOC bouquet of *U. fimbriata* was also highly attractive to *R. ovata*. Because a mixture of the three major C_5 VOCs and the major C_7 compound alone did not attract *R. ovata*, a multicomponent odor is required for the response of the snails. This type of response is remarkably similar to that of the benthic freshwater nematode *B. monohystera* (Höckelmann et al., 2004), which responds to a bouquet of cyanobacterial VOCs, but not to single compounds. In terrestrial systems, multi-component odors are also frequently more effective in eliciting responses in insects than single compounds (Metcalf, 1987). The sesquiterpene β-ionone was a minor constituent of the VOC bouquet released by *U. fimbriata*. It was not tested whether it has an effect on the behavior of *R. ovata* as has been observed for nematodes (Höckelmann et al., 2004). However, as the complete VOC bouquet extracted from *U. fimbriata* and the synthetic mixture of the four most abundant compounds tested showed similar attractant activity, we believe that β-ionone and other minor compounds contributed neither significant attractant nor repellent activity to the observed effect.

In some of the repeated measurement analyses, there was a significant effect of the factor time and/or significant time × treatment interactions (Table 4). This is due to the fact that the response of the snails is neither immediate nor persistent. When the animals are introduced into the aquarium, they need a while to detect and respond to odor cues in their environment. Then, the behavioral response develops gradually, causing the effect of and the interaction with the factor time. In the assays without an attractant odor source, the snails start a so-called "random search" behavior (Streit, 1981), which leads to high fluctuations in the distribution of the animals, and causes the significant interaction terms with the factor time.

For the behavioral assays, we deliberately used the filamentous green alga *U. fimbriata*, as members of the genus *Ulothrix* are commonly found in lake periphyton assemblages (John, 2002), but do not occur in the summer periphyton of the lake where the snails were sampled (M. Kahlert, pers. comm.). Therefore, snails had probably not encountered the odor bouquet of *U. fimbriata* before. These bouquets seem to be species specific (Wendel and Jüttner, 1996; Höckelmann and Jüttner, 2004). This choice allowed us to focus exclusively on genetically fixed abilities for food location, as snails are known to be capable of learning to respond to stimuli they have previously encountered (Croll, 1983). For the terrestrial gastropod *Helix pomatia*, preconditioning to odors even seems to be essential for attraction responses (Teyke, 1995). Thus, snail food preferences are influenced by both innate factors and olfactory learning (Croll and Chase, 1980). Additionally, freshwater gastropods are able to adapt their digestive enzymes to optimally suit the digestion of the most abundant food source (Calow and Calow, 1975; Brendelberger, 1997). Hence, the response in natural systems could be much higher if olfactory learning and conditioning of the array of digestive enzymes play a role. The response observed might be a rather conservative estimate of the potential of VOCs to induce food-finding behavior.

Diatoms are known to produce polyunsaturated straight chain and cyclic hydrocarbons, aldehydes, and alcohols (Pohnert and Boland, 1996; Wendel and Jüttner, 1996), some of which function as pheromones in brown algae (Müller et al., 1971). This interaction can possibly be explained by the phylogenetic relationship between brown algae and diatoms (Pohnert and Boland, 1996). Recently, polyunsaturated aldehydes from diatoms have been shown to act as repellents for crustacean grazers (Jüttner, 2005). To our knowledge, no results have been published either on the possible role of eukaryotic VOCs in interspecific communication or on the VOCs released by (benthic) green algae. Especially green algae

have been largely neglected in the context of biogenic VOCs despite their considerable importance in the field (Stevenson et al., 1996).

The results of our study provide the first indications that VOCs from damaged green algae serve as a food-finding cue for freshwater benthic herbivores. Certainly, VOCs are not the only group of potential infochemicals liberated upon cell damage. Various other organic compounds, e.g., sugars, amino acids, and other short-chain carboxylic acids (e.g., propionic acid and butyric acid), have been described as attractants (Thomas et al., 1980; Thomas, 1986) and feeding stimulants (Thomas et al., 1986, 1989) for (tropical) freshwater snails. Some of these might also play a role in the chemical orientation of gastropod species in temperate latitudes. However, *R. ovata* was not attracted to butyric acid and (chironomid) carrion (P. Fink, unpublished results), probably because *R. ovata* feeds almost exclusively on periphyton and to a lesser degree on detritus (Calow, 1970; Lodge, 1986), and usually not on dead animal tissue that could release significant amounts of carboxylic acids and amino acids. Furthermore, in the heterogeneous benthic environment, many organisms and processes probably release such substances that do not necessarily indicate a food source to *R. ovata*. Algal VOCs are distinguishable from such diverse sources, and might, therefore, be the more appropriate food-finding signal for *R. ovata*. The effectiveness of VOCs as foraging kairomones is further supported by our observation that the concentrations sufficient for a chemotactic response of the snails are about an order of magnitude lower than the effective minimal concentrations of dissolved amino acids (Thomas et al., 1983). This difference is not surprising because, from terrestrial systems, it is known that the detection limit of invertebrates for volatile infochemicals is remarkably low (Harborne, 1995).

In terrestrial plant–herbivore interactions, it has repeatedly been shown that the liberation of volatile lipoxygenase products requires the damage of cells to trigger the enzyme cascade responsible for the release of VOCs. Apart from herbivory (Kessler and Baldwin, 2001), pathogens and senescence (Batten et al., 1995) are known to lead to the liberation of volatile lipoxygenase products. The release of volatile infochemicals from benthic algae under natural conditions can be caused by a variety of mechanisms. In laboratory experiments, intact algal cells do not release any lipoxygenase products (Pohnert, 2000; Jüttner, 2001; this study). However, this is a rather artificial situation, as natural periphyton communities are subject to a constant and high turnover of energy, nutrient, and biomass (Lamberti et al., 1995; Steinman et al., 1995), which contributes to their high productivity and ecological importance for littoral food webs (Klumpp et al., 1992; Pinckney and Zingmark, 1993). This turnover unavoidably involves damage and lysis of benthic algal cells. Thus, senescence and mechanical damage by hydrodynamic forces (Cattaneo, 1990; Watson and Ridal, 2004) and resultant release of VOCs into the water (Watson and Ridal, 2004) are likely to occur in natural environments. Furthermore, algal cells can become infected with parasitic fungi (Van Donk, 1989) or viruses (Reisser, 1993), resulting in increased cell lysis. Thus, a constant release of algal degradation products is expected in any natural biofilm community.

Another potentially important release factor is the grazing of herbivores. Similar to findings from terrestrial plant–herbivore interactions, Durrer et al. (1999) have shown that grazing by herbivorous cladocera on planktonic cyanobacteria leads to the release of significant amounts of VOCs in both the field and the laboratory. Likewise, when snails or other herbivorous invertebrates graze on an algal biofilm in the benthos, algal cells will rupture and release VOCs. Other snails would then be able to detect this food patch. A similar mechanism has been proposed for the mangrove snail *Terebralia palustris*, where active feeding of individual snails on leaf litter leads to the release of odor compounds and,

subsequently, to the attraction of conspecifics (Fratini et al., 2001). In this study, we found such an attraction using a widely distributed (ITIS, 2004) and often highly abundant snail species and a benthic green alga commonly found in lake littoral zones as model organisms. Probably, such a VOC-mediated attraction is not restricted to these species but rather represents a new, so far not investigated means of chemical communication that generally applies to gastropod–algae interactions. Such behavioral response of gastropods to volatile lipoxygenase products (or oxylipins; Pohnert et al., 2002) might also explain the frequently observed patchy occurrence of snails in littoral zones (Lodge, 1986; Klumpp et al., 1992; P. Fink, pers. obs.). The results reported here suggest that VOCs are not only important information-transmitting cues in terrestrial ecosystems (Metcalf, 1987), but also in (benthic) freshwater habitats.

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